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Copper and/or Zinc Oxides Multiwalled Carbon Nanotubes Induced Seed Germination of Two *Solanum lycopersicum* Cultivars.

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Abstract

Contemporary research highlights multiwalled carbon nanotubes (MWCNTs) as a promising and efficient micronutrient delivery system for agricultural applications. The current study adopts a prospective applicant to modulate copper and zinc supply to enhance germination of two tomato (*Solanum lycopersicum*) cultivars: Gana F1 and Alissa F1. This study compared the significance of using multiwalled carbon nanotubes of CuO and ZnO, separately or combined, on seed germination characteristics. The fabricated CuO/MWCNT and ZnO/MWCNT were synthesized and then characterized using TEM and zeta potential measurement. After that, serial concentrations (0.5, 1.0, 1.5, and 2.0cm³/L) of CuO/MWCNTs and ZnO/MWCNTs were prepared from the stock nanomaterials. The combined form CuO /MWCNTs + ZnO /MWCNTs was used at 0.5, 1.0, 1.5, and 2.0 cm³/L, V/V of both nanocomposites. All three treatments were applied to seed irrigation, compared with negative controls irrigated with distilled water. Overall, the germination measurements indicated that both CuO/MWCNTs, ZnO/MWCNTs or the combined formulation, significantly improved seedling performance in Gana F1 and Alissa F1 at lower to moderate (0.5-1.5cm³/L) nano concentrations. At the same time, the inhibitory effect was found to be at the highest nano level (2 cm³/L). Furthermore, the data showed that Gana F1 outperformed Alissa F1 across specific germination indices. The single CuO/MWCNTs treatment exerted its maximum impact at a concentration of 0.5cm³/L in both cultivars. Whereas, ZnO/MWCNTs treatment at 1.5 cm³/L and the combined formula of CuO/MWCNTs + ZnO/MWCNTs (V/V) at 1.0 cm³/L. In general, the CuO/MWCNTs and/-or ZnO/MWCNTs irrigation approach can be beneficial for seed germination. Still vigilant, optimization is crucial to avoid adverse effects resulting from inappropriate concentrations and exposure durations. This optimization requires monitoring the physiological mechanisms involved and modification of nanomaterial applications on a case-by-case basis to achieve sustainable agriculture.

Keywords: Multiwalled carbon nanotubes, *Solanum lycopersicum* cultivars, liquid nanocomposite, germination vigor index.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a member of the family Solanaceae. Tomatoes are among the most extensively cultivated, valuable, and widely consumed vegetables globally, ranking second only to potatoes in terms of consumption (Olaniyi *et al.*, 2010; Liu *et al.*, 2023). Tomatoes are classified as a perennial crop; however, in commercial production contexts, it is regarded as an annual crop (Mohamed *et al.*, 2010; Panno *et al.*, 2021). Tomato is a valuable source of protein, calcium, sodium (Na), iron (Fe), potassium (K), magnesium (Mg), carotenoids, vitamin A, and vitamin C; the latter traits qualify tomato to have a role in reducing cancer risk and degenerative disease (Srividya *et al.*, 2014; Ahmed *et al.*, 2021). Furthermore, tomatoes are considered the largest domestic and industrial commodity, yielding approximately 200 million tons worldwide. Tomatoes are helpful for blood purification, gastrointestinal secretion, and nutrient absorption. Nevertheless, they are highly susceptible to a range of biotic and abiotic stressors agents which collectively reduce crop productivity (Khan *et al.*, 2024).

In this context, various strategies have been implemented to enhance crop production, including the use of chemical inputs (such as commercial fertilizers and pesticides), crop rotation, precision agriculture, urban farming, and genetic modifications through targeted breeding and gene editing. Other traditional and molecular methods have also been employed in crop breeding, including functional genomic tools, genetic selection, mutagenic breeding, physical mapping, somatic clonal variation, and whole-genome sequencing (Das *et al.*, 2011). However, prolonged excessive use of conventional fertilizers results in significant environmental issues, including air pollution, soil quality deterioration, eutrophication of water bodies, and groundwater contamination (Deshpande *et al.*, 2017).

Chemical fertilizers exhibit low efficacy owing to volatilization and leaching, which lead to environmental contamination and increase production costs, thereby constraining the attainment of sustainable agriculture (FAO 2017). Besides, the use of genetic modifications and crop breeding remains challenging due to high costs, ethical constraints and limited efficacy in many applications.

Contrary to this, nanomaterials (NMs) have the potential to transform agriculture by enhancing crop yields, nutrient uptake, agrochemical delivery, disease management, and disease detection, while preserving ecological equilibrium, economic stability, and environmental sustainability (Acharya and Pal, 2020; Khan et al., 2024). Nanomaterials are regarded as an optimal platform for driving the agri-nanotech revolution due to their minimal size (<100 nm), which enables them to traverse biological barriers and penetrate plant tissues through foliar or root application, thereby offering innovative and efficient methods for nutrient and pesticide delivery (Poddar *et al.*, 2018).

Among different carbon-based nanomaterials (CBNs), carbon nanotubes (CNTs) have garnered increased interest in agricultural applications due to their influence on plant growth regulation, utility in innovative delivery systems, capacity to penetrate plant cell walls, nano-transport mechanisms, and function as a medium for biosensors (Siddiqui *et al.*, 2015; Kwak *et al.*, 2019).

Carbon nanotubes can be categorized into two primary types depending on their structures: single-walled carbon nanotubes (SWCNTs), which include a single graphene sheet with diameters ranging from 0.4 to 2 nm, and multiwalled carbon nanotubes (MWCNTs), composed of multiple graphene layers with outer diameters between 2 and 100 nm and inner diameters from 1 to 3 nm. Their lengths vary from 0.2 nm to several microns. Due to their multiple layers of carbon atoms, MWCNTs exhibit greater mechanical strength when compared with SWCNTs. Carbon nanotubes also exhibit higher Young's modulus and greater tensile strength than metals such as steel and iron (Kaur *et al.*, 2018; Rao *et al.*, 2021). Carbons are vaporized from graphite and subsequently deposited onto metal particulates through the application of a laser or an electric arc. Recently, they have been manufactured utilizing the chemical vapor deposition (CVD) technique (Salem *et al.*, 2022; Ragab and Mosaad, 2024).

Copper is a vital micronutrient for plants and is involved in various physiological functions, including photosynthesis, respiration, defence against oxidative stress, and carbon and nitrogen metabolism(Brennan, 2005; Kumar et al., 2018). Zinc, conversely, is a vital micronutrient involved in a broad spectrum of physiological processes in plants, including maintaining the structural integrity of macromolecules and ensuring the functionality of ion transport systems (Broadley et al., 2007); It also functions as a prosthetic component of enzymes within cells and plays a crucial role in the synthesis of plant growth hormones (DalCorso et al., 2014; Lopez-Lima et al., 2021).

Although employing Zn and Cu as chemical fertilizers in agriculture poses considerable challenges, these metals are heavy, increasing their potential toxicity to plants, animals, humans, and the environment. Zn- and Cu-supported MWCNT nanocomposites have demonstrated significant potential across a wide range of applications. Besides expanding its applications in electronics, energy, and structural fields, nanotechnology is now advancing into agriculture, plant biology, and horticulture. Although Cu and Zn play a vital role in metabolic activities, particularly in regulating crop yield and product quality, unadjusted levels of Cu and Zn can negatively interfere with other nutrients and enzyme functions. Therefore, a sufficient supply of copper and zinc to the plant is a crucial factor in the cultivation of various crops (Tripathi et al., 2015; Lone et al., 2025).

