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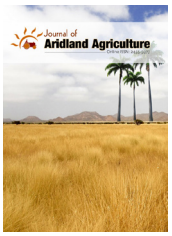

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Carum carvi mediated green synthesis of copper nanoparticles and its effect on *Solanum lycopersicum* seedlings

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ABSTRACT

The present study aims to synthesis the copper nanoparticles (CuNPs) and their effect on the biochemical and physiological characteristics of *Solanum lycopersicum*. The results exhibited the color change in the *Carum carvi* aqueous extract from clear deep brown to a greenish color and this observation indicated the green-biosynthesis of CuNPs by reduction of Cu^{+} to Cu^{0}. Also, the absorbance broadening band for the green-biosynthetic CuNPs appeared at the 340 nm wavelength using UV-Vis but the *C. carvi* aqueous extract not showed any peaks at this wavelength. SEM analysis exhibited the micrographic surface morphology and the shape of the green-biosynthetic CuNPs with a scan area of 50 µm and showed the spherical shape particles of CuNPs aggregation. The three-dimensional image and the surface morphology of green-biosynthesized CuNPs and *C. carvi* aqueous extract were examined using AFM analysis that showed the surface of *C. carvi* aqueous extract was 45.5 nm size with non-homologus and irregular form of distribution, but the green-biosynthesized CuNPs were 12.4 nm size in nanoscale with regular and homogenous distribution form. The results also showed that the effect of bio-synthesized copper nanoparticles was evident on the *S. lycopersicum* seedlings fresh and dry weight according to the different reading times after treatment with nanoparticles. Also, the concentration of 2.5 mg/mL (CuNPs) showed a significant increase in the chlorophyll content (58.51 µg/cm^2) on the 21st day after treatment and a significant increase in the activity of peroxidase enzyme (35.12 U min^{-1} mg^{-1} protein) was obtained at the concentration of 2.5 mg/mL (CuNPs) at 21st day after germination.

KEYWORDS: Copper nanoparticles, *Solanum lycopersicum*, *Carum carvi*, Green-Synthesis

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INTRODUCTION

The techniques that depend on using green biological materials have been applied to manufacture well-known chemical compounds with more sustainable procedures and completely new materials. The range of nanosized particles can be produced by the greener routes, such as nanoparticles of non-metals, metals or their salts and oxides. Some non-contaminating physical methods used in the biosynthesis routes as ultrasound-assisted, microwave heating and ball milling or hydrothermal processes. Non-hazardous solvents were used that including ionic liquids with plant extracts, yeasts, fungi, viruses or bacteria as biological sources (Kharissova *et al.*, 2019). Biosynthesis of green-nanoparticles (NPs) is cost effective, easy, advantageous and ecofriendly in relative to physical or chemical methods used in the biosynthesis procedures, different plant species used to manufacture the copper oxide or copper nanoparticles (Cu/CuO-NPs) due to the presence of the phytochemicals that act as capping agent and as stabilizing agent. These biosynthesized nanoparticles were stable and can use for organic dye degradation as methylene blue and also used for organic compound reduction such as phenols. They were also antioxidant, antibacterial and antifungal agent due to their cytotoxicity effects. They also investigated for their effects on agricultural crops (growth and productivity). Copper nanoparticles were used for increasing the growth of mung bean shoots and roots. These particles were used in the reduction of wheat shoot growth and enhancing the yield of the grain and tolerance to the stress by starch degradation. Copper nanoparticles reduce the chlorophyll, sugar content and carotenoid while anthocyanins and proline were increased in the treated seedlings of *Brassica rapa* (Siddiqi & Husen, 2020). Nanoparticle synthesis using biomaterials is one of the most up to date focuses in recent nanotechnologies or nanosciences. Plants were well known for their high dietary sources of active compounds as flavonoids for humans and in prevention, the coronary of heart disease, also act as anticancer due to having

free radical scavenging capacity and also exhibits anti-HIV functions, antimicrobial agents or chemotaxonomic markers (Amjad *et al*., 2021).

