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Effects of *Hibiscus sabdariffa*, *Carica papaya*, and *Azadiractha indica* Leaves Extract Based Biosynthesized Copper Nanoparticles on the Morphological Characteristics of Salinity Induced Stress on Cowpea (*Vigna Unguiculata*) Seedlings Growth

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ABSTRACT: The objective of this paper is to investigate the effects of *Hibiscus sabdariffa*, *Carica papaya*, and *Azadiractha indica* leaves extract based biosynthesized copper nanoparticles (CuNP) on the morphological characteristics of salinity (NaCl) induced stress on Cowpea (*Vigna unguiculata* (L. Walp) var Ife brown) seedlings growth. Vine length, stem girth, leaf area, number of leaves, and number of primary branches were assessed weekly for seven weeks. The results indicated that salinity stress inhibited cowpea growth. However, the application of biosynthesized copper nanoparticles significantly enhanced growth parameters. Notably, at a low salinity level (625 ppm) and a 50% nanoparticle concentration, a considerable increase in vine length was observed in treated seedlings. Furthermore, leaf area and leaf count exhibited significant improvements compared to the control groups ($p < 0.05$). These findings highlight the potential of biosynthesized copper nanoparticles to improve cowpea growth and productivity under salinity stress, suggesting their importance in developing resilient and high-yield crops to address global food security challenges in changing environmental conditions.

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Salinity-induced stress is a common problem in agriculture, reducing crop development and output in many parts of the world. Among the different crops affected, cowpea (*Vigna unguiculata* (L. Walp)) is

vital as a staple food source, especially in Nigeria with challenging environmental conditions. In this context, the use of nanotechnology, specifically biosynthesized copper nanoparticles (Cu NPs), is a

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potential way to mitigate the negative effects of salt stress on cowpea seedlings. The escalating problem of soil salinity is largely attributed to factors such as excessive irrigation, soil degradation, and climate change, which collectively contribute to elevated levels of sodium chloride (NaCl) and other saline compounds in the soil. On a worldwide basis, soil salinity negatively impacts 950 million hectares of arable land, and of these, 250 million hectares are irrigated land (Ruan *et al.*, 2010), causing a yearly decline of \$27.3 billion (Qadir *et al.*, 2014). Cowpea, also known as the black eye pea and a member of the Fabaceae family, is a significant legume that is raised as a food and cash crop all over the world. The protein-rich cowpea is typically grown for its dry beans and green pods (Hadi *et al.*, 2012). Salinity impairs plants' capacity to absorb water, which decreases development and results in metabolic changes similar to those brought on by drought stress (Munns, 2002). Among the various environmental constraints salinity has stern effects on plant growth and development (Ahanger *et al.*, 2014). Through decreased osmotic potential, increased availability of harmful ions, and impairment of the photosynthetic machinery, salinity may have an impact on seedling germination and growth (Farooq *et al.*, 2015). Nanomaterials are substances that can be applied in stress mitigation through seed priming or foliar spray application. Rossi *et al.*, (2020) reported that Cerium-oxide nanoparticles were found to enhance absorption of minerals as well as augment photosynthetic function in *Brassica canapis* via modifying the cells within the roots. By regulating the NP-induced oxidative strain by lowering the malondialdehyde (MDA) as well as Na⁺ content alongside the lipid peroxidation of the cell structures of the plants subjected to salt stress, Karami-Mehrian *et al.* (2015) observed that the use of Ag NPs boosted the antioxidant enzymatic function in tomato plants. According to El-Sharkawy *et al.* (2017), the administration of K₂SO₄ NPs on *Medicago sativa* L. enhanced the metabolic reaction of the tomato plant to salinity stress by lowering the release of electrolytes, a process that in response raised the concentration of proline and enhanced the function of antioxidant enzymes like CAT. Mubarak *et al.* (2021) corroborated these findings in their work as they demonstrated that Zn nanoparticles can be used to mitigate the impacts of salt while promoting the growth of safflower plants cultivated at various salinity levels. Hence, the objective of this paper is to investigate the effects of *Hibiscus sabdariffa*, *Carica papaya*, and *Azadiractha indica* leaves extract based biosynthesized copper nanoparticles (CuNP) on the morphological characteristics of

salinity (NaCl) induced stress on Cowpea (*Vigna unguiculata* (L. Walp) var Ife brown) seedlings growth.

MATERIALS AND METHODS

Description of study area: The laboratory work for this study was conducted at the Environmental Biotechnology and Sustainability Research (EBSR) Group laboratory, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Field experiments were carried out in a designated shaded area at the senior staff quarters (SSQ) of the same university. The soil used in the study was sourced from a fallow area within the university campus. Flowers of *Hibiscus sabdariffa* were obtained from a commercial botanic garden, while leaves of *Carica papaya* and *Azadiractha indica* used for the biosynthesis of copper nanoparticles were collected from healthy trees within the study area (Ikhajiagbe *et al.*, 2021).

Experimental Design: Topsoil was collected, sun-dried to a constant weight, and measured into nursery bags (5 kg each). The soil in the nursery bags was subjected to the following treatments: NaCl + *Hibiscus sabdariffa* biosynthesized Cu NPs, NaCl + *Carica papaya* biosynthesized Cu NPs, NaCl + *Azadiractha indica* biosynthesized Cu NPs, NaCl alone (negative control), and distilled water alone (positive control).

Preparation of Sodium Chloride (NaCl) Treatment: Sodium chloride (NaCl) solutions at various concentrations were prepared on-site, following the procedures outlined in Mshelmbula *et al.* (2015). Salinity levels of 625 ppm, 1250 ppm, and 2500 ppm were achieved by dissolving 0.625 g, 1.25 g, and 2.5 g of NaCl, respectively, in 1 liter (L) of water. Subsequently, 250 ml of each concentration was applied to labeled bags, which were then left for 24 hours to allow for attenuation. A control experiment, without any sodium chloride treatment, was also established and appropriately labeled.

Sowing of Cowpea Seeds: After attenuation, five (5) cowpea seeds were sown in each bag at 2.5cm uniform depth. Germination was observed three days after sowing and germination readings were taken. The experimental plot was frequently weeded and irrigated with salt treatment every three (3) days.