Consequently, this study aimed to clarify the potential impact of copper and zinc synthesized as multiwalled carbon nanotubes (CuO/MWCNTs and ZnO/MWCNTs) on seed germination, alone and/or in combination. Tomato is considered an ideal species for this purpose, as reported by numerous researchers, due to its reduced root elongation, shortened germination period, and enhanced fruiting, which allow it to mature relatively quickly and activate stress response genes when exposed to CNTs (Cañas et al., 2008; Khodakovskaya et al., 2013; McGehee et al., 2017). Two tomato cultivars, Gana F1 and Alissa F1, were used to test the potential effects of both elements, MWCNTs. The study employs a prospective approach to modulate micronutrient supply, promote tomato cultivar growth, and determine the optimal concentration for each treatment.

2. Materials and Methods

2.1. Plant materials

Pure strains of tomato (*Solanum lycopersicum* L.) seed cultivars (Gana F1 and Alissa F1) were derived from the Agriculture Research Center, Giza, Egypt. The physical integrity of the seeds was assessed and confirmed by immersing them in water for a few minutes; seeds that floated were discarded, and the remaining seeds were utilized for the experiment. The experimental setup comprises Petri dishes and sterilized distilled water.

2.2. Seed viability test

The viability test aimed to evaluate whether the seeds are capable of germination. To estimate viability, five replicates of ten sterilized seeds from each cultivar were sown in Petri dishes. The dishes were irrigated with distilled water when needed and maintained in an incubator at 30°C.

2.3. Determination of the optimum soaking duration test

To determine the appropriate soaking duration, seeds of both cultivars, Gana F1 and Alissa F1, were surface-sterilized with 0.01% sodium hypochlorite for three minutes, then thoroughly washed with several changes of sterile distilled water. The sterilized seeds of both cultivars were soaked in distilled water for different durations. The seeds were divided into five groups (30 seeds per group/cultivar), and soaked at variable times (0, 6, 12, 18, and 24 hours) at room temperature (approximately 25°c). After soaking, 10 seeds were placed on a layer of tissue paper in a sterilized Petri dish, then covered with another layer. Each dish was sealed with a plastic stretch film to maintain warmth and moisture for the seeds. The dishes were then placed in an incubator at 30°C. The seeds were wetted daily, if needed, with 2 mL of water per dish. Seed development was monitored and recorded daily to determine the maximum germination percentage.

2.4. Preparation of CuO/MWCNTs and ZnO/MWCNTs

Based on a previously described methodology (Sapkota et al., 2020), CuO/MWCNTs and ZnO/MWCNTs nanocomposites were separately fabricated. To synthesize CuO/MWCNTs, six grams of copper (II) acetate hydrate were combined with 100 mL of ethanol in a graduated beaker. The mixture was then bath-sonicated at ambient temperature (22°C) for 1 hour to ensure a homogeneous solution. MWCNTs (100 mg) were incorporated into the prepared solution under magnetic agitation for 1 hour. The mixture was allowed to recrystallize undisturbed for 6 hours after the magnetic stirrer was removed. Crystals formed immediately upon removal of the stirrer; however, approximately 6 hours were required for complete, continuous recrystallization of copper (II) acetate within the MWCNTs. The mixture of copper (II) acetate crystals and MWCNTs was isolated from the ethanol solvent by vacuum filtration. The residue was collected and desiccated for 2 hours at 60 °C in a furnace to ensure complete ethanol evaporation. The product was subsequently heated in a muffle furnace (KSL-1100X-S-UL-LD). For this purpose, the desiccated sample was placed in a quartz crucible with a lid, which was then placed in a vacuum chamber. The compartment was hermetically sealed with an oxygen-free copper ring gasket (SUS 314), and the sample was calcined at 500 °C to synthesize the CuO/MWCNT nanocomposites. Similarly, the ZnO/MWCNTs were likewise prepared following the same procedure using Zinc (II) acetate hydrate. Different concentrations of CuO/MWCNTs, ZnO/MWCNTs, and CuO/MWCNTs + ZnO/MWCNTs (0.5, 1.0, 1.5, and 2.0 cm^3/L) were prepared from a stock solution for further investigation in the germination experiment.

2.5. Characterization of CuO/MWCNTs and ZnO/MWCNTs nanocomposites

The crystalline phases of the materials were then subjected to structural and functional characterization to confirm the successful formation and proper dispersion of the metal oxide nanoparticles on the MWCNT framework. Characterization of the synthesized nanomaterials (CuO/MWCNTs and ZnO/MWCNTs nanocomposites) was conducted using Transmission Electron Microscopy (TEM) and Zeta Potential Analysis, which was conducted using a NICOMP ZS3000 particle sizing system (Okil *et al.*, 2019; Singh *et al.*, 2024).

2.6. Treatment pattern and experimental design

Sterilized seeds of both cultivars were pre-soaked in water for 12 hours (the optimal duration as shown in the previous test). After that, every 10 uniform seeds were placed in a clean and oven-dried Petri dish lined with 2 layers of filter paper. The experiment was conducted in 130 glass Petri dishes (10 cm diameter). The dishes were categorized into three groups. Group 1: irrigated with 3 mL/dish of CuO/MWCNTs at concentrations of 0, 0.5, 1.0, 1.5, and 2.0cm^3/L. Group 2: irrigated with 3 mL/dish of ZnO/MWCNTs at concentrations of 0, 0.5, 1.0, 1.5, and 2.0 cm^3/L. Group 3: irrigated with 3 mL/dish of CuO/MWCNTs + ZnO/MWCNTs at concentrations of 0, 0.5, 1.0, 1.5, and 2.0 cm^3/L (V/V). Thus, each group contains 40 Petri dishes (20 for each cultivar; Gana F1 or Alissa F1), in addition to the control group, which includes 10 Petri dishes. The Petri dishes were incubated at 30°C. Throughout the experimental duration, the Petri dishes were observed daily and replenished with distilled water or other treatments as needed. It should be noted that five replicates were used to determine the different germination parameters.

The different germination characteristics, including germination percentage (GP%), plumule length (PL; cm), radicle length (RL; cm), seedling length (SL; cm), seedling fresh weight (SFW; g −1), and germination vigor, were determined in 7-day-old seedlings.

$$\text{Germination (\%)} = \left[\frac{\text{Number of germinated seeds}}{\text{Total Number of tested seeds}}\right] \times 100$$

Seedling Vigor Index (SVI) was assessed according to (Abdul-Baki and Anderson, 1973) using the following formula:

SVI = Germination (%) * Total seedling length (cm), each value is the mean of 5 replicates.

2.7. Statistical analysis

The statistical analysis was carried out using two-way ANOVA using SPSS, ver. 27 (IBM Corp. Released 2013). Data were analyzed using a complete randomization design (Steel and Torrie 1981). Multiple comparisons were carried out applying Duncan's test. The significance level was set at < 0.05. Each value is the mean of 5 replicates.

3. Results and Discussion

3.1. Seed viability test

A seed viability test was conducted to assess the physiological quality of tomato seeds from two different cultivars: Gana F1 and Alissa F1. This test is essential to determine the proportion of seeds that are alive and capable of developing into healthy seedlings under optimal conditions. In this study, seed viability was assessed using a simple germination-based approach with distilled water under controlled conditions. The results (Fig.1) showed that the tomato seeds began germinating three days after sowing (3 DAS) in the Petri dishes with germination percentage 92% and 90% for Gana F1and Alissa F1, respectively. On the fifth day (5 DAS), Fig. 1, all 10 seeds had successfully germinated, with 100% germination for both cultivars.

There was a significant difference ($P>0.05$) in germination percentage between the incubation periods 3 and 5 within the same cultivar (Fig. 1). However, there was no significant difference ($P>0.05$) in the germination percentage between the two cultivars (Gana F1 and Alissa F1), within the same incubation period. A seed was considered viable if it successfully germinated, and a viability rate of 90%, at least (i.e., nine out of ten seeds), was set as the threshold for acceptable physiological quality. This approach aligns with standard seed viability evaluation protocols that consider direct germination performance as a practical indicator of seed health (Copeland et al., 2001).

