



Research article

Effect of zinc and copper oxide nanoparticles on the vegetative growth of *Hibiscus sabdariffa* L

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ABSTRACT

A field experiment was applied at Diyala University, College of Agriculture, Department of Horticulture and Landscape Engineering, during the spring season of 2025, to determine the effect of spraying zinc and copper nanoparticles on the growth and yield of the *Hibiscus sabdariffa* L. The experiment was conducted according to a complete block design (RCBD) with a factorial arrangement and three replications. It included two factors: the first was different concentrations of zinc nanoparticles (0, 75, 100, and 150 mg L⁻¹) and the second was copper nanoparticles (75, 50, 0, and 100 mg L⁻¹). Spraying was carried out two months after planting. The results showed that the 150 mg L⁻¹ concentration of zinc nanoparticles was superior for all traits, while the 100 mg L⁻¹ concentration of copper nanoparticles was superior for plant height and number of branches. The 75 mg L⁻¹ concentration of copper nanoparticles was superior for the number of leaves and the vegetative and dry weight of the vegetative parts.

1. Introduction

Hibiscus sabdariffa L. belonging to the Malvaceae family, is a plant of significant importance and has numerous uses. It is an important food source and a highly valued industrial crop in many countries worldwide, providing a good source of income (Al-Humaidi, 2023). Various parts of the plant are used in a wide range of foods. Fresh and dried parts are used in preparing hot and cold beverages, as well as in the production of condiments, ice cream, and chocolate, adding flavor and color to food. Plant extracts are also used in the manufacture of cosmetics such as lipsticks and perfumes (Azooz, 2009). Furthermore, it plays an important role in treating various human diseases. The extract of calyx leaves has been found to contribute to the treatment of high blood pressure and to reduce blood cholesterol levels. It also reduces blood viscosity. The dry extract of calyx leaves has been shown to have important effects against pollution-induced liver cancer (PCA), and is pharmacologically effective in reducing the carcinogenic effect of dinitrosamine in the liver.

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Nanomaterials are materials with dimensions in the nanoscale range that exhibit distinctive physicochemical properties, such as high surface area relative to volume and altered reactivity. Zinc oxide nanoparticles (ZnO-NPs) and copper nanoparticles (CuO-NPs) are of physiological importance to plant life. Zinc is an essential micronutrient for plants, playing a vital role in plant growth and cell division. It is a crucial cofactor in photosynthetic enzymes, cell membrane efficiency, stomatal opening, hormone regulation, and protein synthesis (Hassan et al., 2020). Copper is also important for plant growth and development, playing a key role in protein synthesis, electron transport in photosynthesis, and enzymes that regulate several vital processes. It contributes to cell wall formation and hormone signaling. At the cellular level, it plays a significant role in oxidation and phosphorylation processes (Kasana et al., 2017). Nanomaterials are among the most commonly used in agricultural research. This study aims to determine the effect of zinc and copper nanoparticles on improving the vegetative characteristics of *Hibiscus sabdariffa* L.

EDITORIAL NOTE – NANOPARTICLE INFORMATION REQUIRED FROM THE ORIGINAL

PRODUCT/SUPPLIER RECORD: manufacturer/source, stated particle size, stated purity, actual preparation method of spraying solutions, and number/timing of spray applications. These details are not provided in the manuscript and have therefore not been invented.

2. Materials and Methods

The experiment was conducted at the College of Agriculture, University of Diyala, during the spring season of 2025. The study investigated the effect of spraying zinc and copper nanoparticles on the vegetative characteristics of *Hibiscus sabdariffa* L. under different treatment conditions. *Hibiscus* seeds were obtained from the *Hibiscus* Development Project in Diwaniyah Governorate, Diwaniyah Agriculture Directorate.