Preparation of Leaf Extract: Leaves of *Hibiscus sabdariffa*, *Carica papaya* and *Azadiractha indica* were rinsed with distilled water to remove dust and particles, oven dried at 100°C for 5 minutes. After

which they were crushed with mortar into tiny pieces and weighed. Ten grams (10g) of each sample was transferred to a beaker containing 100ml of water and boiled for 10 mins. The extract was filtered and kept in a cool dry place as a stock solution for further synthesis of nanoparticles. The stock solution (100%) of each extract produced from the extracts prepared from each of the three leaves earlier on was further diluted with distilled water to achieve the three concentrations (v/v). These concentrations were 5% (5ml of the extracts), 25% (25ml of the extracts) and 50% (50ml of the extracts).

Synthesis of Copper Nanoparticles (Cu NP): Ten (10) ml of the plant leaf extract was added to 90ml of 10mM of copper sulphate solution and was placed on the magnetic stirrer which was stirred constantly under room temperature for 25 mins. The mixing was done until a colour change was observed by visual examination to further confirm the synthesis of Cu NP. The characterization of Cu NP was carried out using ultraviolet-visible (UV-Vis) spectrophotometer at wavelength of 200 - 600nm (Wu *et al.*, 2020).

Foliar Application: Copper NP was applied to the treatments via foliar application. The different concentration of Cu NP was applied to the plants by spraying the leaves of the plants with a known quantity of treatments (100ml) per nursery bag. This application was done to ensure all leaves were properly sprayed with the treatments.

Distribution into Treatments: The following were the treatment regimens in the study; NaCl + *Hibiscus sabdariffa* biosynthesized Cu NPs, NaCl + *Carica papaya* biosynthesized Cu NPs, NaCl + *Azadiractha indica* biosynthesized Cu NPs, NaCl alone (negative control), and distilled water alone (positive control).

Plant Morphological Traits: The following morphological characteristics were observed including the vine length, stem girth, leaf area, number of leaves, number of primary branching, fresh weight, dry weight and number of root nodules.

Data Analysis: Data obtained from the study were subjected to statistical analysis (ANOVA) using Statistical Package for Social Sciences (SPSS 20). Means were separated using Duncan's Multiple Range Test at 0.05 level of significance. Additionally, Graph Pad Prism 5 and Excel were employed to visually present the data in graphical format.

RESULTS AND DISCUSSION

Emergence of seedlings of cowpea was observed from 0 to 9 days after planting in soils treated with varying concentrations of salinity stress. Seedling emergence began three days after planting. Table 1 shows the results gotten from the germination percentage observed in the study prior to the application of nanoparticles. From the table below, reports showed that all treatments had germination, 96% (96.00 ± 2.67) seedling emergence was observed in the control, the lowest was observed in 2500ppm Sodium chloride concentration with a germination percentage of 39% (39.00 ± 6.05). Notably, as salinity concentration increased, seedling emergence percentages decreased, indicating that higher salinity levels negatively affect cowpea seed emergence. For instance, at 625 ppm salinity, emergence percentages increased from Day 3 to Day 5, then remained relatively constant from Day 6 to Day 9, suggesting a changing impact of salinity stress on emergence as seeds develop. In non-saline conditions (0 ppm), the highest emergence percentages were consistently observed; indicating cowpea seeds thrive in such conditions. The data implies a threshold beyond which salinity significantly inhibits cowpea seed emergence, as seen at 2500 ppm. Other studies support the adverse effects of salinity on seed germination and growth, emphasizing the nuanced responses of cowpea seedlings to different salinity stress treatments and the crucial role of salinity stress in shaping early growth stages (Al-saeedi *et al.*, 2017; Maamallan *et al.*, 2020).

Table 1: Effects of salinity stress (NaCl) on seedling emergence (%) of cowpea

NaCl Conc. (ppm)	3	4	5	6	7	8	9
0	23.00±2.13	38.00±1.33	54.00±3.06	58.00±3.59	60.00±2.98	62.00±4.67	96.00±2.67
625	0.00±0.00	14.00±6.70	46.00±9.45	72.00±7.42	74.00±6.70	74.00±6.70	74.00±6.70
1250	6.00±3.06	15.00±5.82	50.00±5.37	70.00±3.33	72.00±4.42	72.00±4.42	72.00±4.42
2500	6.00±3.06	20.00±7.30	32.00±8.00	36.00±7.18	39.00±6.05	39.00±6.05	39.00±6.05

The values expressed are mean $\pm$ SEM.

The findings demonstrated that when exposed to a salinity low salinity conditions (625ppm) for seven

weeks (Fig. 1), the length of the vines of all leaf extracts were significantly higher than the control

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($p < 0.05$), H.S 50% increased from 18.33 ± 0.59 cm to 102.57 ± 12.99 cm, C.P 50% from 15.57 ± 1.25 cm to 136.57 ± 12.31 cm and A.I 50% increased from 19.06 ± 0.49 cm to 154.10 ± 11.74 cm. In comparison, the control groups with saline stress (CTRL-S) and without stress (CTRL-P) saw the vine lengths progress from 14.87 ± 0.92 cm to 28.17 ± 5.42 cm and from 13.7 ± 1.27 cm to 37.56 ± 4.47 cm, respectively. At higher salinity levels, the length of vines of cowpea reduced (Fig. 2-3).

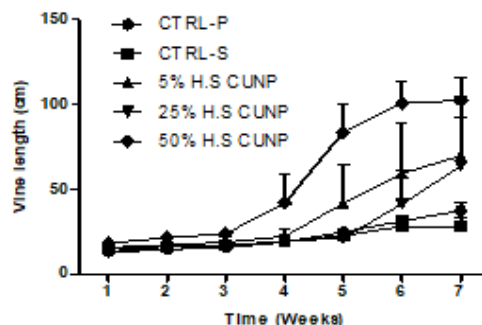


Fig. 1a: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

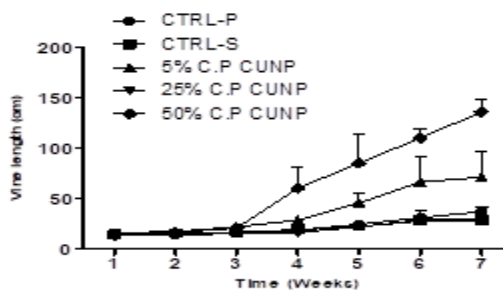


Fig. 1b: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

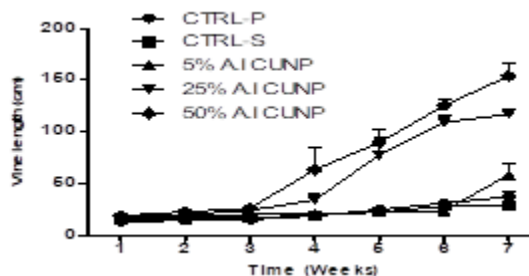


Fig. 1c: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Azadiracantha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