The data also showed that Gana F1 has a higher germination rate than Alissa F1, suggesting superior viability and potential for improved crop yields. These differences in germination rate can significantly affect agricultural productivity, making Gana F1 a more desirable choice for farmers seeking to optimize yields.

3.2. Optimum soaking duration

Generally, the data (Fig. 2) showed a significant difference ($P>0.05$) in germination percentage across the different soaking durations (0, 6, 12, 18, and 24 hours) within the same cultivar. Even though there was no significant difference ($P>0.05$) in the percentage of germination between the two cultivars (Gana F1 and Alissa F1), within the same soaking duration, except for 12 hours. Additionally, Fig. (2) determined 12 hours soaking as the optimal soaking duration as it achieved the highest germination percentage (94% and 90% for Gana F1 and Alissa F1 respectively) and most healthy seedling development compared to other soaking durations (Fig.2). This suggests that 12hrs of soaking provides sufficient water absorption to activate the seeds' metabolic process without causing oxygen deprivation or tissue breakdown that may occur with over-soaking. These findings are consistent with a pervious study (Sabongari and Aliero 2004) indicated that 12-24 hrs of soaking significantly enhanced tomato seed germination, with 24 hrs showing the best results. However, their data also indicated that certain varieties responded equally well to 12 hrs, supporting the variability between genotypes. Similarly, durations of 8-12 hrs were sufficient to stimulate germination in tomato seeds, particularly when organic inputs were used (Arancon et al., 2012). Therefore, the results of this experiment align well with the results of this study and suggest that a 12-hr soaking period is optimal for promoting germination without risking overhydrating or microbial contamination.

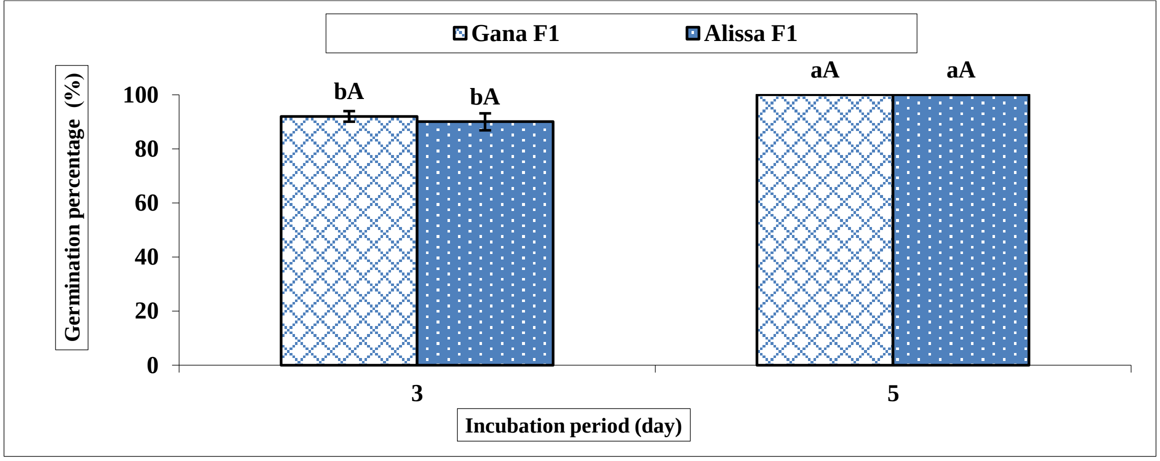


Figure 1: The germination percentage of both tomato cultivars: Gana F1 and Alissa F1. Each value is the mean of 5 replicates.

The small case letters (a, b & c) indicate that there is no significant difference (P>0.05) in germination percentage between the incubation periods (3 and 5 days) for any two cultivars with the same superscript letter within a cultivar.

While the upper-case letters, A, B & C mean there is no significant difference (P>0.05) between any two germination percentage means between the two cultivars (Gana F1 and Alissa F1), have the same superscript letter within the same incubation period.

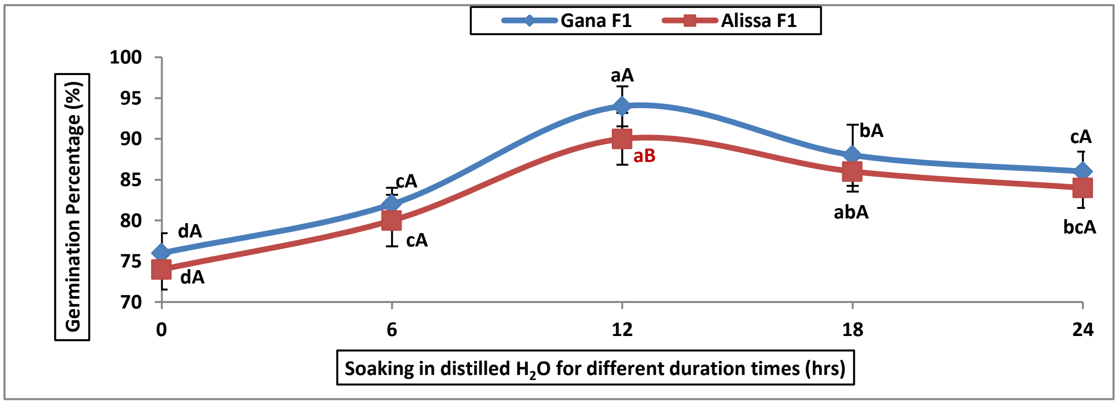


Figure 2: The different soaking durations in distilled H_2O for cultivars, Gana F1 and Alissa F1 cultivars. Each value is the mean of 5 replicates.

The small case letters (a, b & c) indicate that there is no significant difference ($P > 0.05$) in the germination percentage means across the different soaking durations (0, 6, 12, 18, and 24 hours) within the same cultivar.

While the upper-case letters, A, B & C mean there is no significant difference (P>0.05) between any two germination percentage means between the two cultivars (Gana F1 and Alissa F1), have the same superscript letter within the same soaking duration.

3.3. Characterization of CuO/MWCNTs and ZnO/MWCNTs nanocomposites

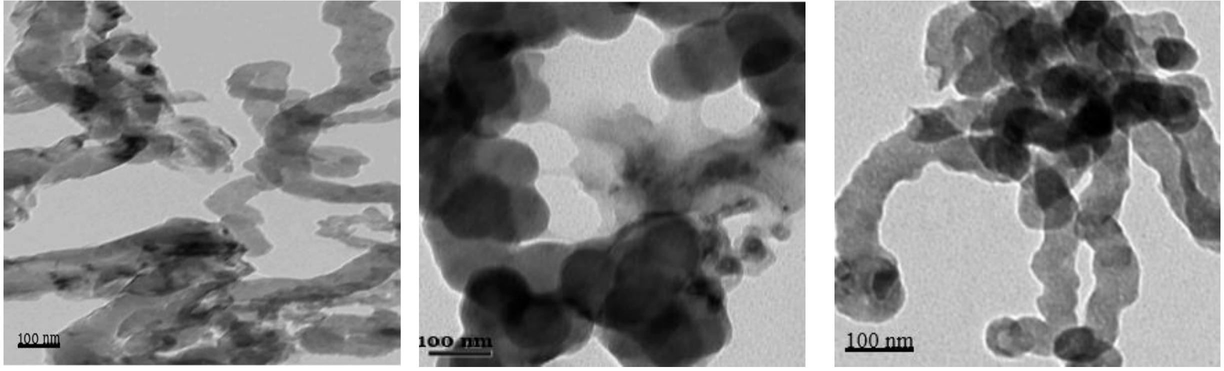
3.3.1. Transmission Electron Microscopy (TEM):

