

Carbon Nanotechnology-Based Sustainable Solutions for Mitigating Drought Stress in Coffee Plants

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ABSTRACT

Carbon nanoparticles (CNPs) have emerged as promising nanomaterials in agriculture because of their unique physicochemical properties. The present study investigates the impact of CNPs on the growth and physiology of a local coffee variety (*Coffea arabica* cv. Shadawi Salibi) in Saudi Arabia under drought conditions. Although several studies have examined the effects of drought on this coffee variety, fewer have focused on the impact of carbon nanoparticles, particularly concerning important factors like gas exchange and proline accumulation in coffee plants under drought conditions. Therefore, various doses of carbon nanoparticles Bulk C (0, 5, 15), CNPs(M) (0, 5, 15), and CNPs(F) (0, 5, 15) were tested to determine their potential effects on the growth and development of coffee plants. Treatment with CNPs including Bulk C, CNPs(M), and CNPs(F) at 5 mM and 15 mM significantly improved root and shoot length, as well as fresh and dry biomass, in coffee plants subjected to drought stress. Typically, drought stress severely reduces photosynthetic pigments (Chl a, Chl b, carotenoids), relative water content, gas exchange (net photosynthesis, stomatal conductance), chlorophyll fluorescence (Fv/Fm), and antioxidant activities. However, CNPs application notably alleviated these negative effects. Additionally, CNPs treatments reduced malondialdehyde (MDA) levels and proline accumulation, indicators of oxidative and osmotic stress, respectively. These findings suggest that treating coffee plants with CNPs enhances their tolerance to drought stress.

Key words: Chlorophyll; malondialdehyde; nanotechnology; photosynthesis; stomatal conductance.

1 INTRODUCTION

The world's population is projected to reach around 9.6 billion by 2050, necessitating a substantial increase in agricultural output to meet the rising food demand (Fukase; Martin, 2020). Plants frequently face a variety of environmental challenges and various abiotic stresses, including drought, at different life stages (Liao et al., 2025). To achieve sustainable agricultural production, it is crucial to enhance plant resilience to stress. Drought stress affects plant growth, reduces crop yields, and endangers food security (Liao et al., 2025). Under drought conditions, several critical plant functions, including morphological, physiological, biochemical, and molecular processes, are adversely affected, leading to issues like nutrient scarcity, ion toxicity, osmotic stress, and oxidative stress, all of which diminish plant growth and yield (Gong et al., 2025; Chandra et al., 2021). Consequently, improving plant drought tolerance is vital for research, especially in Saudi Arabia, to safeguard food supply. In recent decades, significant efforts have been made to promote plant growth and development using phytohormones and chemicals, which may pose risks to human health. Therefore, adopting advanced agricultural technologies is imperative to achieve the sustainable development goals.

Nanotechnology represents a cutting-edge approach for advancing the agricultural sector, providing innovative solutions for enhancing drought stress tolerance (Alsherif et al.,

2023). Among the various nanomaterials, carbon nanoparticles (CNPs) stand out due to their unique physical and chemical properties, positioning them as one of the most promising materials in this field (Alsherif et al., 2023). Notably, CNPs are characterized by their zero-dimensional structure and remarkable attributes (Zheng et al., 2017), including water solubility, stability, low toxicity, biocompatibility, chemical inertness, cost-effectiveness, and the ability to be produced from readily available raw materials through eco-friendly and economical methods. Despite their broad utility in areas such as sensing, bioimaging, defense, photocatalysis, and optoelectronics, their application in agriculture remains widely underexplored. Carbon nanoparticles (CNPs) are utilized in plant systems as they serve multiple purposes, including acting as pesticides, enhancing growth, aiding seed sprouting, and delivering various substances such as DNA, phytohormones, fertilizers, and herbicides to plant cells (Verma et al., 2019). CNPs, in particular, have been shown to facilitate plant photosynthesis and cellular uptake (Wang et al., 2018). Furthermore, the fluorescent properties of carbon dots serve as a valuable tool for tracking their absorption by plants and elucidating their action mechanisms (Jing et al., 2023). The current research aims to develop a method for producing carbon nanostructures from oil fly ash using physical processes like ball-milling and sonication in various media, which has been effective in generating novel nanocrystalline powders. The

enhancement of this synthetic process and the optimization of conditions for desired properties are crucial for their practical use. As such, carbon-rich oil fly ash is identified as a viable source for the mass production of sustainable carbon nanomaterials, with significant implications for agriculture and the food industry (Ahmadi et al., 2025). For an example, CNPs increased the drought tolerance in *Capsicum* plants through improvement of photosynthesis and osmoregulation (Ahmadi et al., 2025).

Coffee stands as one of the most extensively traded goods in the global market, generating substantial income for both developing and developed countries (Tounekti et al., 2018). The significance of coffee to the economies of Saudi Arabia and the world at large is undeniable, as it is widely consumed and holds substantial market value. However, throughout their growth cycle, coffee plants often encounter various environmental challenges, including drought stress (Tounekti et al., 2018). Thus, it is essential to explore how CNPs can support the growth of coffee plants under drought conditions. To the best of our knowledge, no research has yet examined the effect of carbon nanoparticles (CNPs) on coffee seedlings experiencing drought stress. We hypothesize that CNPs can enhance the resilience of coffee seedlings to drought. Our research aims to address the following questions by analyzing physiological and biochemical data in coffee plants: (i) Do CNPs influence the growth and development of coffee seedlings under drought stress? (ii) If CNPs have beneficial effects, which physiological and biochemical mechanisms contribute to enhancing the drought tolerance of coffee plants? Ultimately, our goal is to improve the resilience of drought-affected coffee plants using CNPs, focusing on physiological and biochemical aspects like osmolytes and antioxidant defense mechanisms.

2 MATERIAL AND METHODS

2.1 Experimental site, plant materials and treatments

The studies conducted at the greenhouse and laboratories of Imam Abdulrahman Bin Faisal University and Umm Al-Qura University in Saudi Arabia. A local variety of Saudi Arabian *Coffea arabica*, cv. Shadawi Salibi, was selected for this experiment. Coffee seedlings, three per pot (3/pot) and housed in 16 cm by 20 cm plastic pots containing 3 kg of soil each, were germinated and cultivated. These seedlings were treated with three different concentrations of CNPs (Bulk C, CNPs(M), and CNPs(F) at 5mM, and Bulk C, CNPs(M), and CNPs(F) at 15mM) - which had been extracted from industrial oil fly ash using a high-energy ball-milling technique. This application lasted for 20 days under three varied drought

conditions defined by field capacity (FC): a control group at 100% FC, a moderate drought group at 35% FC, and a severe drought group at 10% FC, with each treatment having three biological replicates. Following the experiment, the roots and shoots of the coffee plants were gathered and cleaned with double-distilled water.