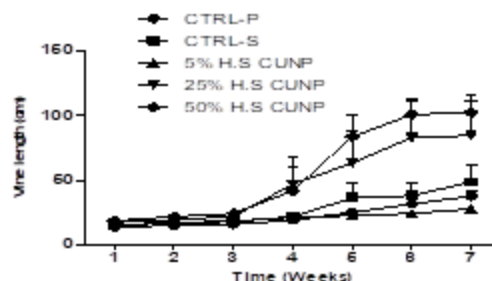


Fig. 2a: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

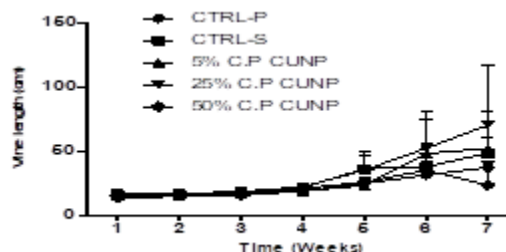


Fig. 2b: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

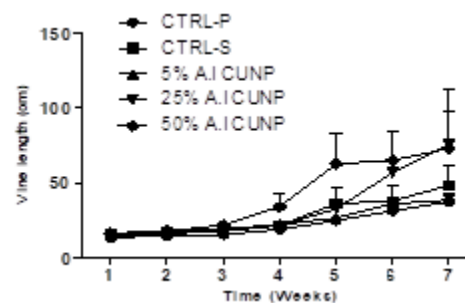


Fig. 2c: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Azadiracantha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

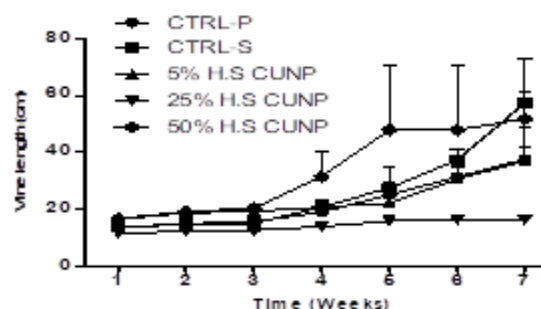


Fig. 3a: Vine length (cm) of cowpea treated with Cupper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

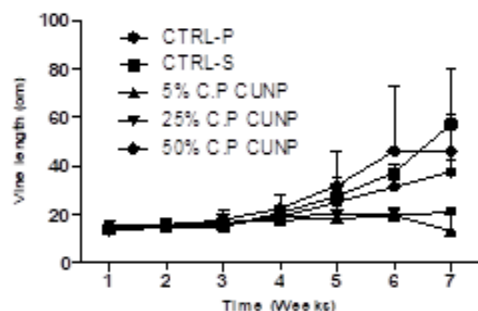


Fig. 3b: Vine length (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

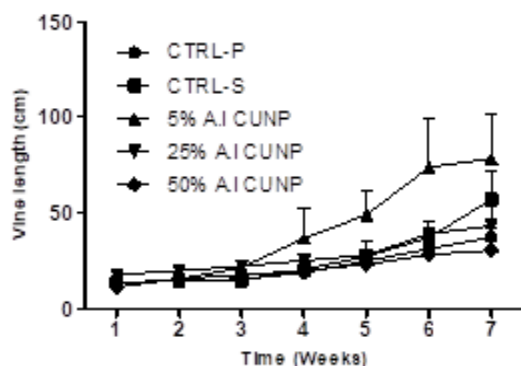


Fig. 3c: Vine length (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Azadirachta indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

In conditions of low salinity (625ppm) (Fig. 4), H.S 25% exhibited a notable increase in leaf area from 19.92 cm² to 66.04 cm². At 1250ppm salinity (Fig. 5), H.S 25% and A.I 25% showed competitive leaf areas of 52.18 cm² and 60.05 cm². Under the highest salinity (Fig 6), H.S 50% displayed the largest leaf area at 56.66 cm², while C.P fell below the control group at 52.65 cm². A.I 25% recorded a notable leaf area of 66.47 cm², surpassing both control groups.

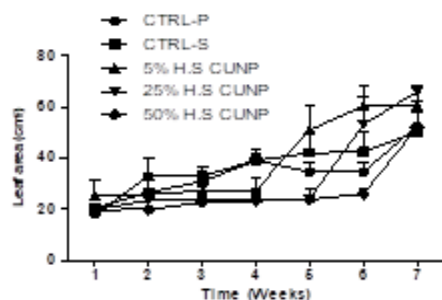


Fig. 4a: Leaf area (cm²) of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

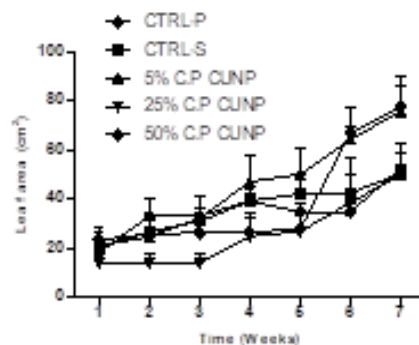


Fig. 4b: Leaf area (cm²) of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

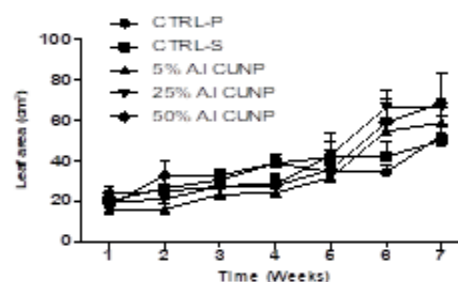


Fig. 4c: Leaf area (cm²) of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Azadirachta indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