TEM analysis was employed to examine the internal structure of the synthesized nanomaterials. TEM image of the as-received MWCNTs (**Fig. 3A)** indicating agglomerated nanotubes in the form of a bundled network with an inner diameter of approximately 50-75 nm. The TEM image (Fig. 3A) showed that the MWNTs are uniformly modified and confirmed the nanoscale nature of the synthesized materials. The CuO/MWCNTs and ZnO/MWCNTs nanocomposites (Fig.3 B and C) and the TEM analysis (Fig.4) revealed a uniform distribution of metal oxide nanoparticles along the surface of the MWCNTs. The nanoparticles appeared well dispersed and firmly attached, indicating successful integration of the metal oxides into the carbon nanotube matrix. The metal oxides are adhering to the external surface of MWCNTs (Chen et al., 2013; Domagała et al., 2020). Furthermore, they firmly adhered to the nanotube tubular structure. Also, the results are in accordance with another investigation (Wahba et al., 2021) confirmed the formation of ZnO hexagonal wurtzite with cabbage-like structure and the good surface-surface contact between the MWCNTs and ZnO particles with homogenous distribution of MWCNTs in the powders using TEM and SEM images. The histogram (Fig. 4 A) showed that the particle size distribution ranged from 40 to 120 nm, giving an average size of approximately 70–80 nm, assuming the histogram peaks within this interval. Such particle dimensions are characteristic of CuO nanoparticles supported on CNTs, enabling efficient surface contact and electron transfer. The uniform dispersion of CuO particles across the CNT network confirms proper anchoring, thereby increasing surface area and the density of active catalytic sites. TEM analysis of the Particle size distribution of ZnO–MWCNTs nanocomposite (Fig. 4 B) and indicated that most of the ZnO nanoparticles are distributed within the range of 60–200 nm. These results yielded an average particle size of about 140 nm, as indicated by the histogram peak in this range. These dimensions are typical for ZnO nanoparticles loaded on CNTs, exhibiting effective surface contact and enhanced electron transfer. The uniform distribution of ZnO particles across the CNT network demonstrates successful anchoring, increasing surface area, and the density of active catalytic sites.

3.3.2. Zeta Potential Measurement

Zeta potential analysis was used to evaluate the surface charge and suspension stability of the nanocomposites. Both CuO/MWCNTs and ZnO/MWCNTs showed zeta potential values within the range, indicating moderate to good colloidal stability. This suggests that the nanocomposites possess a suitable surface charge that resists aggregation, making them promising candidates for biological or environmental applications where solution stability is essential.

The zeta potential measurement for the CuO nanocomposite showed a value of +11.53 mV (Table 1 and Fig.5 A). The positive potential value can be attributed to the presence of Cu^{2+} ions and surface CuO groups covering the MWCNT surface, which shifted the charge from negative (-14.58 mV in pure MWCNTs) to positive. This positive surface charge indicated that the nanoparticles possess a moderately stable colloidal dispersion (Wang et al., 2009). CuO and related copper species or other metal oxides can uniformly coat MWCNTs (Sivkov et al., 2021). At the same time recent studies confirmed the formation of Cu–O–C bonds on the nanotube surface, providing a mechanistic basis for the observed change in surface charge.



A B C

Figure 3: TEM image of (A): MWCNTs, B: the morphology of decorated CuO/MWCNTs nanocomposite, and C: the morphology of decorated ZnO/MWCNTs nanocomposite.

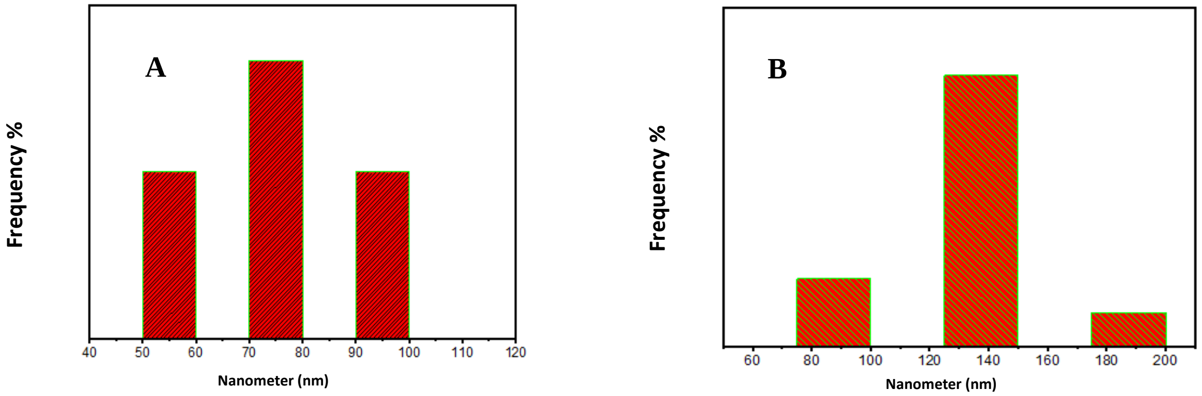


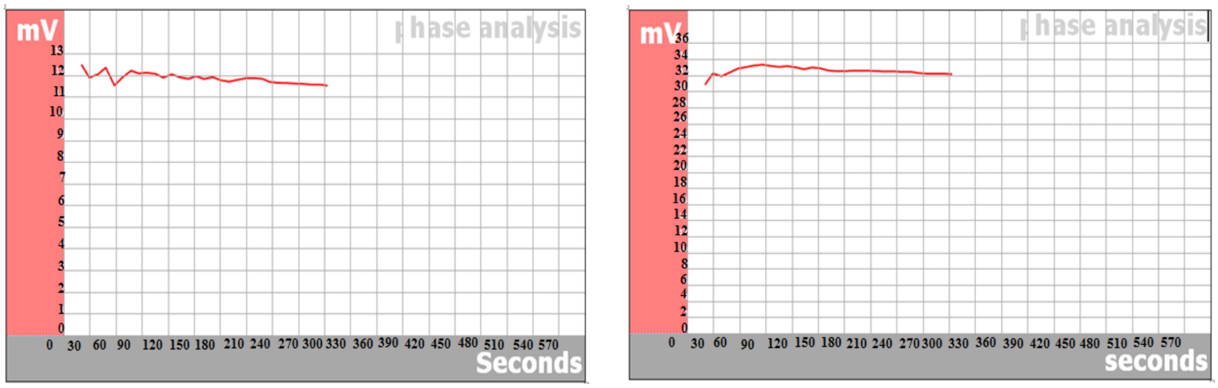
Figure 4: TEM images indicating the deposition of (A): CuO and (B): ZnO nanocrystals along the walls of the tubes.

Table 1: The zeta potential measurements for CuO/MWCNTs and ZnO/MWCNTs nanocomposites.

Sample	Zeta potential (mV)
MWCNTs	- 14.58
CuO/MWCNTs	+11.53
ZnO/MWCNTs	+32.21

Generally, it is recognized that aggregation takes place within ±15 mV of the zeta potential, dispersion becomes unstable within ±10 to ±30 mV, and sustained dispersion is maintained when the zeta potential exceeds ±30 mV. CuO nanocomposites exhibited a zeta potential of +11 mV, indicating incipient instability (Bae et al., 2009).

The Zeta Potential of ZnO nanocomposite measured at +32.21 mV, which indicated a moderately stable suspension (Bae et al., 2009). The positive potential value can be attributed to the presence of Zn^{2+} ions and surface ZnO groups covering the MWCNT surface, which shifted the charge from negative (-14.58 mV in pure MWCNTs) to positive (Wang et al., 2009). The ZnO nanoparticles possess a reasonably high positive zeta potential, indicating good dispersion and stability in their suspension (Table 1 and Fig. 5 B), which is favorable for various applications, especially in nanotechnology, medicine, and materials science.



A B

Figure 5: Zeta potential analysis of A: CuO/MWCNTs and B: ZnO/MWCNTs.