2.2 Processing of carbon nanoparticles (CNPs)

In this study, carbon-rich fly ash powder was obtained from a local water desalination facility in Rabigh, Saudi Arabia. This powder was used to produce graphitic carbon nanoparticles, starting with 10 g of the raw ash, utilizing a high-energy ball-milling technique. The ball-milling process lasted 45 minutes in a ceramic-coated chamber, employing ceramic balls and conducted under a flow of nitrogen gas. The use of an inert gas like nitrogen during ball milling was crucial to prevent any unwanted chemical reactions that could alter the material's characteristics. The milling itself was performed using a horizontal oscillatory mill (PM 400; Retsch, Hann, Germany), set to 25 Hz. Due to the tendency of carbon nanoparticles to form clusters, driven by repulsive electrostatic forces, a dispersion process involving hydrogen peroxide followed by centrifugation was employed to break up these clusters and stabilize the nanoparticles (H_2O_2 + centrifuge method).

2.3 Characterization of carbon nanoparticles (CNPs) by TEM and XRD

Transmission electron microscopy (TEM) images of carbon nanoparticles (CNPs) were taken using a JEM1010 microscope (JEOL, Japan) at an 80 kV acceleration voltage, and the size distributions were analyzed with Nano Measurer software (version 1.2.5). Additionally, X-ray diffraction (XRD) patterns were obtained using a Philips X-ray diffractometer with a PW 1930 generator and a PW 1820 goniometer. The diffractometer used cobalt K-alpha ($CoK\alpha$) and copper K-alpha ($CuK\alpha$) radiation sources, operating at 45 kV and with a wavelength of 0.15418 nm, respectively. These analyses helped determine the crystallinity and phase purity of the CNPs.

2.4 Growth parameters

The lengths of the roots and shoots were measured in centimetres (cm) using the metric system to ensure precise measurements. Additionally, the weights of the coffee seedling roots and shoots were accurately determined using a digital balance (model HR-200), which provided precise readings for further analysis. To measure the dry weight, fresh coffee seedlings were dried overnight in an oven at 65°C, and the weight of the dried seedlings was recorded.

2.5 Relative water content (RWC)

To measure the relative water content (RWC) of the leaves, samples were collected from the most recently fully developed leaf of each plant across all treatments. The sampling was performed early in the morning at 8:00 am to minimize the effects of diurnal variation. The fresh weight was recorded in the laboratory, after which the samples were soaked in double distilled water at room temperature (~25°C) for 24 hours. The turgid weight was then measured, followed by drying the leaves in an oven at 70°C for 24 hours to determine their dry weight. The RWC was calculated using the standard formula.

$$\text{RWC (\%)} = (\text{Turgid Weight} - \text{Dry Weight}) / (\text{Fresh Weight} - \text{Dry Weight}) \times 100$$

2.6 Photosynthetic pigments

The photosynthetic pigments were extracted following the method described by Arnon (1949). A mature leaf weighing 0.25g was harvested and subsequently ground in 5 ml of 80% acetone to extract the photosynthetic pigments. The resulting leaf extract was then centrifuged at $3,000 \times g$ for 15 minutes at a temperature of 4°C. After centrifugation, the supernatant containing the dissolved pigments was collected for analysis. The concentrations of Chl a, Chl b, and carotenoids were determined by measuring the absorbance of the supernatant at specific wavelengths (663nm, 645nm) using a spectrophotometer.

2.7 Leaf gas exchange and chlorophyll fluorescence

A portable photosynthesis measurement device (LI-6400; LI-COR) was employed to accurately determine key physiological parameters, including net photosynthesis, stomatal conductance, and chlorophyll fluorescence. During the measurements, the CO₂ concentration within the leaf chamber was precisely maintained at 400 $\mu\text{mol mol}^{-1}$ to ensure consistency across samples. Additionally, the temperature within the chamber was carefully regulated and kept constant at 25°C to accurately reflect the environmental conditions during the data collection process. A constant photosynthetically active radiation (PAR) level of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was provided using a red/blue LED light source to simulate natural sunlight and ensure uniform light conditions across all measurements.

2.8 MDA content measurements

The MDA content was measured following the method outlined by De Vos (1989). A fresh tissue sample weighing 0.3 g was homogenized in 5 mL of 5% trichloroacetic acid (TCA). Following homogenization, the mixture was subjected to centrifugation at $8,000 \times g$ for 15 minutes to separate the solid tissue debris from the supernatant. To this supernatant,

2.5 mL of thiobarbituric acid (TBA) was added. TBA reacts with MDA to form a colored complex, allowing for spectrophotometric quantification. The mixture of supernatant and TBA was then heated at 100°C in a water bath for 20 minutes. Immediately after heating, the mixture was rapidly cooled on ice to halt the reaction. To further purify the solution, it was centrifuged again at $10,000 \times g$ for 5 minutes, which helped remove any remaining particulates that could interfere with the spectrophotometric measurements. The absorbance of the final supernatant was measured at two wavelengths: 532 nm and 600 nm. The MDA concentration was calculated using the difference in absorbance between these wavelengths and applying the molar extinction coefficient for MDA, which is $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.9 Proline content determination

The proline content, an important indicator of plant stress tolerance, was determined following the method described by Bates et al. (1973). The procedure began with the collection of fresh leaf tissue, from which a 0.2 g sample was precisely weighed. This sample was then homogenized in 10 mL of 3% (w/v) sulfosalicylic acid. Once homogenized, the mixture was subjected to centrifugation at $15,000 \times g$ for 10 minutes. From this supernatant, 2 mL were carefully transferred to a new test tube, where it was combined with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin reagent. These test tubes were then incubated in a boiling water bath for 1 hour. After the incubation, the tubes were rapidly cooled, and 4 mL of toluene were added to each tube. The absorbance of the toluene layer was then measured at 520 nm using a spectrophotometer.

2.10 Antioxidant enzymes

A fresh leaf sample, weighing precisely 0.3 g, was collected and immediately immersed in 5 mL of a 50 mmol/L phosphate buffer solution adjusted to pH 7.0. Additionally, the buffer contained 10 g/L of polyvinylpyrrolidone (PVP). This homogenate was subsequently centrifuged at $15,000 \times g$ for 20 minutes to separate the solid tissue debris from the liquid phase. After centrifugation, the collected supernatants served as the initial extract for assessing enzyme activity.