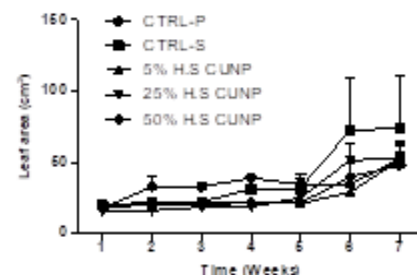


Fig. 5a: Leaf area (cm²) of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

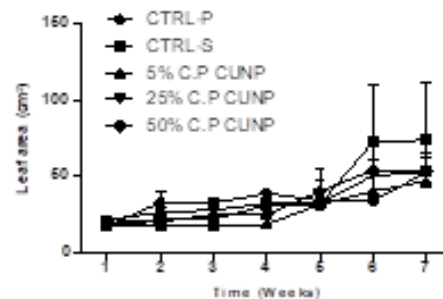


Fig. 5b: Leaf area (cm²) of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

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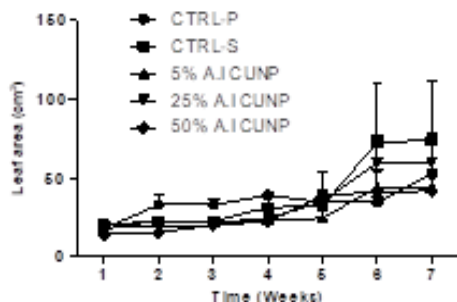


Fig. 5c: Leaf area (cm^2) of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

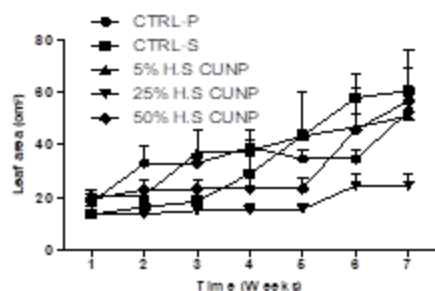


Fig. 6a: Leaf area (cm^2) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

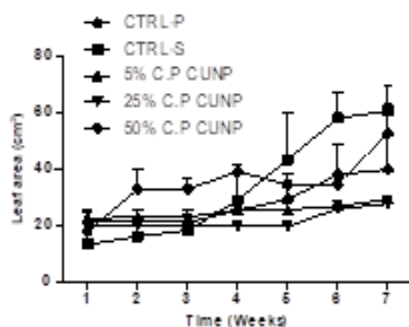


Fig. 6b: Leaf area (cm^2) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

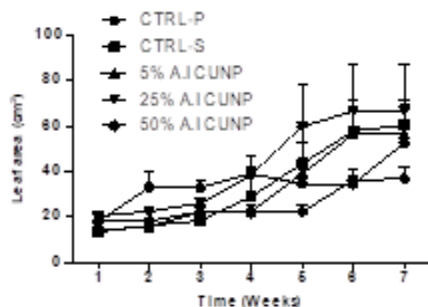


Fig. 6c: Leaf area (cm^2) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

In low salinity conditions (625 ppm), sorrel leaf extract (H.S 50%), copper nanoparticles (C.P 50%), and neem leaf extract (A.I 50%) treatments resulted in impressive vine length increases, surpassing control groups. At 1250 ppm salt concentration, 25% nanoparticle application led to the highest vine lengths. At 2500 ppm salt concentration, only A.I 5% showed significantly longer vine length. The concentration of nanoparticles from various leaf extracts consistently influenced cowpea vine length, with biosynthesized copper nanoparticles yielding the best results. Under conditions of low salinity (625ppm) (Fig. 7), both H.S (5% and 50%) experienced a substantial increase in average leaf count, reaching 15.33 ± 2.91 and 15.33 ± 2.40 , respectively, surpassing the control groups (CTRL-S and CTRL-P) which had average leaf count increases of 8 ± 1.45 and 9.66 ± 1.45 , respectively. A.I 50% also exhibited a high average leaf count of 15.67 ± 3.48 , whereas C.P 25% showed an average leaf count of 13.33 ± 3.93 , exceeding the control groups. At a higher salinity level of 1250ppm (Fig. 8), a 25% nanoparticle application led to increased leaf counts for H.S (18.67 ± 6.57) and A.I (10.33 ± 3.38), while all C.P concentrations remained lower than the control (13.67 ± 2.19). However, at 2500ppm salinity (Fig. 9), none of the nanoparticle treatments improved leaf count, possibly due to stress-induced leaf shedding during the study.