3.4. Seed germination characteristics in response to the different treatments

In this experiment, representative Gana F1 and Alissa F1 samples of the different treatments CuO/MWCNTs and ZnO/MWCNTs, separately or in combination, were collected 7 days after sowing (7 DAS), when apparent variations in germination characteristics could be observed. In these samples the germination percentage radicle length, plumule length, seedling length and fresh weight, and the germination vigor were determined (Plate 1). Seed germination and seedling growth are widely used to evaluate the phytotoxicity of chemical substances, including engineered nanomaterials, because seed germination, as well as shoot and root elongation measurements, are relatively rapid indicators for detecting acute phytotoxicity (Munzuroglu and Geckil, 2002)**,** offering several advantages: sensitivity, simplicity, low cost, and compatibility with reactive compounds and contaminated soil samples.

3.4.1. Seed germination percentage in response to the different treatments.

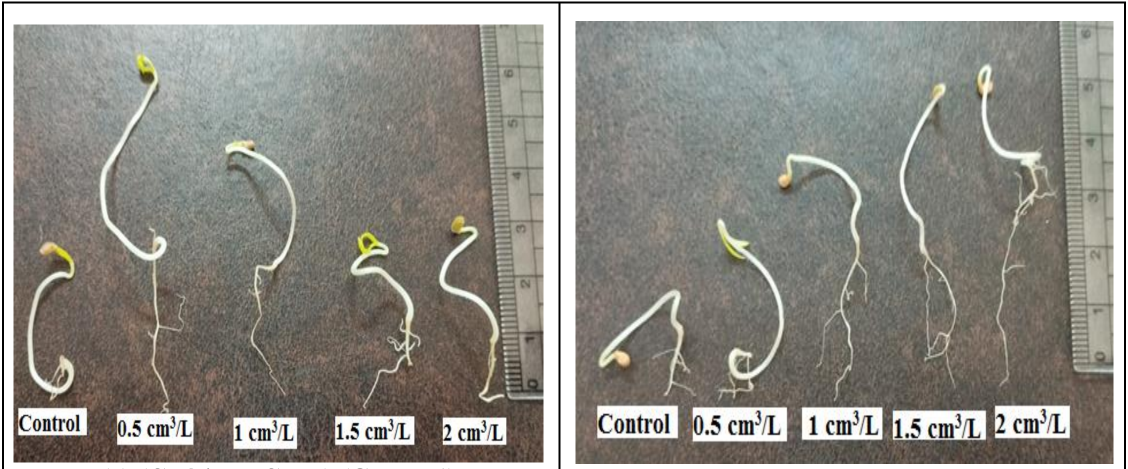
Overall, seed germination significantly changed ($P>0.05$) on the third day (3 DAS) among the different concentrations (0.5, 1.0, 1.5, and 2.0 cm^3/ L) of CuO/MWCNTs, ZnO/MWCNTs, or CuO/MWCNTs + ZnO/ MWCNTs (V/V) as well as between the two cultivars; Gana F1 and Alissa F1 plants (Fig. 6).

The separate CuO/MWCNTs treatment significantly augmented seed germination at 0.5 and 1.0 cm^3/L for Gana F1 and 0.5, 1, and 1.5 cm^3/L for Alissa F1 plants achieving 100% germination at 0.5cm^3/L in both cultivars (Fig. 6). The other single treatment of ZnO/MWCNTs particularly increased seed germination significantly at 0.5, 1, 1.5 and 2.0cm^3/L ZnO/MWCNTs as compared for both Gana F1 and Alissa F1 control plants. Besides, these changes were cultivar-dependent at all tested concentrations except for 1.5 cm^3/L ZnO/MWCNTs. The maximum seed percentage (100%) was obtained at 1.5 cm^3/L ZnO/MWCNTs for both cultivars. Besides, the mixed treatments, CuO/MWCNTs and ZnO/MWCNTs (V/V), significantly enhanced tomato seed germination at 1, 1.5, and 2 cm^3/L compared to Gana F1 and Alissa F1 control plants, one-to-one. Superior germination induction was achieved at 1.0 cm^3/L and was evaluated at 100% for both cultivars (Fig. 6). By the fifth day, all treatments and concentrations, as well as the control plants, reached 100% seed germination. This trend continues: a full germination percentage through the seventh day, then it begins to drop off. This outcome is supported by a previous investigation (Asmat-Campos et al., 2022) which recorded the same tendency in tomato seeds treated with ZnO NPs. These findings suggest that none of the investigated nanomaterials, either individually or in combination, had any inhibitory effect or were phytotoxic to the germination of both cultivars, regardless of concentration. Moreover, uniform germination across treatments and concentrations at the 5th and 7th days also indicated that all concentrations maintained full viability and germination potential in both cultivars.

The results also revealed that the maximum germination was induced at the lowest concentration of CuO/MWCNTs (0.5 cm^3/L) and reduced at higher concentrations, is in accordance with a previous work (Kadri *et al.*, 2022) who recorded increased germination parameters at lower concentrations of Cu NPs, and decreasing values at higher concentrations in Barley (*Hordeum vulgare* L.) seedlings.

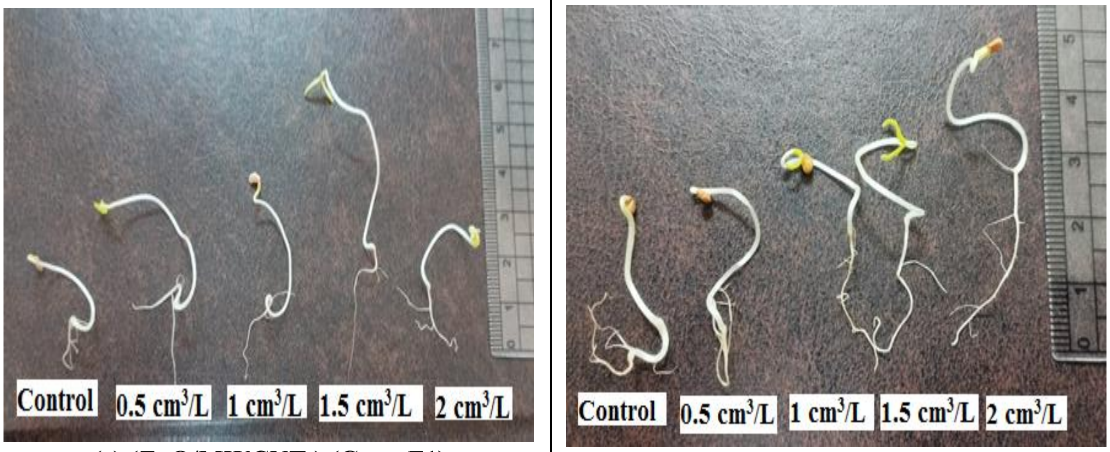
For ZnO/MWCNTs, 1.5 cm^3/L was determined as the optimal concentration, as it induced 100% germination on the 3rd day for both cultivars. Moderate and near-high concentrations of ZnO-based nanomaterials have been documented to improve seed germination performance compared to lower or excessive doses. For instance, a study (Włodarczyk and Smolińska, 2022) found that in tomato (*Solanum lycopersicum* L.), the suspension of ZnO nanoparticles (<50 nm) was optimal, yielding a germination rate index about 45.7 % higher than the control. Similarly, a Zn-supported multiwalled carbon nanotube (ZnO/MWCNT) nanocomposite at an intermediate concentration (15 µg mL^{-1}) produced the best seedling growth and germination rate, demonstrating a synergistic micronutrient-release effect that promoted seed germination under arid conditions (Kumar et al., 2018). Additional studies reinforce the concept that ZnO nanoparticles at medium to moderate–high concentrations can significantly enhance germination outcomes. For example, in wheat, priming with ZnO NPs up to 250 ppm/L markedly increased germination rate compared to both the control and lower concentrations, indicating an optimal middle-range effect (Pandya et al., 2024). Likewise, in tomato seeds treated with ZnO NPs across a concentration gradient (10-500 ppm/L), dose-dependent inhibition began at relatively low levels (≈ 20 ppm). However, intermediate concentrations still outperformed both lower and very high treatments (Emam *et al.*, 2022).