Carum carvi (caraway) plant was traditionally used in the treatment of pneumonia, diarrhea, appetizer, indigestion, stomach disorders and galactagogue, also many active compounds were extracted from caraway as a fixed oil, essential oil, monoterpenes, sesquiterpenes and many other valuable with industrial applications compounds (Mahboubi, 2019). Caraway aqueous extract was used for biosynthesizing green FeNPs and CuNPs under optimal conditions. These biosynthesized nanocomposites were detected and characterized using FTIR, UV-Vis, EDX, SEM and XRD, techniques. Also, their antibacterial activity was assessed (Heydari *et al*., 2019). So due to the importance of green nanotechnology and its applications this article aimed to Green-Synthesis of copper nanoparticles using the *Carum carvi*, and studying its effects on some of the biochemical and physiological characteristics of *Solanum lycopersicum*.

MATERIAL AND METHODS

Plant Sample Collection and Classification

Carum carvi and *Solanum lycopersicum* seeds were collected from the local market in Baghdad city and its classification was confirmed in the National Herbal Commission/General Commission for Agricultural Research.

Preparation of the Aqueous Extract

C. carvi aqueous extract was prepared through the traditional procedure that the plant materials were surface sterilized by washing repeatedly with tap water then followed by washing with sterilized $DDWH_2O$ and dried at room temperature for two weeks, then ground by a grinding-machine. The aqueous extract was prepared by maceration of 50 g of the *C. carvi* powdered in a 500 mL conical flask containing 250 mL of sterilized $DDWH_2O$. The mixture was shaked using shaker incubator for 24 hrs and warmed at 45°C using magnetic stirrer, then cooled down at room temperature. The prepared mixture was filtered using Whatman No.1 filter paper. The filtrated extract stored at 4°C for future use (Murthy *et al*., 2020).

Biosynthesis of Copper Nanoparticles

During the synthesis of copper Nanoparticles, 1 mM Cu $(NO_3)_2{\cdot}3H_2O$ solution and leaf extract were prepared. For Cu^{++} ions reduction, 30 mL of *C. carvi* aqueous extract was added dropwise to 20 mL of 1 mM Cu $(NO_3)_2{\cdot}3H_2O$ solution, with continuous stirring using a magnetic stirrer. The color change was observed from a very brown color to green after 12 hrs under 45°C. The mixture was then centrifuged, the pellet was washed with water and centrifuged again and the resultant pellet was taken and dried in the oven. The prepared CuNPs were stored in a refrigerator in an airtight glass bottle (Andualem *et al*., 2020).

Preparation the Concentrations of Green-manufactured CuNPs and *C. carvi* Aqueous Extract

Five different concentrations of each of the *C. carvi* aqueous extract and the biosynthesized CuNPs were prepared as follows (0.0, 0.75, 1.5, 2.5 and 3.5 mg/mL) sequentially. The aqueous extract and the biosynthesized CuNPs were dried well and the concentrations were kept till use for the following experiments.

Characterization of CuNPs

The prepared CuNPs were firstly diagnosed visually through the observation of the color change of the mixture during the reaction and then characterized using different methods such as Field Emission Scanning Electron Microscopy (FE-SEM) that the French MIRA3 FE-SEM Scanner used to determine the size and shape of green-synthesized CuNPs. This done by placing 5 µL approximately of the ready-to-examine solutions onto a gold-carbon electron microscope holder clipped, and leaving it till drying at room temperature, then tested using different magnifying powers. Also, Atomic force microscopy (Angstrom Advanced AA2000) was used to determine the surface morphology and roughness of the prepared CuNPs, also for determine their size and diameter, that a small drop of the sample was placed on a 1x1 cm glass slide and left to dry at room temperature and ready to examine. Ultraviolet-Visible spectrometer was used in the diagnosis procedures that a sample was taken 48 hrs after preparing the green nanoparticles, and it was examined by a UV spectrometer with a wavelength of 200-1100 nm.

Sterilization of *S. lycopersicum* Seeds

S. lycopersicum plant seeds were randomly chosen and taking into account, that they have the same size, then running water was used for seed washing, soaked in 90% Ethanol alcohol for 5 sec, then 2.5% sodium hypochlorite solution (NaOCl) was used for further surface sterilization for 20 min with a continuous stirring through magnetic stirrer instrument, then seeds were washed five times with sterilized $DDWH_2O$ for 5-10 min each time with continuous (Oraibi *et al*., 2022).