2.10.1 Superoxide dismutase (SOD) activity measurements

The activity of superoxide dismutase was measured following the procedure outlined by Hasan et al. (2020), with slight modifications to enhance accuracy and adaptability. 0.1 mL of riboflavin was added as a mixture component. Additionally, 1.5 mL of L-methionine was used, serving as a reducing agent, and 1.0 mL of nitroblue tetrazolium (NBT) was included. The mixture also contained 0.75 mL of

Triton-X, a surfactant that aids in the uniform distribution of the components in the reaction mixture. Furthermore, 2 mM of ethylenediaminetetraacetic acid (EDTA) was added to the base of the reaction mixture was 27.0 mL of sodium potassium phosphate (NaKPi) buffer at pH 7.8. To this pre-prepared mixture, 0.5 mL of enzyme extract was added, making the final volume of the assay mixture 3.0 mL. The assay tubes containing the complete mixture were incubated at 25°C for 10 minutes. The reaction was initiated by exposing the mixture to white light from a pre-heated 15-watt, 6500 K fluorescent lamp. The absorbance of the reaction mixture was measured at 560 nm using a spectrophotometer.

2.10.2 Catalase (CAT) activity measurements

Catalase activity was determined using the spectrophotometric method described by Aebi (1984). 20 µL of the plant sample extract, which contained catalase, was mixed with 830 µL of a potassium phosphate buffer solution (50 mM, pH 7.0). This buffer was used to maintain a stable pH. The enzymatic reaction was initiated by adding 150 µL of hydrogen peroxide (H₂O₂) to the mixture. The decrease in H₂O₂ concentration was measured spectrophotometrically by monitoring the absorbance at 240 nm.

2.11 Statistical analysis

In this experiment, all collected data were subjected to statistical analysis using Minitab-17 statistical software. To compare the treatment means, Fisher's Least Significant Difference (LSD) test was used. The LSD test was applied with a significance level set at $p \leq 0.001$.

3 RESULTS

To analyze the morphological and structural characteristics of the raw milled powder and newly synthesized CNPs, TEM and XRD techniques were employed. Additionally, particle size distribution was estimated. Figure 1(a) displays the TEM image of the ball-milled fly ash sample, showing randomly distributed large clusters of agglomerated particles at the nanoscale. Figure 1(b) presents the XRD pattern of this sample, with notable peaks at 25.3° and 43.1°, corresponding to C (002) and (100) reflections, indicative of its hexagonal graphitic structure. Figure 1(c) illustrates the TEM image of CNTs prepared by an oxidation method followed by centrifugation, showing smaller and irregularly sized CNPs. Figure 1(d) shows an improved XRD pattern with a sharper C (002) peak, suggesting increased graphitization and higher translational order. The average particle size of the CNPs is approximately 19.8 nm.

The XRD profile (Fig. 1a) displays distinct peaks at 22° and 43°, characteristic of activated carbon material. These peaks indicate a low degree of graphitization and structural

regularity. The peaks correspond to the (002) and (100) reflection planes for DP-AC. The broad peak suggests the presence of amorphous carbon, while the sharper peak at 22° reflects increased translational order at elevated temperatures. Overall, these findings confirm the successful synthesis of activated carbon material.

Drought stress greatly hampers the growth of root and shoot lengths in coffee plants, resulting in stunted development. However, treatment with carbon nanoparticles (CNPs) led to a marked improvement in growth, with significant increases in both root and shoot lengths compared to plants under drought stress without treatment (Figure 2).

Similarly, drought stress also significantly ($p \leq 0.001$) reduces the root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight of coffee plants. The highest root and shoot lengths were recorded when CNPs-F (5mM) were applied to the coffee plants. The application of CNPs-F at a concentration of 5mM had a significant positive effect on increasing the root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight of coffee plants (Figure 3)

Drought stress significantly reduced the RWC, Chla, Chlb, and carotenoids content in coffee plants. However, when coffee plants were subjected to CNPs (Bulk C, CNPs(M), and CNPs(F) at 5mM, and Bulk C, CNPs(M), and CNPs(F) at 15mM) RWC increased by 24%, 30%, 38%, 4%, 18%, 30% and 93%, 57%, 63%, 25%, 48%, 48%, 58%, Chl a increased by 36%, 58%, 78%, 12%, 56%, 76% and 43%, 67%, 83%, 17%, 56%, 81%, Chl b increased by 40%, 63%, 77%, 23%, 51%, 73% and 44%, 71%, 81%, 28%, 65%, 80%, carotenoids increased by 54%, 37%, 85%, 37%, 18%, 82% and 44%, 75%, 94%, 25%, 25%, 92%, respectively, in compared to that observed in drought-stressed (35% and 10% FC) coffee plants (Figure 4).

The application of drought treatments (35% and 10% FC) resulted in significant decreases in A, gs, Fv/Fm, amounting to 37%,67%; 38%,76% and 60%,83%, respectively, in comparison to the control. However, the application of CNPs treatments (Bulk C, CNPs(M), and CNPs(F) at 5mM, and Bulk C, CNPs(M), and CNPs(F) at 15mM) led to increases in A by 23%, 33%, 54%, 8%, 31%, 51% and 19%, 34%, 56%, 5%, 25%, 52% and gs by 29%, 40%, 40%, 57%, 7%, 28%, 49% and 73%, 54%, 79%, 50%, 65%, 76%, as well as Fv/Fm by 58%, 73%, 84%, 54%, 64%, 81%, respectively, in comparison to coffee plants under drought stress at 35% and 10% FC (Figure 5).

Compared to the control seedlings, drought treatments at 35% and 10% FC resulted in significant increases in MDA and proline content in coffee seedlings, with increases of 29%, 34%, and 29%, 48%, respectively. Additionally, treatment with CNPs (Bulk C, CNPs(M), and CNPs(F) at 5mM, and Bulk C, CNPs(M), and CNPs(F) at 15mM) led to reductions in MDA and proline content by 20%, 32%, 52%, 13%, 22%, 46%; 15%, 30%, 50%, 14%, 23%, 47%; and 19%, 30%, 41%, 9.7%, 0.27%, 45%; 20%, 27%, 34%, 3%,

11%, 38% at 35% and 10% field capacity (FC), respectively, compared to the control (Figure 6).