These findings collectively suggest that medium to moderately high concentrations of ZnO and ZnO–MWCNT nanomaterials can provide optimal conditions for seed germination and early seedling development. Regarding the mixed treatment CuO/MWCNTs + ZnO/MWCNTs, the 1 cm^3/L concentration showed the highest germination



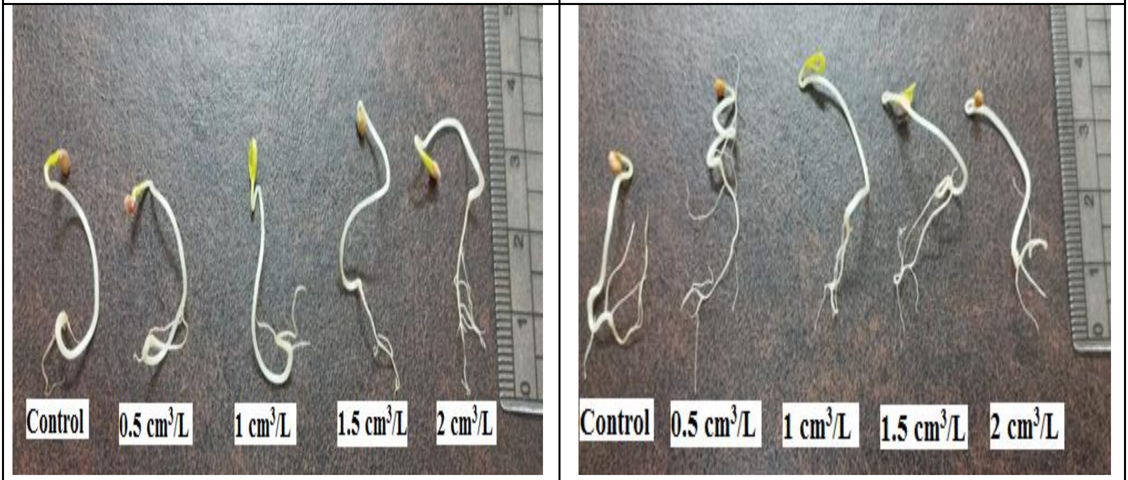
(a) (CuO/MWCNTs) (Gana F1)

(b) (CuO/MWCNTs) (Alissa F1)



(c) (ZnO/MWCNTs) (Gana F1)

(d) (ZnO/MWCNTs) (Alissa F1)



e) (CuO/MWCNTs) + (ZnO/MWCNTs) (Gana F1)

f) (CuO/MWCNTs) + (ZnO/MWCNTs) (Alissa F1)

Plate 1: Representative Gana and Alissa samples of the different treatments, CuO/MWCNTs and ZnO/MWCNTs separately or in combination (0.5, 1, 1.5, and 2 cm^3/L) were collected (7 DAS) when apparent morphological variations could be seen as compared to control plants of both cultivars.

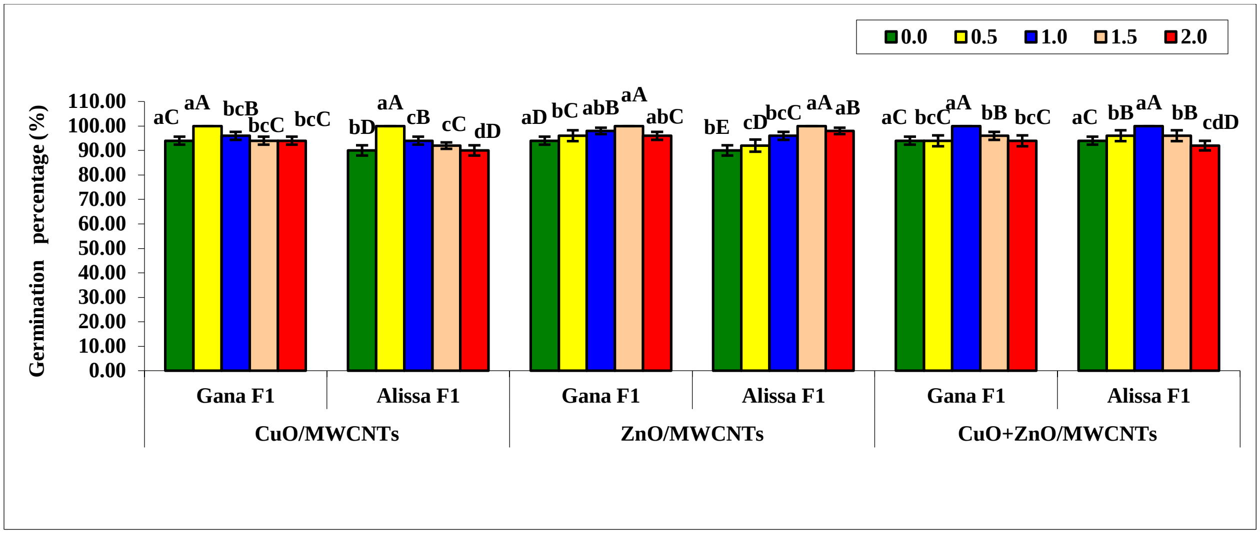


Figure 6: The germination percentage of tomato seeds (Gana F1 and Alissa F1) Soaked 12/ h in water and irrigated with 3 mL of H_2O_2, CuO/MWCNTs, ZnO/MWCNTs and CuO/MWCNTs+ZnO/MWCNTs nanocomposites treatments three days after sowing (3 DAS).

The small case letters; a, b & c mean there is no significant difference ($P>0.05$) in the germination percentage between any two means that have the same superscript letter within the same cultivar.

While, the upper-case letters; A, B & C mean there is no significant difference ($P>0.05$) in the germination percentage between any two means that have the same superscript letter within the same concentration.

percentage. As reported previously, low concentrations of CuO/MWCNTs and moderate levels of ZnO/MWCNTs were found to promote seed germination and early seedling growth. Therefore, it can be inferred that the combined treatments CuO/MWCNTs and ZnO/MWCNTs are likely to exert their most significant effects at moderate concentrations, where the synergistic interaction between the nanocomposites enhances physiological performance without inducing toxicity (Ji et al., 2022)**.**

3.4.2. Radical length in response to the different treatments

Regarding the consequences of different nano treatments on radicle expansion, the single treatment with CuO/MWCNTs significantly increased radicle length at 0.5 and 2.0 cm^3/L for Gana F1 and at 0.5, 1.5, and 2 cm^3/L for Alissa F1 plants. Furthermore, this enhancement was cultivar-dependent. The uppermost radicle length achieved at this treatment was recorded at 0.5cm^3/L, with an average length of (3.22±0.79), (3.57±0.60) cm compared to (2.67±0.37^{bB}, 3.17±0.37) cm for both Gana F1 and Alissa F1 control plants in the same order. On the other side, the single treatment of ZnO/MWCNTs significantly increased the radical length at all concentrations for Gana F1 plants and 0.5, 1.5, and 2.0 cm^3/L in Alissa F1 cultivar. Besides, these changes were not cultivar-dependent at the concentrations of 1 and 2 cm^3/L. The maximum increment in radicle length was 28.46% and 20.5% over the control group at 1.5 cm^3/L for Gana F1 and Alissa F1, respectively, in the one-to-one comparison.

Concerning the mixed treatment, CuO/MWCNTs and ZnO/MWCNTs (V/V), this combination significantly induced root elongation at 0.5, 1, 1.5, and 0.5 and 1 cm^3/L for Gana F1 and Alissa F1, respectively. The higher induction was achieved at 1.0 cm^3/L and evaluated by 33.3% and 33.75% for Gana F1 and Alissa F1 compared to the control group **(**Fig. 7). In accordance, a recent study (Hoe *et al.*, 2018) revealed that lower concentrations of 25 and 50 mg l^{-1} copper nanoparticles treatments significantly stimulated the primary roots elongation in soybean and similar results were recorded in barely plants (Kadri et al., 2022).