Germination of *S. lycopersicum* Seeds

Sterilized *S. lycopersicum* seeds were soaked in different green-synthesized CuNPs concentrations for 25 min and sterilized $DDWH_2O$ was used as a control. Where sterilized soil was used for the germination of seeds treated with biosynthetic copper nanoparticles with 15 *S. lycopersicum* seeds for each treatment.

Fresh and Dry Weight Determination

S. lycopersicum seedlings were dried after 21 days of germination, using an oven at a temperature of 45 °C, fresh and dry weights were measured using a sensitive balance.

Total Chlorophyll Content Estimation (Shoot) µg Cm²

Total chlorophyll content in shoots (vegetative parts) was determined using Chlorophyll-meter/Spad 502 by taking the average readings of ten random leaves of the germinated *S. lycopersicum* seedlings.

Determination the Peroxidase Enzyme Activity (U min^{-1} mg^{-1} Protein)

Peroxidase enzyme activity was determined according to Bergmeyer (1974). That this procedure used hydrogen peroxide degradation rate by means of POD enzyme with Goaicol that gave hydrogen, it's determined by measuring the color evolution rate using a spectrophotometer at a wavelength of 470 nm.

Statistical Analysis

The statistical program SPSS was used for analyzing the resulting data according to the factorial experiment. The experimental design was a Completely Randomized Blocks Design (CRBD) with 30 replicates for each treatment (p=0.05).

RESULTS AND DISCUSSION

Physical and Chemical diagnosis of Green-synthesized CuNPs

Results in Figure 1 exhibited the color change in the *C. carvi* aqueous extract from clear deep brown to greenish color after the addition of 20 mL of 1 mM Cu $(NO_3)_2 \cdot 3H_2O$ under stirring conditions for 12 hrs. This observation indicated the green-biosynthesis of CuNPs by reduction of Cu^+ to Cu^0. The reduction reaction allowed the presence of the active groups in the secondary chemical compounds of the plant in the aqueous extract and entry into the growth phase, leading to the formation of CuNPs that differ in their chemical and physical properties from the compounds involved in the reaction.

While the results in Figure 2 showed the absorbance broadening band for the green-biosynthetic CuNPs that appeared at the 340 nm wavelength but the *C. carvi* aqueous extract not showed any peaks at this wavelength.

While Figure 3 exhibited the micrographic surface morphology and the shape of the green-biosynthetic CuNPs indicated using scanning electron microscopy (SEM) with a scan area of 50 µm, the green-biosynthetic CuNPs showed aggregation and spherical shape particles, this method of nanoparticles characterization showed that *C. carvi* aqueous extract has a capability for the biosynthesis of CuNPs that were distributed uniformly and their shape were spherical.

Also Atomic Force Microscopy (AFM) instrument was used for the diagnosis of the characteristics of green-biosynthesized CuNPs and the results in Figure 4 revealed the three-dimensional image and the surface morphology of green-biosynthesized CuNPs and *C. carvi* aqueous extract, that surface of *C. carvi* aqueous extract was 45.5 nm size in with non-homologues and irregular form of distribution, but the green-biosynthesized CuNPs were 12.4 nm size in nanoscale with regular and homogenous distribution form.

The above results that showed the mechanism of diagnosing of the green-biosynthetic copper nanoparticles were in line with those obtained by Wu *et al.* (2020) who reported that the green-approach of metal-nanoparticles is a cost-effectiveness and eco-friendly procedures, and CuNPs was bio-synthesized using *Cissus vitiginea* plant, also their results were showed the characterization methods that used in the diagnosis of the bio-synthetic CuNPs that included using of UV-spectroscopy absorbance around 370 nm while the Scanning electron microscopy data showed the distribution of biosynthetic-nanoparticles and the particles sizes were found to be in the range of 5-20 nm.