In coffee plants subjected to drought stress at 35% and 10% FC, there were significant increases of 57% and 68%, and 51% and 67%, respectively, in SOD and CAT content compared

to the control. In addition, CNPs treatment (Bulk C, CNPs(M), and CNPs(F) at 5mM, and Bulk C, CNPs(M), and CNPs(F) at 15mM) further increased the 7%, 22%, 28%, 12%, 25%, 35%; 14%, 21%, 39%, 13%, 20%, 36% and 6%, 16%, 30%, 2%, 12%, 23% at 35% and 10% FC, respectively when compared to the control (Figure 7).

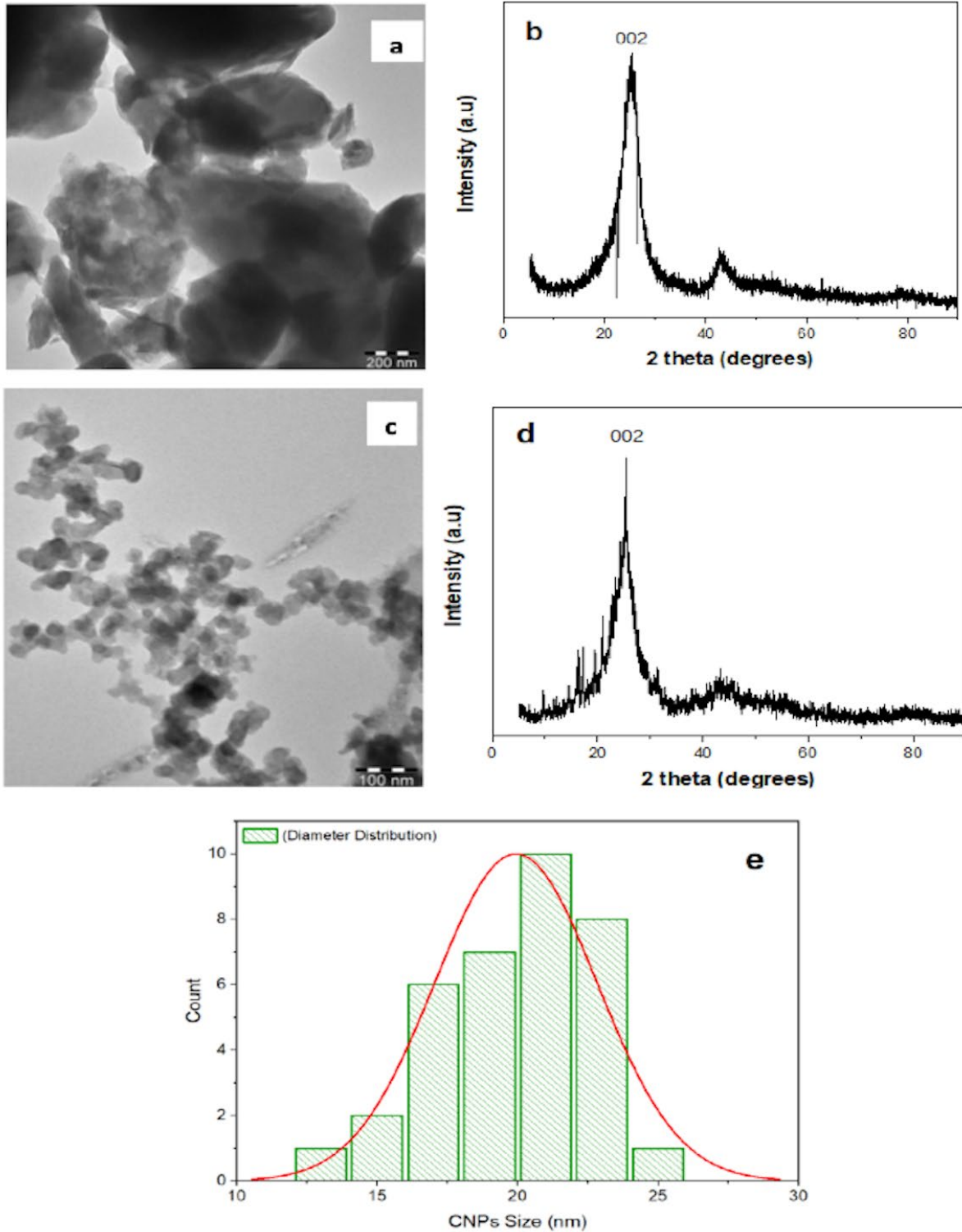


Figure 1: Analysis of TEM (a) MB powder, XRD patterns (b) BM powder, TEM (c) produced CNPs, XRD patterns (d) produced CNPs and (e) size of obtained CNPs.

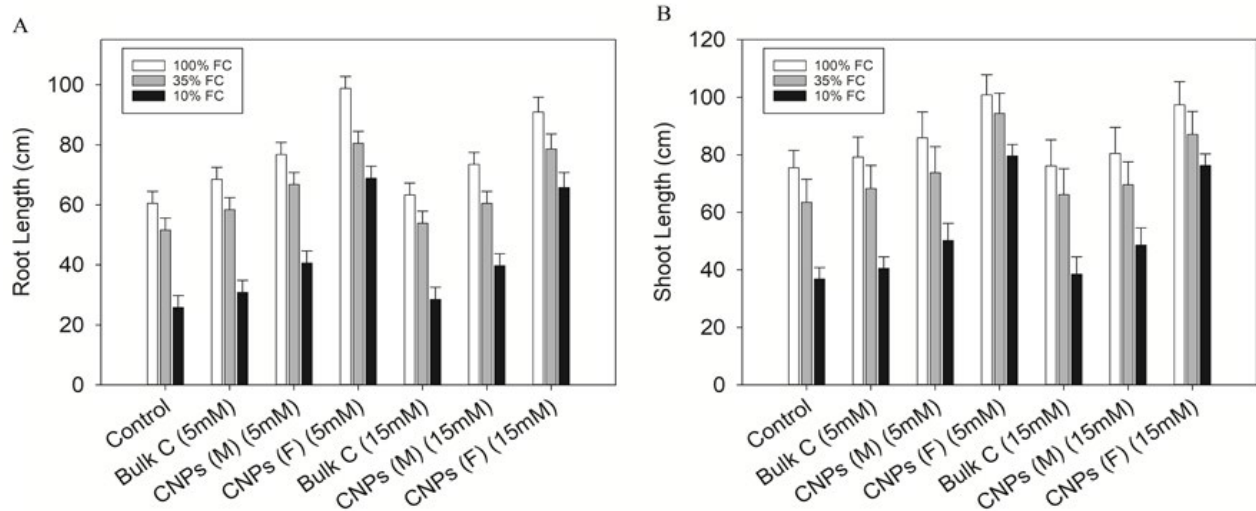


Figure 2: Impact of carbon nanomaterials on the root and shoot length of coffee plants (*Coffea arabica* cv. shadawi salibi) under drought stress (35% FC and 10% FC). Bars marked with distinct letters denote statistically significant differences between groups, as determined at the $p \leq 0.05$ significance level.