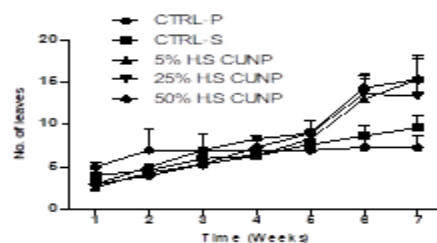


Fig. 7a: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

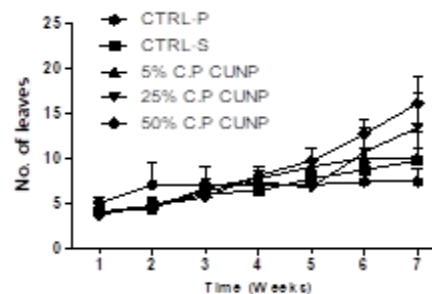


Fig. 7b: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

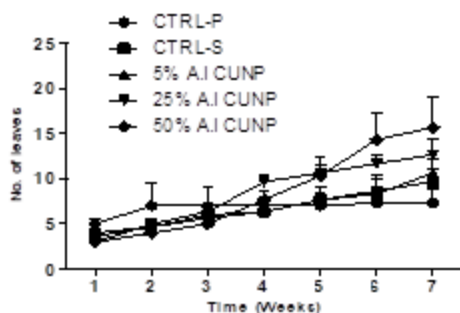


Fig. 7c: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

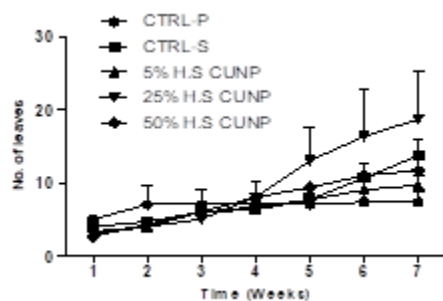


Fig. 8a: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

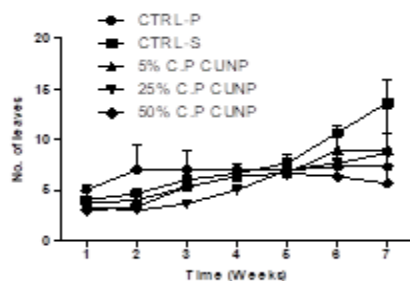


Fig. 8b: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

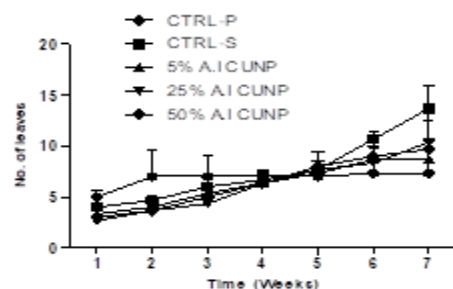


Fig. 8c: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

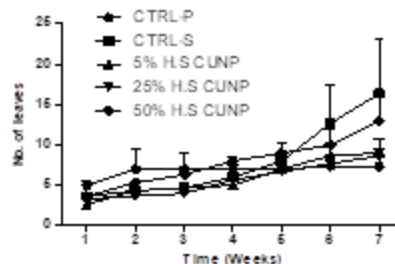


Fig. 9a: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

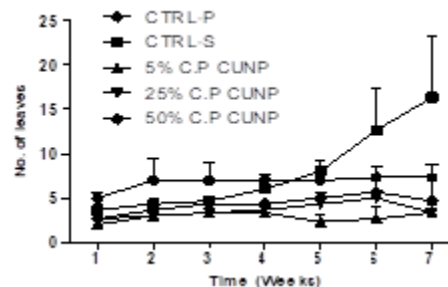


Fig. 9b: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

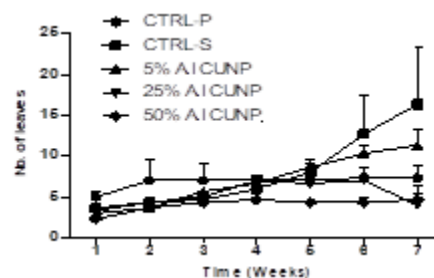


Fig. 9c: Number of leaves of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

Under low salinity conditions (625ppm) (Fig. 11), H.S 25% and C.P 50% showed higher average primary branching counts of 11.33 ± 2.96 and 13.33 ± 0.67 , respectively, compared to control groups (CTRL-P and CTRL-S) with counts of 6 ± 0.58 and 8 ± 1.16 . Conversely, A.I at 25% and 50% concentrations achieved counts of 11.67 ± 1.67 , surpassing the control count. At 1250ppm salinity, a 25% nanoparticle application led to the highest primary branching count for H.S (10.67 ± 3.18), while C.P and A.I recorded lower values than the control. At 2500ppm salinity, the impact of nanoparticle applications on primary branching was limited, except for A.I 5%, which exceeded the control count with an average of 10 ± 2.33 .

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Over the initial seven weeks post-planting, a minor increase in stem girth was observed across all treatments. Under low salinity conditions of 625ppm (Fig. 13), H.S at 25% ($2.37 \pm 0.03\text{cm}$), C.P at 5% ($2.67 \pm 0.18\text{cm}$), and A.I at 25% ($2.3 \pm 0.27\text{cm}$) exhibited average stem girths, while the control groups (CTRL-P and CTRL-S) had average stem girths of $1.5 \pm 0.12\text{cm}$ and $2.17 \pm 0.09\text{cm}$, respectively. When the salt concentration was increased to 1250ppm (Fig. 14), all nanoparticle applications showed lower values compared to the CTRL-S control, with H.S at 5% ($2.27 \pm 0.09\text{cm}$), 25% C.P ($2.1 \pm 0.21\text{cm}$), and A.I at 25% ($2.3 \pm 0.30\text{cm}$) exhibiting increases, while CTRL-S increased to $2.6 \pm 0.15\text{cm}$, which was also greater than the positive control, CTRL-P, that went from $0.5 \pm 0.12\text{cm}$ to $1.5 \pm 0.12\text{cm}$. Under the 2500ppm salt concentration (Fig. 15), the impact of nanoparticle applications on stem girth was limited, except for A.I at 5%, which exhibited an increase in average stem girth from $1.5 \pm 0.06\text{cm}$ at the first week after planting to $2.63 \pm 0.24\text{cm}$ at the seventh week, surpassing the control value for CTRL-S ranging from $1.47 \pm 0.13\text{cm}$ to $2.17 \pm 0.22\text{cm}$.

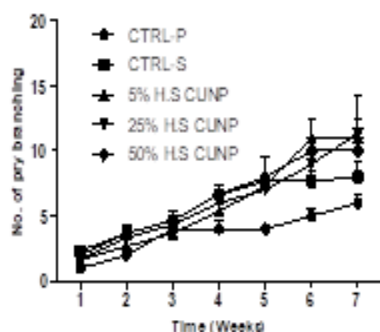


Fig. 10a: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

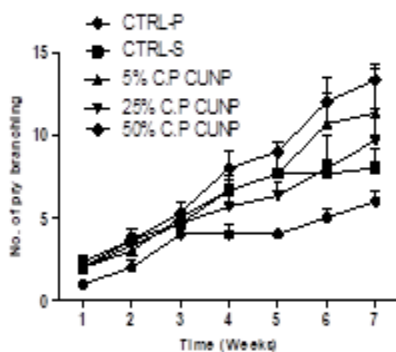


Fig. 10b: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

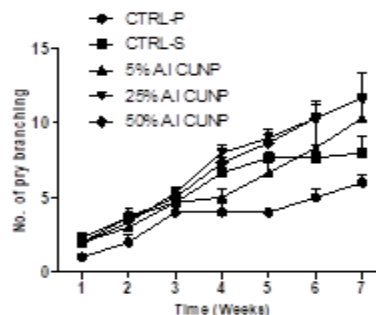


Fig. 10c: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

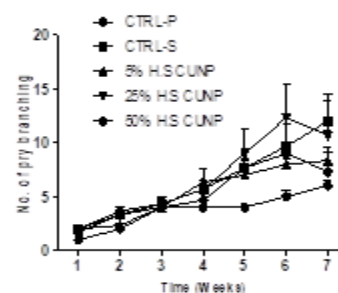


Fig. 11a: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

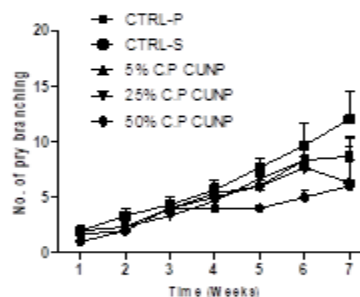


Fig. 11b: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

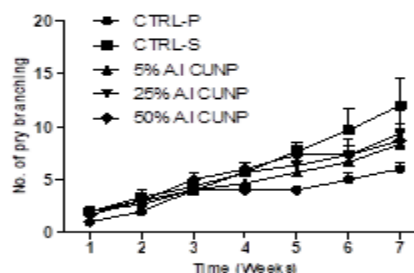


Fig. 11c: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Azadiractha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