While Mali *et al.* (2020) confirmed that the biosynthesis of CuNPs was first indicated by the mixture (plant extract and metal salt solution) color change from yellow to green color that the interaction between the conduction electrons of metal-NPs and the incident photons was responsible for color change phenomena. Also, bio-synthetic CuNPs were confirmed by the characteristic peaks obtained at 269 nm which was assessed by UV-Vis spectroscopy. They also characterized the biosynthetic-CuNPs using SEM analysis that revealed that presence of spherical particles with some agglomeration and the particles size was calculated by SEM analysis and was found to be in the range of 2-10nm size in the nanoscale with 5 nm average

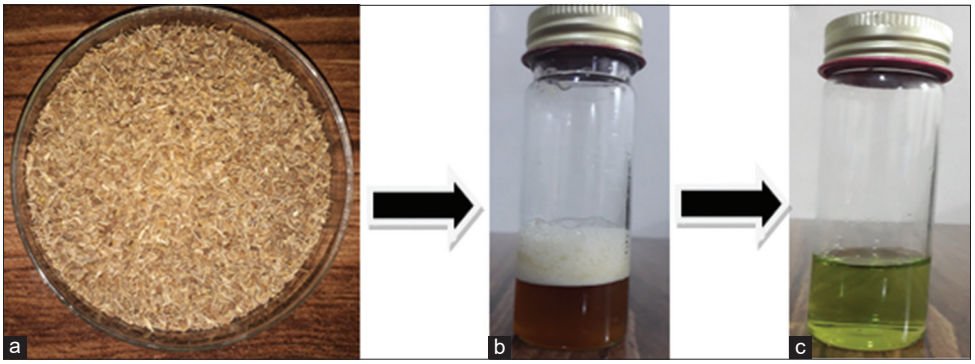

Figure 1: The Color change of *C. carvi* aqueous extract after addition of Cu (NO3)2•3H2O. a) Showed *C. carvi* seeds,b) Shown *C. carvi* seeds aqueous extract and c) Shown the green-synthesized CuNPs

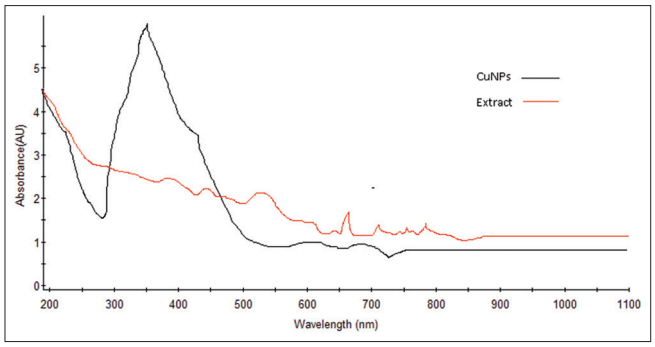

Figure 2: UV-Visible spectroscopy of CuNPs biosynthesized from *C. carvi* aqueous extract

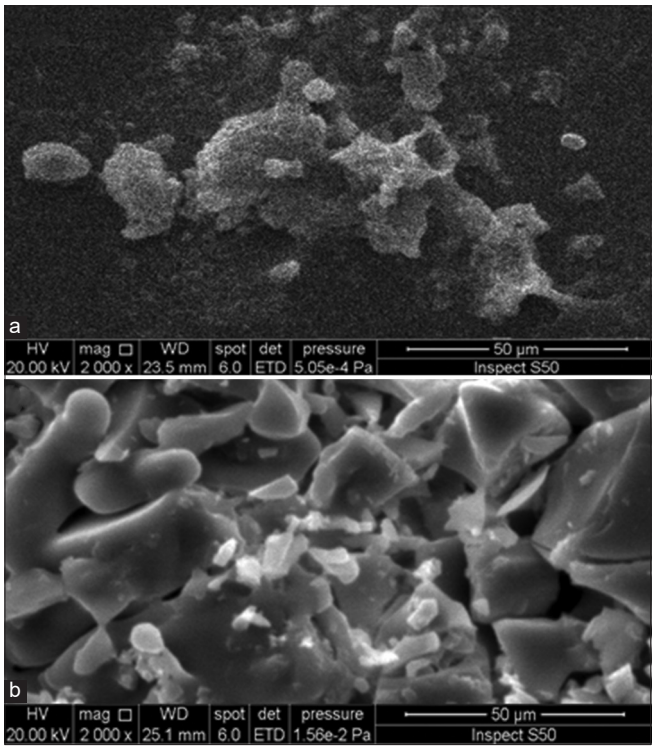

Figure 3: SEM images of green-biosynthesized CuNPs from *C. carvi* aqueous extract at 50 µm scan area: a) *C. carvi* extract; b) green-biosynthesized CuNPs