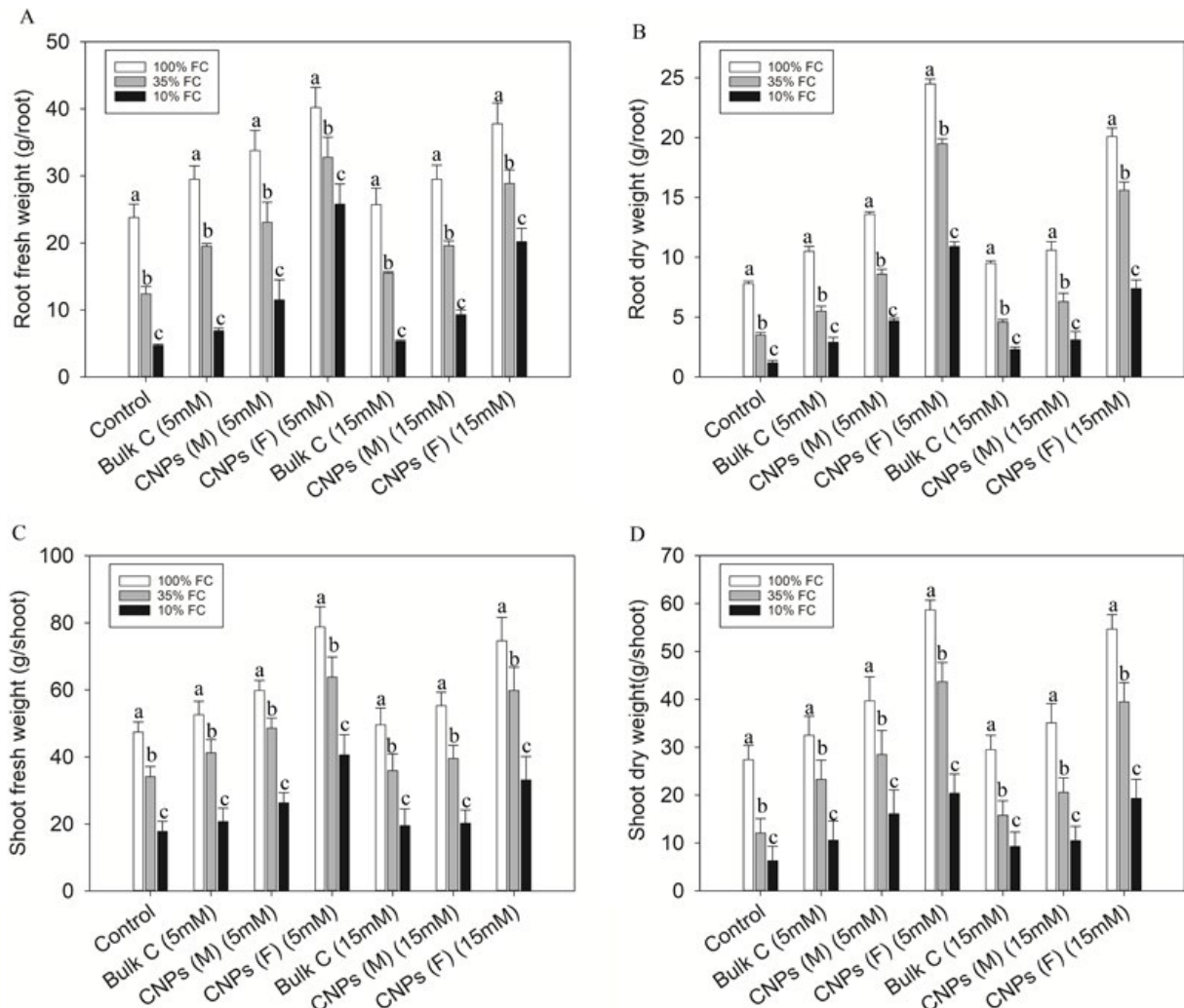


Figure 3: Impact of carbon nanomaterials on the fresh and dry weight of coffee plants (*Coffea arabica* cv. shadawi salibi) under drought stress (35% FC and 10% FC). Bars marked with distinct letters denote statistically significant differences between groups, as determined at the $p \leq 0.05$ significance level.