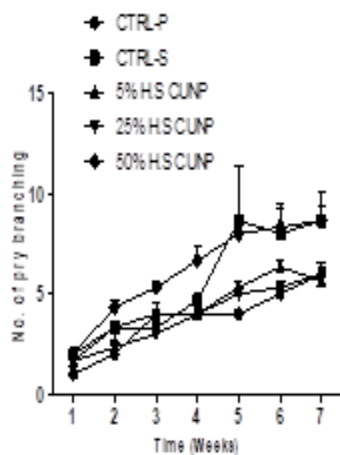


Fig. 12a: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

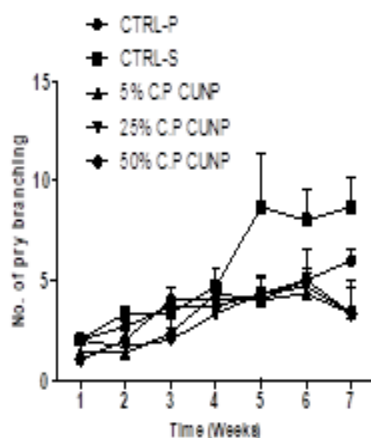


Fig. 12b: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

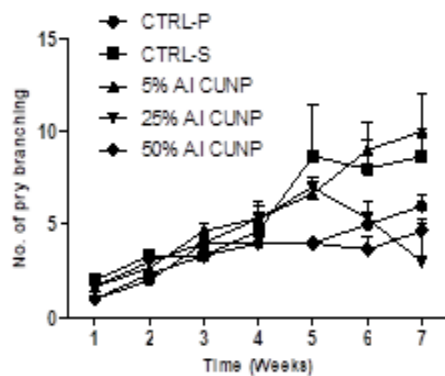


Fig. 12c: Number of primary branching of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Azadirachta indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

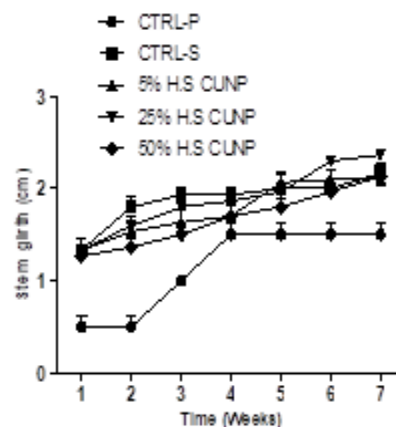


Fig. 13a: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

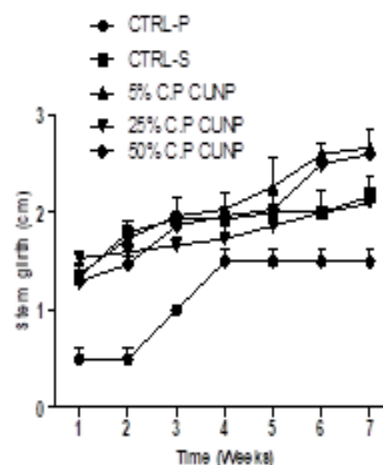


Fig. 13b: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

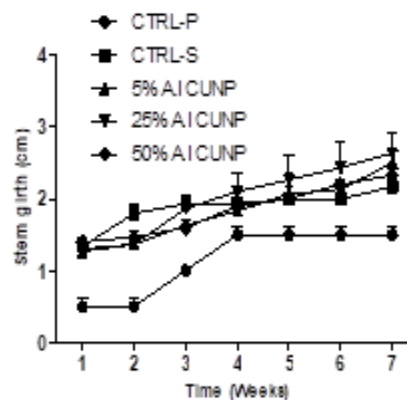


Fig. 13c: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 625 ppm NaCl concentration to form biosynthesized *Azadirachta indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

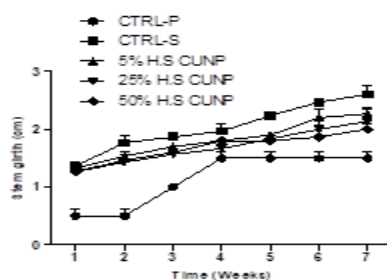


Fig. 14a: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

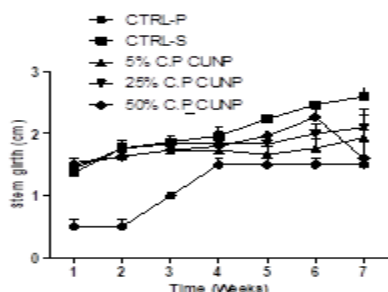


Fig. 14b: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

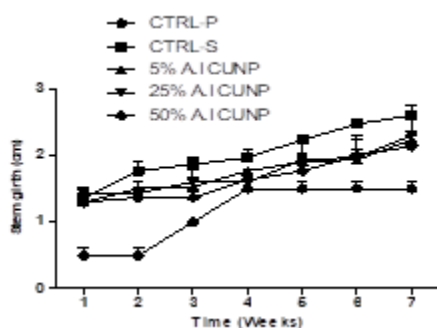


Fig. 14c: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 1250 ppm NaCl concentration to form biosynthesized *Azadiracantha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

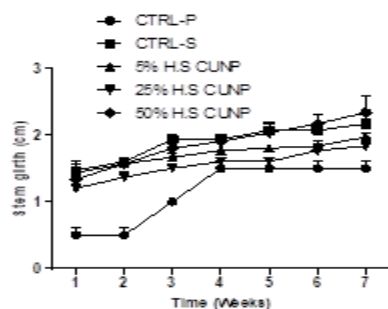


Fig. 15a: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Hibiscus sabdariffa* biosynthesized (HSCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

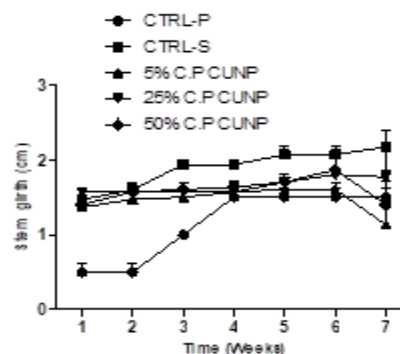


Fig. 15b: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Carica papaya* Copper Nanoparticles (CPCuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