particle diameter. But, Murthy *et al.* (2020) investigated the green-biosynthesis of CuNPs using *Hagenia abyssinica* (Brace) JF leaf aqueous extract and these particles (CuNPs) were characterized using UV visible spectroscopy, SEM and more than one other technique, and showed that the maximum UV visible absorbance was found to be in 403 nm for CuNPs due to their surface plasmon resonance, they also reported that SEM micrographs showed a mix of hexagonal, spherical, cylindrical, triangular, and irregularly shaped CuNPs and the average CuNPs size was found to be 34.76 nm.

Green synthesized copper nanoparticles have attracted much attention due to their wide applications in different fields. These applications depend on their chemical and physical properties. The aqueous extract of medicinal fruit *Piper retrofractum* can be used as ecofriendly reagent in the biosynthesis of CuNPs with copper sulfate as a starting material in the reaction. *P. retrofractum* aqueous extract was employed as a capping agent and bio-reduction in the biosynthesis of CuNPs. The stirring and sonication were used for assisted the reaction process and the characteristics and structure of bio-synthesized CuNPs were diagnosed using FT-IR, UV-Vis, TEM, XRD, and SEM-EDS. UV Vis measurement showed surface plasmon resonance peaks at 234-255nm, While FTIR peaks of Cu-O were diagnosed in the range of 550-570 cm^{-1} and Cu-O-H bonds that led the bending absorptions in the region of 870-880 cm^{-1}. Bio-synthesized CuNPs have spherical shapes and high copper content recording 70.3 % as confirmed by SEM. Using a TEM-micrograph, the CuNPs particle size distribution could be seen with high uniformity and a size of 2–10 nm, the bio-synthesized CuNPs had good stability, and these results proved that *P. retrofractum* fruit aqueous extract could be applied for CuNPs green-synthesis with high uniformity of particle sizes (Amaliyah *et al.*, 2020).

Also, the use of biochemical materials in the green-synthesis of nanoparticles was one of the most up to date focuses in modern nanoscience and nanotechnologies and more researchers were focused on the green methods of manufacturing metal nanoparticles with an important goals to overcome the toxic chemicals and possible dangers for an innocuous and safe environment. Copper nanoparticles were green-biosynthesized

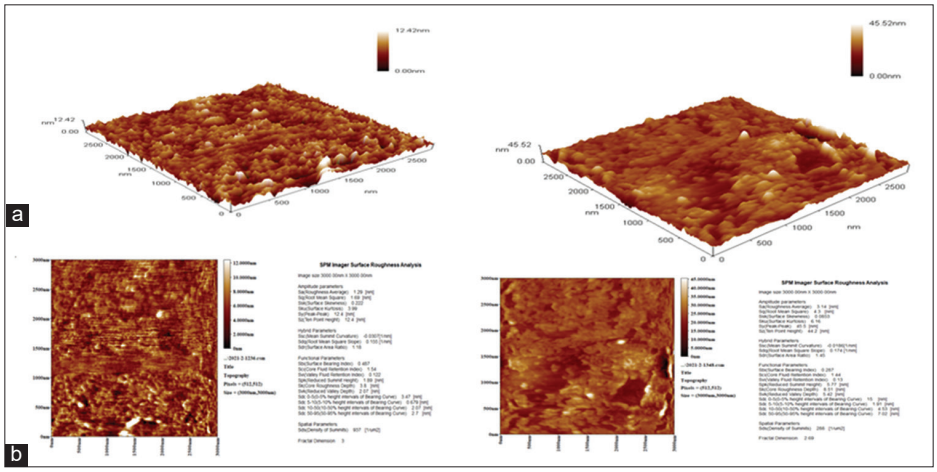

Figure 4: Three dimentional image on the surface morphology of CuNPs. a) green-biosynthesized CuNPs; b) *C. carvi* extract using AFM

using *Fortunella margarita* leaves aqueous extract and that reflected the novelty in the nano-sciences field. The visual observation of the color change from dark-green to bluish-green was clearly showed the spontaneous biosynthesis of copper nanoparticles, at which the *F. margarita* phytochemicals interact with Cu^{+2} ions. The CuNPs were characterized using UV-Vis spectroscopy, SEM and XRD. The UV-Vis analysis showed the CuNPs surface plasmon resonance properties and the characteristic absorption peak were at 679 nm, while SEM analysis exhibited the spherical and agglomerated shape of green-synthesized CuNPs with 51.26-56.66 nm size range (Amjad *et al*., 2021).