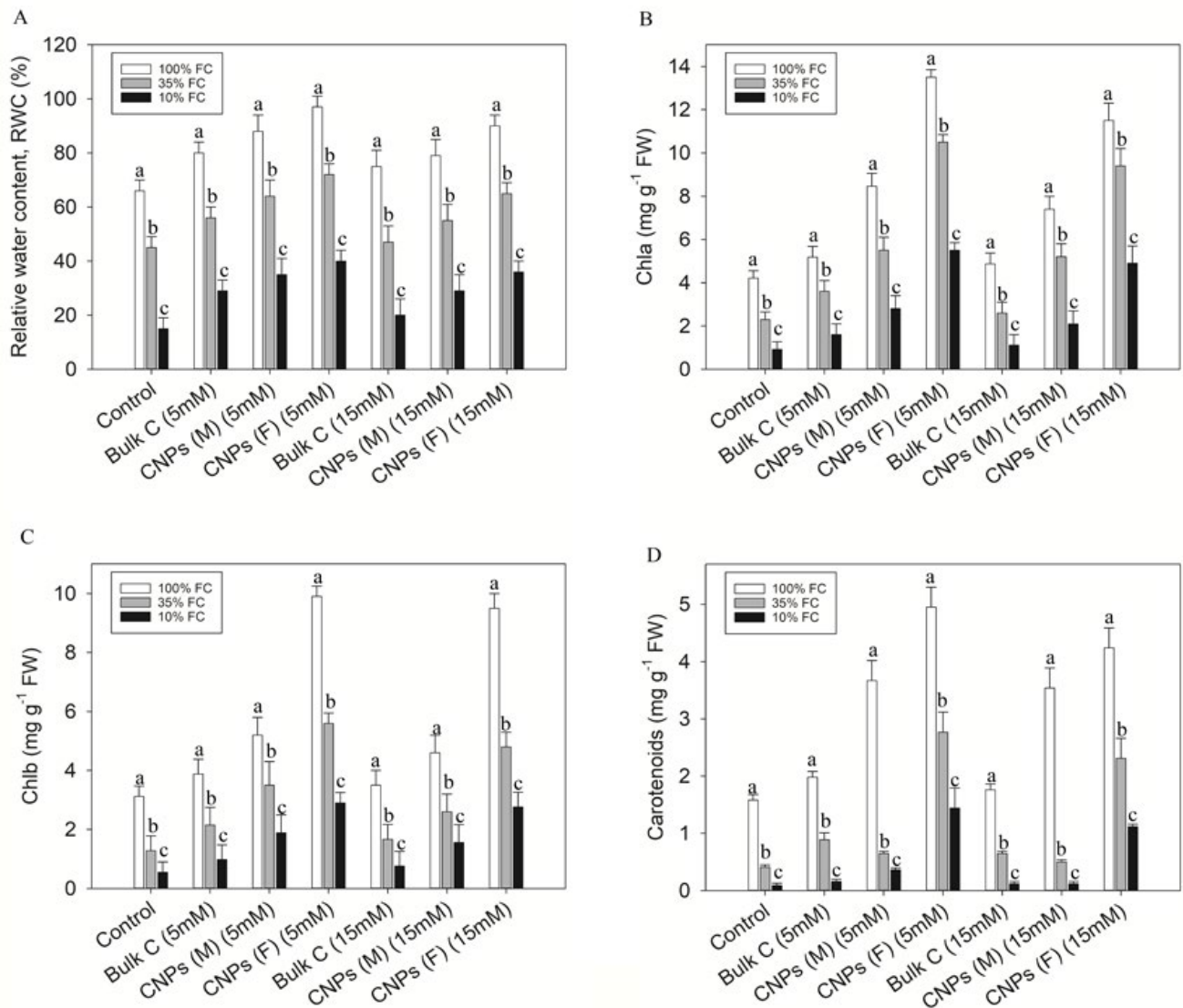


Figure 4: Impact of carbon nanomaterials on the relative water content, Chl a, Chl b, and carotenoids of coffee plants (*Coffea arabica* cv. shadawi salibi) under drought stress (35% FC and 10% FC). Bars marked with distinct letters denote statistically significant differences between groups, as determined at the $p \leq 0.05$ significance level.

4 DISCUSSION

The impact of drought stress on plants is well-established, with its detrimental effects observed from the roots to the shoots. Every growing part of the plant is affected by drought stress. In this experiment, drought stress inhibited root and shoot length as well as the fresh and dry weight of the shoots and roots. These findings align with previous studies conducted on coffee plants, which have consistently demonstrated the adverse effects of drought stress on plant growth and physiological functions. The newly obtained CNPs, characterized by TEM and XRD, have been shown to be effective, possibly due to their unique size (Figure 1). In this study, carbon nanoparticles (CNPs) were employed to explore their significance in the growth and physiological processes of coffee plants under drought conditions. Coffee

plants served as a model plant for this research. The CNPs were found to have a beneficial effect on both root and shoot length, as well as on the fresh and dry weight of the shoots and roots. The influence of CNPs on plant tissues and their ability to penetrate them are heavily dependent on the particles' size and chemical properties [3], making their characterization essential. The current research identified that the optimal concentration of CNPs-F (5 mM) significantly enhances the growth parameters of coffee plants. However, concentrations exceeding 5 mM did not contribute positively to the growth of coffee plants, suggesting that lower concentrations of CNPs are more advantageous. Similar research by Li et al. (2016) demonstrated that a low concentration (0.4 mg/mL) of fluorescent carbon dots increased the shoot and root length of *Vigna radiata*.