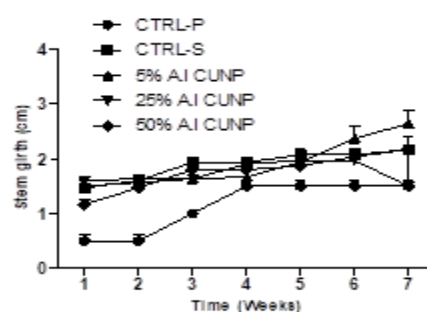


Fig. 15c: Stem girth (cm) of cowpea treated with Copper Nanoparticles (CuNP) in 2500 ppm NaCl concentration to form biosynthesized *Azadiracantha indica* Copper Nanoparticles (AICuNP) for 7 weeks. The values expressed are means $\pm$ SEM.

After harvest, various parameters including the count of primary root branches, nodules per plant, and fresh and dry root weights were examined (Table 2). Cowpea treated with 25% copper nanoparticles synthesized from *Hibiscus sabdariffa*, combined with a low salt concentration of 625ppm (H.S 25%), displayed the highest count of primary root branches at 103.33 ± 6.09 , while the untreated control without salt treatment had the lowest count at 15 ± 1.73 . In the case of cowpea treated with 50% copper nanoparticles from *Carica papaya* and exposed to a salt concentration of 1250ppm, the observed number of roots was 45 ± 0.01 . Regarding root length, H.S 25% at 625ppm exhibited the highest value of 52.73 ± 1.39 cm, while C.P 5% at 2500ppm had the lowest at 6.5 ± 0.78 cm. Differences were significant across treatments ($p < 0.05$). C.P 50% at 1250ppm had the highest nodule count (100 ± 0.01), whereas H.S 50% at 2500ppm had the lowest (2.33 ± 0.33), and nodules were absent in A.I 25% and 50% at 1250ppm. In terms of fresh weight, C.P 50% at 1250ppm recorded 7.52 ± 0.86 g, while C.P 5% at 2500ppm had 0.75 ± 0.01 g. The highest dry weight was in the untreated control (4.27 ± 0.15 g), while C.P 5% at 2500ppm had the lowest (0.14 ± 0.01 g).

Table 2(a): Root parameters of salinity-induced (625ppm) cowpea occasioned by the application of copper nanoparticles.

NaCl Treatment (625ppm)	No. of primary root branches	Root length (cm)	No. of nodules per plant	Fresh weight (g)	Dry weight (g)
CTRL-P	15.00±0.58 ^a	14.67±1.55 ^{abc}	4.67±0.33 ^{bc}	6.90±0.06 ^e	4.27±0.15 ^f
CTRL-S	87.67±18.93 ^c	24.67±2.75 ^{cd}	9.00±0.58 ^e	4.12±0.07 ^c	2.51±0.29 ^e
H.S 5%	38.00±1.16 ^{ab}	7.90±0.52 ^a	7.00±0.58 ^{de}	1.17±0.01 ^a	1.33±0.44 ^{cd}
H.S 25%	103.33±6.55 ^c	52.73±3.90 ^e	7.33±0.33 ^e	4.57±0.30 ^d	2.30±0.01 ^e
H.S 50%	40.00±5.77 ^{ab}	33.33±6.41 ^e	3.33±0.89 ^{ab}	2.01±0.10 ^b	0.75±0.11 ^{ab}
C.P 5%	25.00±2.89 ^a	15.73±0.85 ^{abc}	2.67±0.33 ^{ab}	2.20±0.06 ^b	0.87±0.01 ^{abc}
C.P 25%	31.33±5.07 ^{ab}	22.13±1.86 ^{abcd}	8.33±0.33 ^e	3.62±0.01 ^c	1.41±0.01 ^d
C.P 50%	35.00±0.57 ^{ab}	6.93±0.84 ^a	3.00±0.58 ^{ab}	2.54±0.03 ^b	1.56±0.06 ^d
A.I 5%	54.00±2.31 ^b	23.53±2.15 ^{bcd}	8.33±0.33 ^e	4.64±0.67 ^d	1.68±0.06 ^d
A.I 25%	28.33±3.60 ^{ab}	13.50±2.02 ^{ab}	5.33±1.33 ^{cd}	2.53±0.04 ^b	1.16±0.01 ^{bcd}
A.I 50%	26.33±1.64 ^a	20.00±6.67 ^d	2.00±0.58 ^a	1.83±0.10 ^{ab}	0.55±0.02 ^a
F-statistics	11.538	18.380	16.295	53.890	38.094
P-value	<0.001	<0.001	<0.001	<0.001	0.002
LSD (0.05)	5.987	2.486	0.469	0.168	0.126

Means on the same row with similar alphabets do not differ from one another. Values expressed are mean±SEM. At 0.05 level of significance, all treatments are significantly different from one another (p-value < 0.05)

Table 2(b): Root parameters of salinity-induced (1250ppm) cowpea occasioned by the application of copper nanoparticles.

NaCl Treatment (1250ppm)	No. of primary root branches	Root length (cm)	No. of nodules per plant	Fresh weight (g)	Dry weight (g)
CTRL-P	15.00±0.58 ^a	14.67±1.55 ^a	4.67±0.33 ^b	6.90±0.06 ^f	4.27±0.15 ^e
CTRL-S	35.00±2.89 ^{ab}	19.50±0.12 ^{abc}	4.00±0.58 ^b	2.64±0.17 ^b	1.60±0.06 ^{bc}
H.S 5%	41.33±0.67 ^b	16.53±0.15 ^{ab}	10.00±0.58 ^c	2.23±0.16 ^{ab}	0.93±0.02 ^{ab}
H.S 25%	35.67±1.67 ^{ab}	14.83±2.48 ^a	16.67±1.67 ^d	2.30±0.06 ^{ab}	2.00±0.58 ^c
H.S 50%	95.00±2.89 ^c	31.33±0.67 ^{ef}	11.67±1.20 ^c	3.80±0.12 ^{cd}	1.31±0.01 ^{bc}
C.P 5%	42.33±3.76 ^b	34.87±2.89 ^f	5.67±0.33 ^b	4.62±0.01 ^{de}	1.50±0.06 ^{bc}
C.P 25%	41.33±0.67 ^b	30.00±2.89 ^{cde}	11.67±1.67 ^c	2.64±0.01 ^b	1.54±0.21 ^{bc}
C.P 50%	45.00±0.01 ^b	14.60±0.31 ^a	78.33±1.67 ^e	7.52±0.86 ^f	2.86±0.14 ^d
A.I 5%	35.00±0.01 ^{ab}	26.60±2.39 ^{def}	4.33±0.33 ^b	2.98±0.37 ^{bc}	1.44±0.72 ^{bc}
A.I 25%	100.00±0.01 ^c	23.20±0.01 ^{bcd}	0.00±0.00 ^a	5.43±0.02 ^e	3.67±0.18 ^e
A.I 50%	41.00±1.00 ^b	18.50±0.29 ^{ab}	0.00±0.00 ^a	1.55±0.13 ^a	0.48±0.01 ^a
F-statistics	13.127	10.551	488.575	41.846	28.831
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (0.05)	5.402	1.680	0.740	0.228	0.160