The experimental soil was plowed twice perpendicularly with a moldboard plow, then leveled and divided into three sections. Each section contained 16 experimental units measuring 1 x 5 m². The physical and chemical properties of the soil were measured, as shown in Table 1. Each experimental unit contained four plants, with a spacing of 75 cm between rows. The soil was fertilized with phosphate fertilizer in the form of superphosphate at a rate of 160 kg ha⁻¹ in a single application before planting, followed by the addition of 100 kg/ha⁻¹ of nitrogen fertilizer, soil analysis was carried out at the Scientific Research Authority, Baghdad, Iraq.

The nitrogen fertilizer was applied in two applications: the first after germination and the second before flowering. Seeds were sown on March 29, 2025, and the field was irrigated without flooding until germination was complete. When the plants reached a height of 10–15 cm, thinning and patching were carried out. The plants were then sprayed as needed, along with all necessary maintenance practices such as hoeing and weed control. The factorial design was implemented according to a randomized complete block design (RCBD). The first factor included zinc oxide nanoparticles at four concentrations (0, 75, 100, and 150 mg L⁻¹), respectively. The second factor was copper oxide nanoparticles at four concentrations (0, 50, 75, and 100 mg L⁻¹), respectively. The lowest significant difference was measured at a probability level of 5% using the Genstat statistical software (Al-Rawi and Khalafallah, 1980).

The following traits were studied: plant height, number of branches, number of leaves, and fresh and dry weight of the plant.

Table 1. Physical and chemical properties of the experimental soil.

Ph	EC (ds/ m)	Available Elements (mg kg ⁻¹)					Organic Matter %	Soil Separators %			Soil Texture
		Nitrogen	Phosphorus	Potassium	Zinc	Copper		Clay	Silt	Loam	
8.12	0.85	0.025	11.05	358.9	4.55	2.15	2.05	19	41	40	Mixture

3. Results and Discussion

Effect of Nanoconcentrations of Zinc Oxide and Copper Oxide and Their Interactions on Vegetative Growth Characteristics of *H. sabdariffa*

3.1 Plant Height (cm)

The results of the statistical analysis in Table 2 showed that the 150 mg L⁻¹ concentration of nano zinc oxide was significantly superior, recording the highest average plant height of 149.49 cm, while the control treatment recorded the lowest average for this characteristic at 143.15 cm. Meanwhile, the 100 mg L⁻¹ concentration of nano copper oxide was significantly superior, giving the highest average plant height of 148.03 cm, while the control treatment recorded the lowest average height at 142.53 cm. As for the interaction between the levels of nano zinc and nano copper, the interaction treatment of 150 mg L⁻¹ nano zinc with 100 mg L⁻¹ nano copper achieved the highest average plant height of 154.06 cm, while the interaction treatment at the control level for both factors resulted in the lowest average plant height of 139.33 cm.

Table 2. Effect of zinc oxide and copper and their interactions on the average height of *H. sabdariffa* (cm).

zinc oxide nanoparticle (mg L ⁻¹)	copper nanoparticle (mg L ⁻¹)				Average
	0	50	75	100	
0.0	139.33	143.73	144.16	145.36	143.15
75	142.0	144.0	145.26	145.46	144.18
100	142.7	143.1	144.3	147.23	144.33
150	146.1	146.66	151.13	154.06	149.49
Average	142.53	144.37	146.21	148.03	

LSD 0.05: zinc oxide nanoparticle = 1.01 / copper nanoparticle = 1.01 / interactions = 2.02

3.3 Number of Leaves (leaves plant⁻¹)

The results showed that the concentration of 150 mg L⁻¹ of nano-zinc oxide was significantly superior, recording the highest number of leaves at 294.22 leaves plant⁻¹, while the control treatment recorded the lowest average for this characteristic at 280.20 leaves plant⁻¹. Similarly, the concentration of 75 mg L⁻¹ of nano-copper oxide was significantly superior, giving the highest average number of leaves at 286.05 leaves plant⁻¹, while the 50 mg L⁻¹ treatment of nano-copper oxide recorded the lowest average number of leaves at 282.95 leaves plant⁻¹. Regarding the interaction between the levels of zinc and nano-copper, the interaction treatment of 150 mg L⁻¹ nano-zinc with the control treatment of

nano-copper achieved the highest average number of leaves, reaching 301.90 leaves plant⁻¹, while the interaction at the control level for both factors resulted in a lower average number of leaves, reaching 276.66 leaves per plant (Table 4).