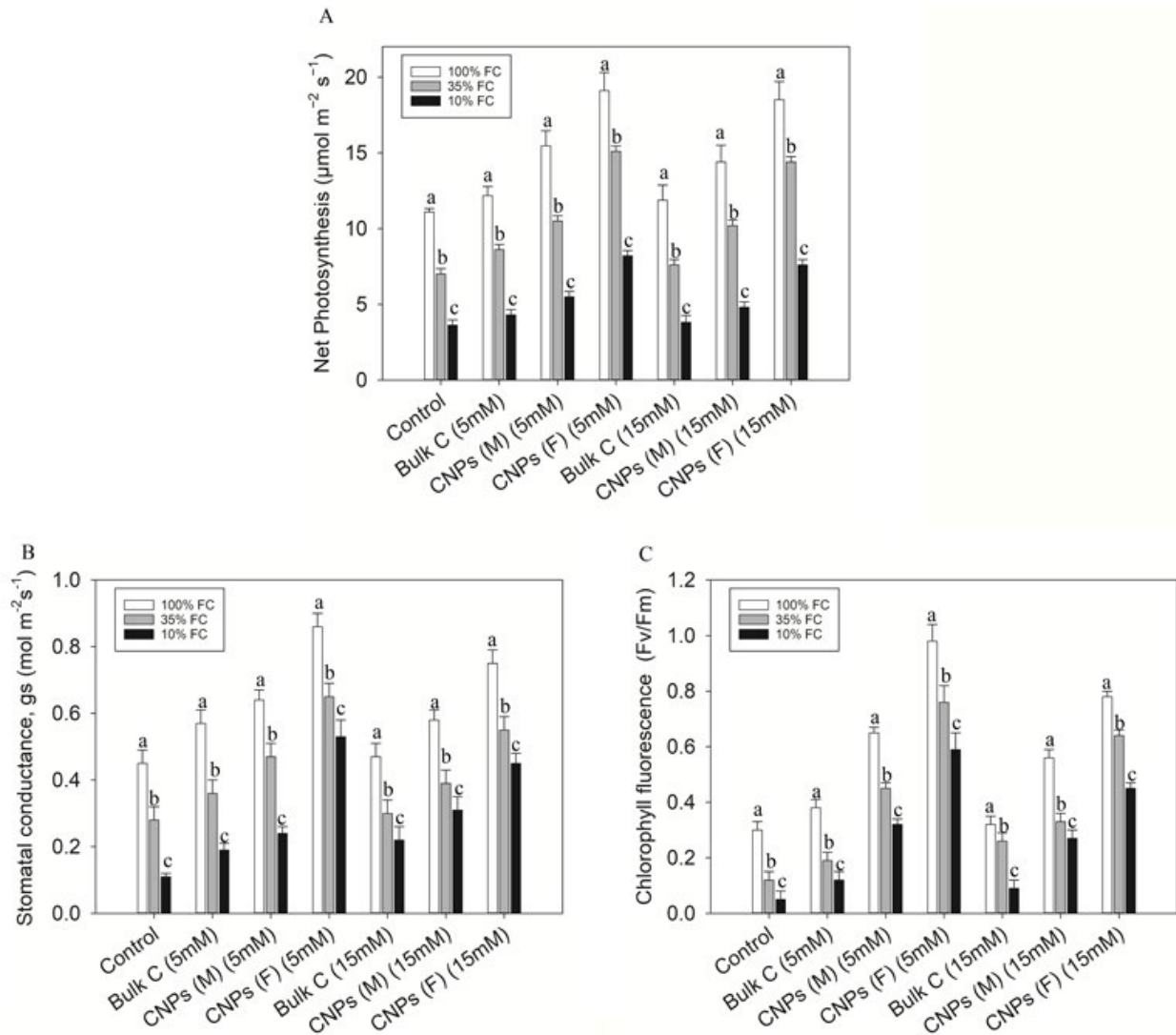


Figure 5: Impact of carbon nanomaterials on the net photosynthesis (A), stomatal conductance (gs), and chlorophyll fluorescence (Fv/Fm) of coffee plants (*Coffea arabica* cv. shadawi salibi) under drought stress (35% FC and 10% FC). Bars marked with distinct letters denote statistically significant differences between groups, as determined at the $p \leq 0.05$ significance level.

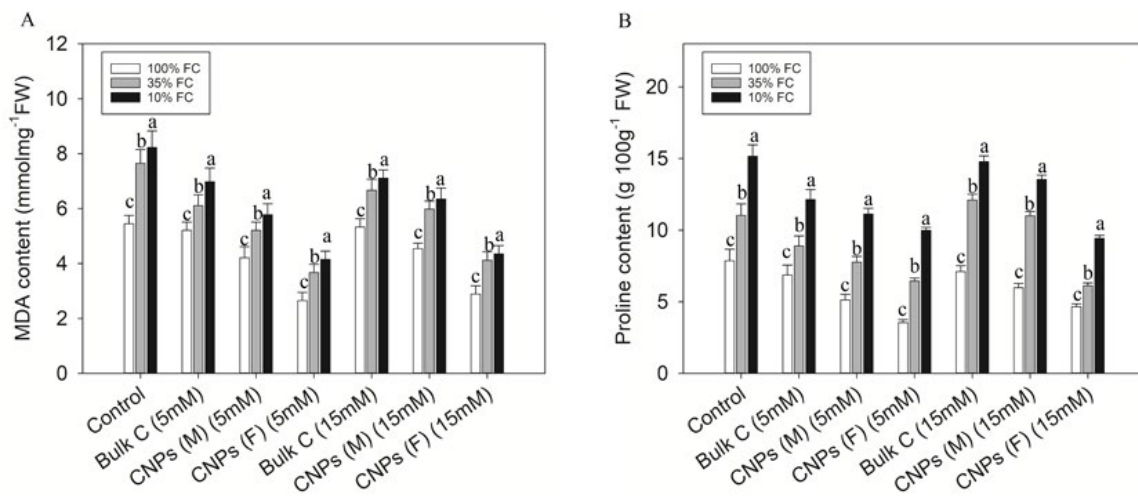


Figure 6: Impact of carbon nanomaterials on the MDA and proline content of coffee plants (*Coffea arabica* cv. shadawi salibi) under drought stress (35% FC and 10% FC). Bars marked with distinct letters denote statistically significant differences between groups, as determined at the $p \leq 0.05$ significance level.

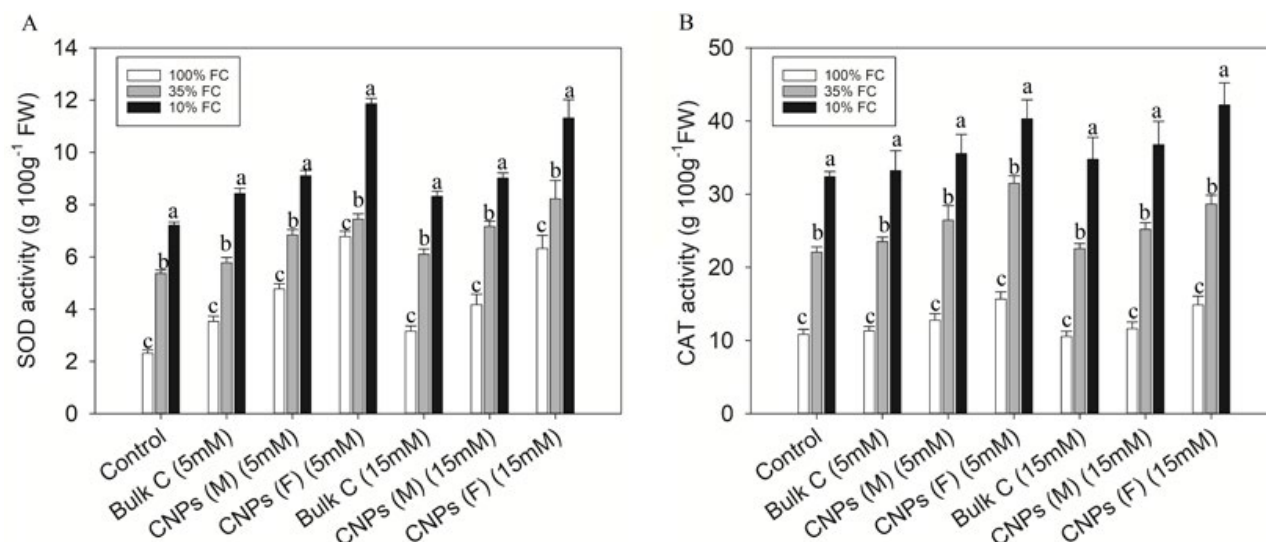


Figure 7: Impact of carbon nanomaterials on the antioxidant activities (SOD and CAT) of coffee plants (*Coffea arabica* cv. shadawi salibi) under drought stress (35% FC and 10% FC). Bars marked with distinct letters denote statistically significant differences between groups, as determined at the $p \leq 0.05$ significance level.

CNPs treatments increased the RWC of drought-stressed coffee seedlings, suggesting that CNPs enhanced water balance. This improvement is likely due to the high surface area and uptake capacity of CNPs (Ahmadi et al., 2025). The impact of CNPs dosage on crops is primarily observed through changes in photosynthetic pigment, which is a critical factor in determining plant growth and development. Chlorophyll is essential for photosynthesis, and its levels can directly influence a plant's ability to thrive, especially under stress conditions. In our study, approximately all dosages of CNPs resulted in increased levels of chlorophyll, including Chl a, Chl b, and carotenoids, particularly in coffee plants subjected to drought stress. Interestingly, the 5 mM concentration of the CNPs-F treatment proved to be the most effective in increasing chlorophyll content compared to other dosages evaluated.