Effect of Green-biosynthesized CuNPs on Some *S. lycopersicum* Physiological and Biochemical Characteristics

The results (Table 1 & 2, Figure 5) showed a significant increase with the increase in growth days after treatment with green-biosynthetic CuNPs, where day 21 recorded a significant increase in the *S. lycopersicum* plantlets fresh weight (521.4 mg) compared to the day 10 and day 15 of plant growth after treatment, which recorded 488.8 and 441 mg respectively and regarding the effect of different concentrations of green-biosynthetic CuNPs on the *S. lycopersicum* fresh weight, the concentrations 2.5 and 3.5 mg/mL recorded a significant increase (575.6 and 477 mg, respectively) compared to the control and other concentrations of CuNPs (Control, 0.75, 1.5 mg/mL) recording 454, 442.6, 469.3 mg respectively. While the concentration of 2.5 mg/mL of CuNPs recorded a significant increase in the *S. lycopersicum* dry weight (104 mg) compared to other CuNPs concentrations and the control. Also, the interaction between the number of plant growth days after treatment with CuNPs and the different concentrations of the CuNPs recorded the highest fresh weight at the concentration 2.5 mg/mL on day 21 after treatment, where it reached 649 mg, and the highest dry weight was recorded at the same concentration on day 10 after treatment with a value of 125 mg. The effect of bio-manufactured copper particles was evident on the fresh and dry weight of the plant according to the different reading times after treatment with nanoparticles.

While the results in Table 3 showed no significant differences regarding the number of plant growth days after treatment with green-biosynthetic CuNPs and day 21 recorded the highest increase recording 52,794 μg/cm², but the effect of different concentrations of green-biosynthetic CuNPs on the chlorophyll content showed a significant increase of chlorophyll content (2.5 mg/mL) green-biosynthetic CuNPs concentration, which recorded 54.99 μg/cm², compared with 0.75, 1.5, 3.5 mg/mL for green-biosynthetic CuNPs, which recorded 47.46, 51.85 and 47.14 μg/cm² content of chlorophyll in *S. lycopersicum* plant respectively, and regarding the interaction between the effect of different concentrations of green-biosynthetic CuNPs and the number of plant growth days after treatment, the concentration of 2.5 mg/mL showed a significant increase in chlorophyll content, reached 58.51 μg/cm² in the day 21 after treatment.

Table 1: Effects of different green-biosynthesized CuNPs concentrations on *S. lycopersicum* fresh weights throughout 21 days of germination (n=30)

Days	Green-biosynthesized CuNPs concentrations (mg/mL)					Mean
	Fresh weight (mg)					
	Control	0.75	1.5	2.5	3.5	
10	501	362	610	489	482	488.8
15	374	494	372	589	376	441
21	487	472	426	649	573	521.4
Mean	454	442.6	469.3	575.6	477	
L.S.D=0.05	Days=11.6 CuNPs concentrations=15.1 Days*CuNPs concentrations=43.21					

Table 2: Effects of different green-biosynthesized CuNPs concentrations on *S. lycopersicum* dry weights throughout 21 days of germination (n=30)

Days	Green-biosynthesized CuNPs concentrations (mg/mL)					Mean
	dry weight (mg)					
	Control	0.75	1.5	2.5	3.5	
10	79	101	93	125	84	96.4
15	119	82	89	100	89	95.8
21	96	85	99	87	93	92
Mean	98	89.3	93.6	104	88.6	
L.S.D=0.05	Days=0.61 CuNPs concentrations=12.3 Days*CuNPs concentrations=41.6					

Table 3: Effects of different green-biosynthesized CuNPs concentrations on *S. lycopersicum* chlorophyll contents (μg/cm²) throughout 21 days of germination (n=30)