Table 4. Effect of zinc oxide and copper oxide and their interactions on the number of leaves of *H. sabdariffa* (leaf plant⁻¹).

zinc oxide nanoparticle (mg L ⁻¹)	copper nanoparticle (mg L ⁻¹)				Average
	0	50	75	100	
0	276.66	282.5	278.16	283.5	280.2
75	284.3	286.16	281.16	278.56	282.55
100	277.16	277.5	277.73	299.66	283.01
150	301.9	285.66	307.16	282.16	294.22
Average	285.01	282.96	286.05	285.97	

LSD 0.05: zinc oxide nanoparticle = 1.98 / copper nanoparticle = 1.98 / interactions = 3.97

3.4 Fresh Weight (kg/plant⁻¹)

The concentration of 150 mg L⁻¹ of nano-zinc oxide was significantly superior, recording the highest average fresh weight of the vegetative parts at 2.67 kg plant⁻¹, while the control treatment recorded the lowest average for this characteristic at 2.02 kg plant⁻¹. Similarly, the concentration of 75 mg L⁻¹ of nano-copper oxide was significantly superior, giving the highest average fresh weight of the vegetative parts at 2.36 kg plant⁻¹, while the control treatment recorded the lowest average at 2.14 kg plant⁻¹. Regarding the interaction between the levels of zinc and nano-copper, the interaction treatment of 150 mg L⁻¹ nano-zinc with 75 mg L⁻¹ nano-copper achieved the highest average fresh weight of the vegetative parts, reaching 2.92 kg plant⁻¹, while the interaction treatment at the control level for both factors gave the lowest mean fresh weight of the plant's vegetative parts, at 1.39 kg plant⁻¹ (Table 5).

Table 5. Effect of zinc oxide and copper oxide and their interactions on the fresh weight of the vegetative parts of *H. sabdariffa* (kg/plant⁻¹).

zinc oxide nanoparticle (mg L ⁻¹)	copper nanoparticle (mg L ⁻¹)				Average
	0	50	75	100	
0	1.39	2.45	2.13	2.14	2.02
75	2.45	2.33	2.19	1.92	2.22
100	1.95	2.07	2.19	2.13	2.08
150	2.77	2.28	2.92	2.73	2.67
Average	2.14	2.28	2.36	2.23	

LSD 0.05: zinc oxide nanoparticle = 0.06 / copper nanoparticle = 0.06 / interactions = 0.12

3.5 Dry Weight (g/plant⁻¹)

The results showed that the concentration of 150 mg L⁻¹ of nano-zinc oxide was significantly superior, recording the highest average dry weight of the vegetative parts at 461.01 g/plant⁻¹, while the control treatment recorded the lowest average for this characteristic at 430.35 g plant⁻¹. Similarly,

the concentration of 75 mg L⁻¹ of nano-copper oxide was significantly superior, giving the highest average dry weight of the vegetative parts at 462.23 g plant⁻¹, while the 100 mg L⁻¹ treatment recorded the lowest average at 424.08 g/plant⁻¹. Regarding the interaction between the levels of nano-zinc and nano-copper, the interaction treatment of 150 mg L⁻¹ nano-zinc with 75 mg L⁻¹ copper achieved the highest results. The nano-concentration resulted in a higher average dry weight of the vegetative parts, reaching 495.66 g plant⁻¹, while the interaction treatment at the control level for both factors yielded the lowest average dry weight of the vegetative parts, at 385.06 g plant⁻¹ (Table 6).