Our findings align with those of Shekhawat et al. (2021), who also observed that CNPs positively affected photosynthesis by increasing the levels of chlorophyll a and b. This increase in chlorophyll content was associated with a consistent increase in biomass accumulation in *Vigna radiata*. Specifically, Shekhawat et al. (2021) reported that the highest chlorophyll content was achieved at a CNPs concentration of 100 μM , which was 1.9 times higher than that observed in the control group. This evidence further supports the conclusion that CNPs can significantly enhance photosynthetic efficiency and chlorophyll fluorescence and overall plant growth, particularly under stress conditions. CNPs deposit more on stomatal cell surfaces compared to other nanoparticles, entering the plant cell wall and altering metabolic processes (Nel et al., 2009; Ponti et al., 2010; Ahmadi et al., 2024). CNPs enhanced stomatal conductance in coffee plants under drought stress, likely due to the noticeable changes in stomatal cells induced by CNPs. Similarly, Ahmadi

et al. (2024) found that applying 1000 mg/L of multi-walled carbon nanotubes (MWNTs) increased both photosynthesis and stomatal conductance in *Capsicum annuum* L. under both stress and non-stress conditions.

Oxidative stress caused by abiotic conditions can lead to irreversible damage through lipid peroxidation, disrupting membrane integrity and increasing MDA content (Hasan et al., 2018; Bakir et al., 2022). Consequently, monitoring MDA accumulation is crucial, as it serves as a widely used marker of oxidative stress in plants. In drought-stressed coffee plants treated with CNPs, MDA levels were low, suggesting reduced oxidative stress. Similarly, treatments with Carbon Quantum Dots (CQDs) and Advanced Carbon Quantum Dots (Pro-CQDs) at 50 and 100 mg L^{-1} significantly lowered MDA content in grapevines, consistent with the present findings (Gohari et al., 2021). Excessive reactive oxygen species (ROS) production generally harms plant cells, disrupting homeostasis and increasing cell membrane permeability (Rahman et al., 2024; Das et al., 2024). The observed increase in proline content with decreasing CNP dosages in this study indicates a linear relationship between ROS formation and its mitigation through proline. We assessed the activities of CAT and SOD, key ROS scavenging enzymes associated with drought stress. In coffee plants treated with CNPs under drought conditions, both SOD and CAT activities were elevated compared to control plants. This suggests that CNPs enhance the activity of these enzymes in plants. Similar patterns of antioxidant activity were observed in tomatoes treated with CNPs (Ahmadi et al., 2025). The reduced ROS accumulation in CNP-treated plants, as compared to controls, likely results from increased ROS scavenging. This enhanced scavenger activity helps protect cells from damage, contributing to a healthier seedling (Figure 8).

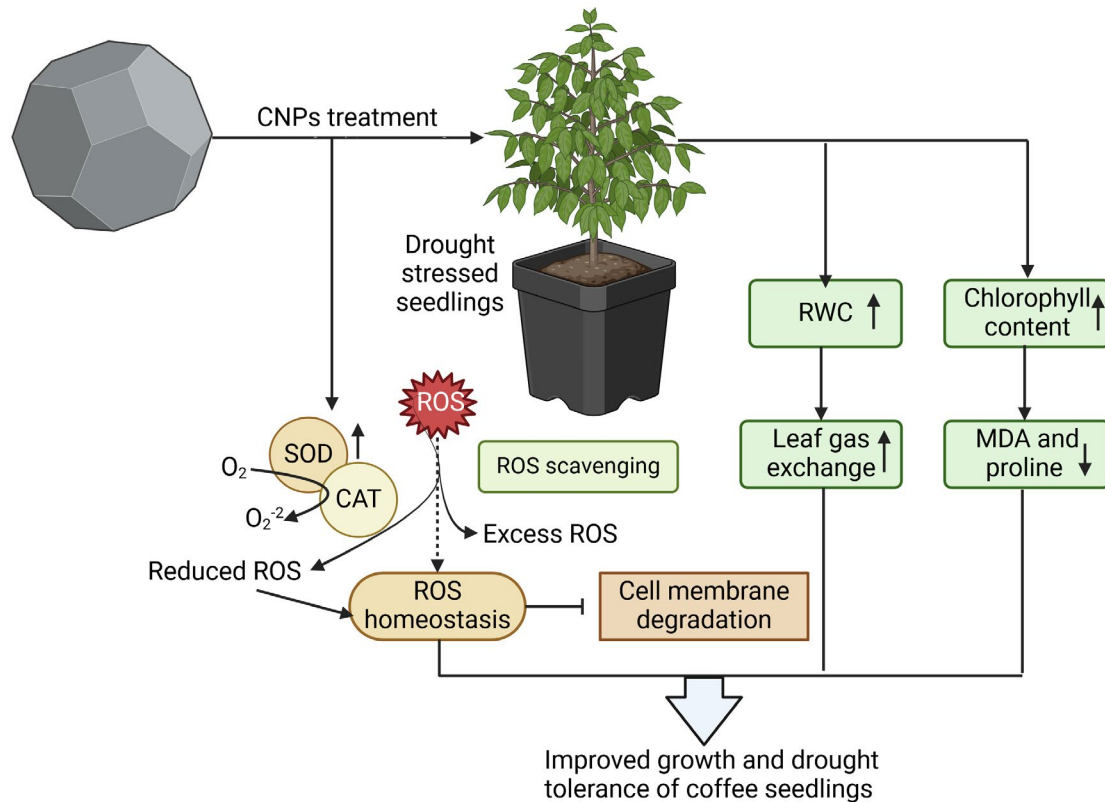


Figure 8: A schematic representation of carbon nanoparticles (CNPs) induced drought tolerance in coffee seedlings.

5 CONCLUSIONS

This study demonstrates that applying carbon nanoparticles (Bulk C, CNPs-M, and CNPs-F) at 5 mM and 15 mM can mitigate drought stress in coffee plants, with CNPs-F (5 mM) producing the strongest enhancement in growth, photosynthetic, and antioxidant performance. The results indicate a dose-dependent response and highlight the potential of CNPs as a sustainable approach for improving drought tolerance. Further research should assess long-term environmental effects and validate these findings across other crop species.

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7 AUTHORS' CONTRIBUTION

Conceptual Idea: Nadiyah, M.A.; Methodology design: Nadiyah, M.A.; Saleh, M.A.; Data collection: Nadiyah, M.A.; Data analysis and interpretation: Nadiyah, M.A.; Saleh, M.A.; and Writing and editing: Nadiyah, M.A.; Saleh, M.A.

8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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