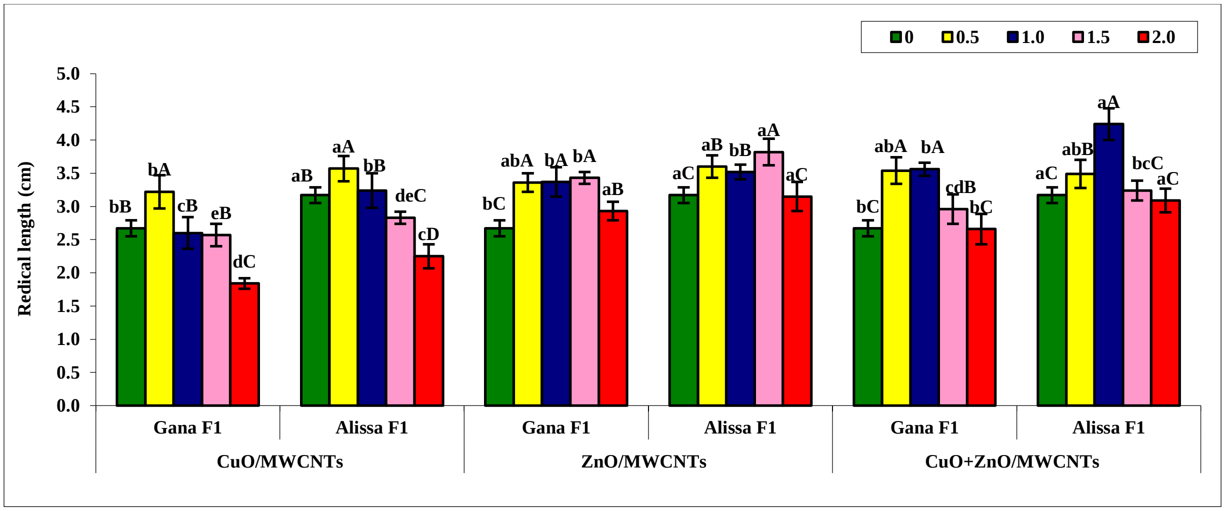


Figure 7: Gana F1 and Alisa F1 cultivars' radical length, under the irrigation treatment using Cu/MWCNTs and Zn/MWCNTs either separately or in combination at different concentrations (0.5, 1, 1.5, 2 cm^3/L). Each value is the mean of 5 replicates. (mean± SE).

The small case letters (a, b & c): There is no significant difference ($P > 0.05$) in radical length between any two means within the same column (cultivar) that have the same superscript letter.

For the uppercase letters A, B & C, there is no significant difference ($P > 0.05$) between any two means within the same row (concentrations) that have the same superscript letter.

Additionally, other studies have reported that zinc oxide-based nanomaterials can significantly influence the germination and early seedling growth of tomato (*Solanum lycopersicum* L.). The application of ZnO nanoparticles at low to moderate concentrations has been shown to enhance root elongation by improving water uptake, enzymatic activity, and micronutrient availability (Dimkpa *et al.*, 2012; Włodarczyk and Smolińska, 2022). The mixed ZnO and CuO+ZnO NPs treatment also slightly increased root length (3%) at concentrations below 10 mg/L. But, this elevation in root length was not significantly different from the control (Singh and Kumar, 2019).

3.4.3. Plumule length in response to the different treatments

Remarkably, the data (Fig. 8) revealed significant variations across different treatments, concentrations, and cultivars. The CuO/MWCNTs nanocomposite significantly promoted plumule elongation at 0.5 and 1 cm³/L, only in Gana F1. The most inducible plumule length, 3.16 cm, was achieved at 0.5 cm³/L compared to 2.63 cm for the Gana F1 control. In contrast, the same treatment significantly suppressed Alissa F1 plumule elongation. The inhibition observed in Alissa F1 plumule was confirmed to be dose-dependent, with a minimum length of 2.51 cm at 2.0 cm³/L, compared to 3.30 cm for the Alissa F1 control.

Conversely, ZnO/MWCNTs significantly stimulated plumule elongation in both cultivars except for 0.5 and 1 1.5cm³/L for Alissa F1 only. This stimulation was generally cultivar-dependent. The maximum recorded plumule length was 1.5 cm³/L ZnO/MWCNTs for both cultivars, with an increase of 41.8% and 11.21% over control plants for Gana F1 and Alissa F1, respectively.

The mixed treatment CuO/MWCNTs + ZnO/MWCNTs (V/V) significantly increased plumule length at all concentrations, except 2 cm³/L, compared with the control in Gana F1 plants. It is worth mentioning that the same treatment non-significantly elongated Alissa F1 plumule at 1.5 cm³/L only and significantly depressed plumule elongation at 0.5, 2 cm³/L, while non-significantly depressed it at 1 cm³/L compared to Alissa control. This mixed treatment yielded the highest plumule length at 1.0 and 1.5 cm³/L, reaching 3.45 and 3.27 cm, respectively, compared to 2.63 and 3.30 cm for Gana F1 and Alissa F1 control plants, respectively (Fig. 8).

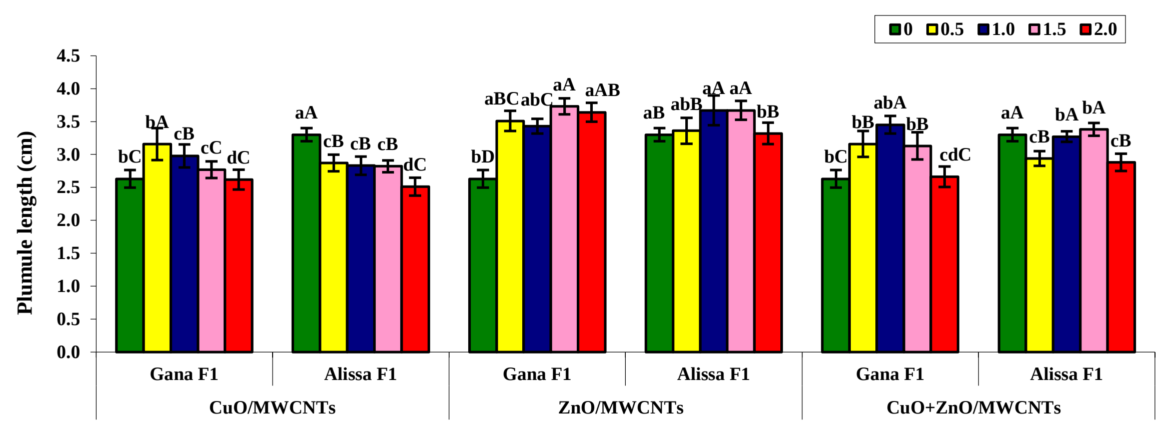


Figure 8: The plumule length of Gana F1 and Alisa F1 cultivars irrigated with Cu/MWCNTs and Zn/MWCNTs either separately or in combination with different concentrations (0.5, 1, 1.5, 2 cm^3/L). Each value is the mean of 5 replicates. (mean± SE).

The small case letters (a, b, & c): There is no significant difference ($P > 0.05$) between any two means within the same column (cultivar). Those with the same superscript letter are not significantly different.

For the uppercase letters A, B & C, there is no significant difference ($P > 0.05$) between any two means within the same row (concentration), and the same superscript letter is used.

These results align with those of (Khaldari *et al.*, 2021), who demonstrated that root and shoot development of tomato and lettuce seedlings was effectively promoted by both green and chemically synthesized CuO NPs at the lowest concentration; however, at higher concentrations, the NPs exerted a limiting effect on all examined germination factors. Additionally, concentrations of copper oxide nanoparticles exceeding 100 ppm markedly inhibited root and shoot elongation in two key plant species, Glycine max and Cicer arietinum (Adhikari *et al.*, 2012).