Days	Chlorophyll contents (μg/cm²)					Mean
	Green-biosynthesized CuNPs concentrations (mg/mL)					
	Control	0.75	1.5	2.5	3.5	
10	51.09	49.1	39.89	53.92	49.2	48.64
15	48.13	42.82	57.39	52.54	53.82	50.94
21	58.31	50.47	58.27	58.51	38.41	52.794
Mean	52.51	47.46	51.85	54.99	47.14	
L.S.D=0.05	Days=6.31 CuNPs concentrations=3.1 Days*CuNPs concentrations=12.3					

The results in table 4 exhibited that there were no significant differences in the number of germination days after treatment with green-biosynthetic CuNPs concentration, and the day 10 after germination recorded the highest value of 25.404 U min^{-1} mg^{-1} protein activity of peroxidase enzyme activity. But, the concentrations of green-biosynthetic CuNPs showed a significant increase in the activity of peroxidase enzyme at concentrations 2.5 and 3.5 mg/mL, recoding 30.18 and 28.34333 U min^{-1} mg^{-1} protein respectively. Compared with the control and other concentrations of nanoparticles (0.75 and 1.5 mg/mL), which were recorded as 18.23, 21.9 and 26.1 U min^{-1} mg^{-1} protein respectively, and with regard to the interaction between germination days after treatment and the different concentrations of green-biosynthetic CuNPs, the concentration of 2.5 mg/mL recorded the highest significant increase in the activity of peroxidase enzyme

Table 4: Effects of different green-biosynthesized CuNPs concentrations on *S. lycopersicum* peroxidase enzyme activity (U min^{-1} mg^{-1} protein) throughout 21 days of germination (n=30)

Days	Peroxidase enzyme activity (U min^{-1} mg^{-1} protein)					Mean
	Green-biosynthesized CuNPs concentrations (mg/mL)					
	Control	0.75	1.5	2.5	3.5	
10	22.82	19.27	31.29	27.93	25.71	25.404
15	13.89	28.01	25.18	27.49	29.49	24.812
21	17.98	18.42	21.83	35.12	29.83	24.636
Mean	18.23	21.9	26.1	30.18	28.34333	
L.S.D=0.05	Days=1.6 CuNPs concentrations=6.32 Days*CuNPs concentrations=13.2					

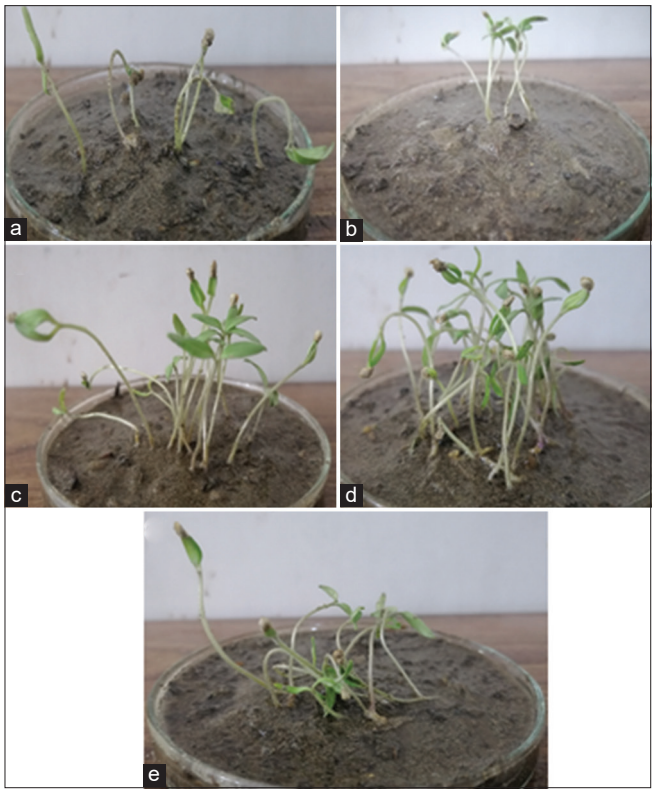

Figure 5: Effect of the CuNPs on *S. lycopersicum* growth. a) *S. lycopersicum* seeds treated with water only; b) *S. lycopersicum* seeds treated with 0.75 mg/mL CuNPs; c) *S. lycopersicum* seeds treated with 1.5 mg/mL CuNPs;d) *S. lycopersicum* seeds treated with 2.5 mg/mL CuNPs and e) *S. lycopersicum* seeds treated with 3.5 mg/mL CuNPs

on 21st day after germination, recording 35.12 U min^{-1} mg^{-1} protein.