Table 6. Effect of zinc oxide and copper oxide and their interactions on the dry weight of the vegetative parts of *H. sabdariffa* (g plant⁻¹).

zinc oxide nanoparticle (mg L ⁻¹)	copper nanoparticle (mg L ⁻¹)				Average
	0	50	75	100	
0	385.06	479.06	432.6	424.66	430.35
75	425.86	444.96	445.8	453.73	442.59
100	485.56	385.33	474.86	384.66	432.6
150	489.13	426.0	495.66	433.26	461.01
Average	446.4	433.84	462.23	424.08	

LSD 0.05: zinc oxide nanoparticle = 3.23 / copper nanoparticle = 3.23 / interactions = 6.46

The results for the above vegetative growth characteristics show that a concentration of 150 mg L⁻¹ of nano-zinc was superior for all characteristics, while a concentration of 100 mg L⁻¹ of nano-copper was superior for plant height and number of branches. A concentration of 75 mg L⁻¹ of nano-copper was superior for the number of leaves and the vegetative and dry weight of the vegetative system. The interaction treatment of 150 mg L⁻¹ of nano-zinc with 100 mg L⁻¹ of nano-copper was superior for plant height and number of branches, and a concentration of 150 mg L⁻¹ of nano-zinc was superior to a concentration of 75 mg L⁻¹ of nano-copper for the number of leaves and the vegetative and dry weight of the vegetative system.

This is attributed to the fact that using appropriate amounts of micronutrients such as zinc leads to an increase in growth characteristics. This is due to improved absorption and translocation of plant nutrients to different parts of the plant, contributing to increased photosynthesis, which in turn promotes growth and enhances vegetative characteristics. This finding is consistent with More et al. (2018). Furthermore, nano-zinc acts as a biostimulant for the synthesis of the amino acid tryptophan, which is responsible for the synthesis of the hormone auxin. This leads to increased cell division and elongation, directly impacting plant height. Additionally, nano-copper is involved in the synthesis of the protein plastocyanin, an essential electron carrier in photosynthesis. This leads to increased leaf efficiency, dry matter production, and leaf area. The small size of the nanoparticles results in high permeability through leaf stomata compared to traditional fertilizers, thus quickly providing the plant with its needs during active growth stages (Al-Jubouri & Muhammad, 2021).

Spraying with nano-copper as a copper source also contributed to enhanced plant growth because it is more active and can penetrate the leaf epidermis, ensuring controlled release (Kumar et al., 2021). Furthermore, the plant's response to nano-copper spraying in terms of growth characteristics can be

explained by the importance of nano-fertilizers and their crucial role in plant nutrition. They increase crops' ability to withstand various stress conditions and resist diseases, in addition to achieving a better nutritional balance of these nutrients within the plant. This promotes improved plant growth due to the clear role of these nutrients in enhancing the plant's ability to utilize growth factors more effectively (Yang et al., 2016).

The significant superiority of the interaction coefficients between zinc and nano-copper in growth characteristics and the result of the cooperative effect of both elements, as zinc contributed to stimulating the building of auxins and proteins necessary for cell elongation and increasing leaf area (Al-Jubouri and Muhammad, 2021). Copper also has an important role in photosynthesis and enzyme activity, which may contribute to vegetative growth. This is consistent with what Dimkpa et al. (2019) found, that nano-elements work in functional integration to improve the overall physiological performance of the plant.

4. Conclusions

The study indicates that zinc oxide nanoparticles at 150 mg L^{-1} were the most effective zinc treatment for the measured vegetative growth traits of *Hibiscus sabdariffa* L. Copper oxide nanoparticles at 100 mg L^{-1} were most effective for plant height and number of branches, whereas 75 mg L^{-1} was most effective for number of leaves, fresh weight, and dry weight. The interaction between zinc and copper oxide nanoparticles provided an advantage for several traits, with $150 \text{ mg L}^{-1} \text{ ZnO} + 100 \text{ mg L}^{-1} \text{ CuO}$ giving the highest plant height and number of branches, and $150 \text{ mg L}^{-1} \text{ ZnO} + 75 \text{ mg L}^{-1} \text{ CuO}$ giving the highest number of leaves, fresh weight, and dry weight. These findings are limited to the conditions of the 2025 field experiment and the treatments evaluated, and should not be generalized beyond those conditions.

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