Means on the same row with similar alphabets do not differ from one another. Values expressed are mean±SEM. At 0.05 level of significance, all treatments are significantly different from one another (p-value < 0.05)

Table 2(c): Root parameters of salinity-induced (2500ppm) cowpea occasioned by the application of copper nanoparticles.

NaCl Treatment (2500ppm)	No. of primary root branches	Root length (cm)	No. of nodules per plant	Fresh weight (g)	Dry weight (g)
CTRL-P	15.00±1.73 ^a	14.67±3.84 ^{bc}	4.67±0.33 ^{def}	6.90±0.06 ⁿ	4.27±0.15 ⁱ
CTRL-S	45.00±2.89 ^{cd}	20.70±0.65 ^{cd}	3.33±0.89 ^{cd}	3.17±0.01 ^f	1.25±0.06 ^g
H.S 5%	52.67±2.03 ^d	25.13±2.61 ^d	6.00±0.58 ^f	1.27±0.2 ^b	0.37±0.09 ^b
H.S 25%	46.00±2.0 ^{cd}	16.33±0.17 ^{bc}	3.67±0.33 ^{cde}	1.65±0.03 ^{cd}	0.72±0.01 ^{de}
H.S 50%	45.33±2.91 ^{cd}	20.97±0.55 ^{cd}	2.33±0.33 ^{bc}	2.11±0.57 ^e	1.02±0.01 ^f
C.P 5%	16.67±2.85 ^a	6.50±0.78 ^a	1.33±0.33 ^{ab}	0.75±0.01 ^a	0.14±0.01 ^a
C.P 25%	26.00±0.02 ^{ab}	14.77±2.83 ^{bc}	2.67±0.67 ^{bc}	1.58±0.01 ^c	0.72±0.09 ^{de}
C.P 50%	25.00±0.02 ^{ab}	12.07±0.07 ^{ab}	0.00±0.00 ^a	1.36±0.06 ^b	0.60±0.09 ^{cd}
A.I 5%	58.00±0.01 ^d	35.33±1.45 ^e	3.67±0.67 ^{cde}	4.83±0.03 ^g	2.98±0.01 ^h
A.I 25%	35.00±0.01 ^{ab}	17.00±0.50 ^{bc}	5.00±0.58 ^{ef}	0.89±0.06 ^a	0.49±0.06 ^{bc}
A.I 50%	35.67±1.33 ^{bc}	18.50±0.29 ^{bd}	8.67±0.33 ^g	1.86±0.08 ^d	0.88±0.05 ^{ef}
F-statistics	11.619	9.759	20.908	632.495	312.003
P-value	0.001	0.001	0.001	0.001	0.001
LSD (0.05)	3.202	1.768	0.379	0.056	0.052

Means on the same row with similar alphabets do not differ from one another. Values expressed are mean ± SEM. At 0.05 level of significance, all treatments are significantly different from one another (p-value < 0.05). KEY: CTRL-P- positive control without any treatment. CTRL-S- control with NaCl concentration. H.S, C.P and A.I- 5% - NaCl treated with *H. sabdariffa*, *C. papaya* and *A. indica* Cu nanoparticles at 5% concentration. H.S, C.P and A.I 25% - NaCl treated with *H. sabdariffa*, *C. papaya* and *A. indica* Cu nanoparticles at 25% concentration. H.S, C.P and A.I 50% NaCl treated with *H. sabdariffa*, *C. papaya* and *A. indica* Cu nanoparticles at 50% concentration.

The impact of various nanoparticles and plant extracts on cowpea vine length, a crucial factor influencing plant growth, was extensively studied. Abdelfattah *et al.* (2023) found that 200 ppm MgO nanoparticles significantly increased shoot length,

shoot dry weight, and root dry weight of cowpea plants. Similarly, Samsoun *et al.* (2022) reported substantial plant growth, increased photosynthetic pigments, and enhanced antioxidant activity when MgO nanoparticles were applied via foliar spray.

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Increased leaf area and leaf number were observed in treated plants, surpassing the control group, suggesting enhanced growth and productivity. This explains the role of biosynthesized nanoparticles in mitigating the adverse effects of salinity stress on cowpea growth, a critical consideration due to salinity's disruptive impact on plant physiological processes (Abdul Qados, 2010; Ikhajiagbe and Musa, 2020).

Conclusion: The study demonstrates the remarkable resilience and growth acceleration capabilities of biosynthesized nanoparticles, as cowpea plants treated with these nanoparticles not only thrived under saline stress but also outperformed the positive control. This aligns with broader agricultural research trends that explore innovative approaches, such as nanotechnology, to boost crop yields, especially in challenging environmental conditions. The substantial improvements in growth parameters observed in nanoparticle-treated cowpea plants emphasizes the pivotal role of these particles in promoting growth and productivity, particularly in the face of salinity stress. These findings signify the potential of nanotechnology-based strategies to contribute to the development of robust, high-yielding crops, addressing global food security concerns in an evolving environmental context. Further investigations are needed to unravel the molecular mechanisms underlying these interactions and optimize nanoparticle applications for enhancing phytonutrient production in crops.

Declaration of Conflict of Interest: The authors declare that no known conflict of interest or personal relationships that could have influence the work reported in this paper.

Declaration of Data Availability: Data are available upon request from the corresponding author.

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