The obtained results were in the line of Singh *et al.* (2017) who reported that the *Brassica oleracea* var and *S. lycopersicum* seedlings were exposed to different concentrations of biosynthesized CuNPs (10, 50, 100, and 500 mg/L^{-1}) and the Cu bioaccumulation was investigated by atomic absorption spectroscopy. Plants exposure to 100 and 500 mg/L^{-1} of CuO-NPs has showed a significant reduction in the sugar content and total chlorophyll contents in the two tested plants while the concentration of 10 mg/L^{-1} of Cuo-NPs increased the sugar content and pigment slightly in tomato plants only. Antioxidant enzyme activity was observed also in a dose dependent manner upon plants exposure to CuO-NPs. Lignin deposition in the roots of both plants treated with CuO-NPs highest concentration was observed also. Green-biosynthesized CuO-NPs were carried out using *Morus alba* leaf aqueous extract and the CuO-NPs accumulated more actively by *S. lycopersicum* plants as compared to cauliflower plant which may due to the difference in morphology of root system.

While Khaldari *et al.* (2021) reported that the biological methods for synthesizing the metal nanoparticles were conventional methods that use the biological resources as stabilizing and reducing (caping) agents and ecofriendly methods. They used the *Camellia sinensis* L. (green tea) leaves and *Lavandula anguistifolia* (lavender) for one step and low cost procedures for green-biosynthesis of CuO-NPs (Copper oxide nanoparticles). Also the X-ray Diffraction results (XRD), FESEM (field emission scanning electron spectroscopy) and TEM (transmission electron microscopy) revealed that the lavender was more productive in the bio-synthesis of uniform and pure CuO-NPs that recorded 50 nm. And these green-biosynthesized CuO-NPs had little inhibitory effects on some crops seed germination parameters such as germination rate, germination percentage, root and shoot length) of *Lactuca sativa* L. (lettuce), and *Solanum lycopersicum* seeds. They also reported that the green-synthesized CuO-NPs had a very effective role in the shoot and root expansion of tomato and lettuce seedlings at 4 μg/mL^{-1} (lowest concentrations).

It is very necessary for plants to uptake different nutrient elements and minerals for their growth and development and there were two classes of plant nutrients divided into micronutrients and macronutrients. Macronutrients were required generally in high concentrations and micronutrients were comparatively required in low concentrations. Both of these nutrients were essential for maintaining the normal growth and structural integrity in plants and deficiency of each causes death and disease in different parts of the plant. Copper in plants required in low concentrations due to it is a micronutrient and the high Cu concentration promotes hampered growth and toxicity in plants. Also, the chloroplast in plants contained a sufficient number of Cu, so it helps in the synthesis of chloroplast and other different plant pigments. CuNPs could stimulate shoot and root growth in *Triticum aestivum* and *Phaseolus radiaes* and this response varied typically with the varying CuNPs concentrations. When *Triticum aestivum* were treated with different CuNPs concentrations (20, 25, 30 and 35 ppm) it showed better yields and growth and the concentrations above 1000 ppm showed a growth reduction in *Triticum aestivum* and followed by such decrease in the plant yield. And it was noticed that when *Allium cepa* plant was treated with CuNPs (20 μg/mL), it enhanced the mitotic index and growth. Also, treatment of *Arabidopsis thaliana* plant seedlings with higher concentrations of CuONPs showed shoot and root growth inhibition and decreased chlorophyll contents. Recently, it was showed that the treatment with CuO-NPs increased the total phenol, defense enzymes and other defense parameters

significantly along with *Lens culinaris* plant (Chakraborty *et al*., 2022).

CONCLUSIONS

This study concluded by the synthesis of copper nanoparticles from the plant extract efficiently and with high quality properties, and the effect of these biosynthesized nanoparticles on the *S. lycopersicum* was promising through its positive effect on the various physiological and biochemical characteristics of the plants that were used in the study.

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