On the other hand, moderate ZnO NP concentrations (25–250 mg·L^{-1}) improved root and plumule length. In comparison excessive doses (≥500 mg·L^{-1}) inhibited growth due to ion accumulation and ROS generation (de la Rosa *et al.*, 2013; Basith *et al.*, 2018) . Also, ZnO nanoparticles stimulate seed metabolic processes at sub-toxic levels, whereas higher concentrations induce oxidative stress and reduce elongation (Dimkpa *et al.*, 2012).

Interestingly, the use of ZnO-MWCNTs nanocomposites provides a synergistic advantage by facilitating nutrient transport through the nanotube matrix and enhancing oxidative enzyme activity, thereby promoting radicle emergence and shoot development (Khaldari *et al.*, 2021). These results collectively confirm that ZnO-MWCNTs, when applied within an optimal concentration range, can positively modulate tomato germination and early seedling growth by balancing nutrient enhancement and oxidative stability.

3.4.4. Seedling length in response to the different treatments

In general, the observed changes in seedling length were not cultivar-dependent. In contrast, seedling length showed significant fluctuations across treatments and concentrations, except within narrow limits. This study (Fig. 9) revealed that single CuO/MWCNT treatment showed a substantial surge at 0.5 and 1 cm^3/L in both cultivars. After that, the seedling length declined with increasing CNT dose. The most considerable seedling length averaged 7.22 and 6.94 cm compared to the control group of 6.59 and 6.48 cm for Gana F1 and Alissa F1, respectively. The single treatment of ZnO/MWCNTs showed a continuous increase in seedling length, reaching a maximum at 1.5 cm^3/L. This increment was estimated at 26.1% and 18.83% over the Gana F1 and Alissa F1 controls, respectively. The combination of CuO/MWCNTs and ZnO/MWCNTs V/V also significantly induced seedling elongation at lower to moderate concentrations, achieving the highest length at 1.0 cm^3/L in both cultivars. This increase in seedling length was 17.45% and 17.44% over the control group for Gana F1 and Alissa F1, respectively. Later, the seedling length declined and was even inhibited at 2 cm^3/L in Alissa F1 plants.

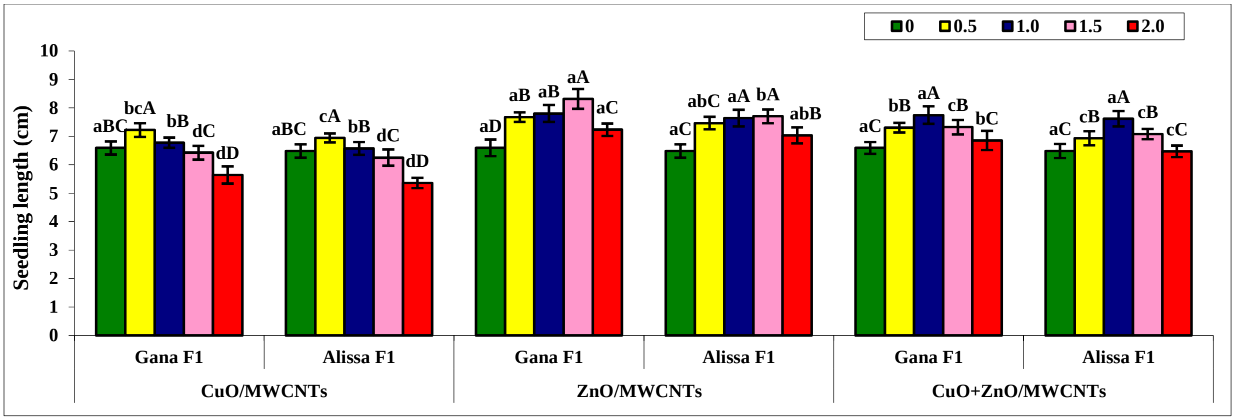


Figure 9: The seedling length of Gana F1 and Alisa F1 cultivars irrigated with Cu/MWCNTs and Zn/MWCNTs either separately or in combination with different concentrations (0.5, 1, 1.5, 2 cm^3/L).

The small case letters (a, b & c): There is no significant difference ($P > 0.05$) between any two means within the same column (cultivar); those with the same superscript letter are not significantly different.

For the upper-case letters A, B & C, there is no significant difference ($P > 0.05$) between any two means within the same row (concentration), and the same superscript letter is used.

Given that both root and shoot lengths have been observed to increase in response to CuO /MWCNTs, ZnO/MWCNTs and their combination, it is expected that the overall seedling length will also increase. This enhancement is due to the synergistic effect of MWCNTs as carriers of metal nanoparticles, which facilitate nutrient and water uptake, ultimately promoting the growth of the entire seedling (Ji *et al.*, 2022; Włodarczyk and Smolińska 2022).

3.4.5. Seedling weight in response to the different treatments

Regarding the potential effects of the two tested MWCNTs on tomato seedling weight, the data in **Figure 10** showed significant variation across treatments and concentrations and to a certain extent across the two cultivars. The CuO/MWCNTs displayed the heaviest seedling at 0.5cm³/L CuO/MWCNTs, with an average of 0.036, 0.035g compared to 0.030, 0.031g for Gana F1and Alissa F1 control plants, respectively. The second treatment, ZnO/MWCNTs, showed a significant increase in seedling weight at all concentrations in both cultivars, except at 0.5 in Alissa F1. The maximum seedling weight was observed at 1.5 cm³/L for both cultivars, with average increases of 16.67% and 12.9% over the control group for Gana F1 and Alissa F1, respectively. The mixed CuO/MWCNTs and ZnO/MWCNTs (V/V) treatment achieved the highest seedling weight of 0.031 and 0.038 g, respectively, compared to the control group (0.030 and 0.031 g for Gana F1 and Alissa F1, respectively) at 1.0 cm³/L CuO/MWCNTs + ZnO/MWCNTs (V/V).

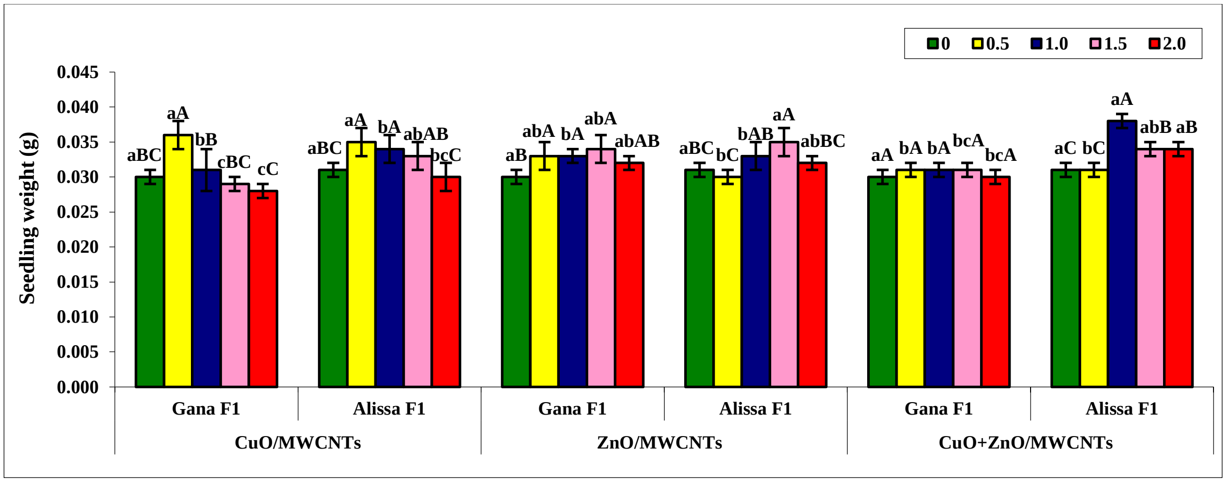


Figure 10: The seedling weight of Gana F1 and Alisa F1 cultivars sprayed with Cu/MWCNTs and Zn/MWCNTs either separately or in combination with different concentrations (0.5, 1, 1.5, 2 cm^3/L)

The small case letters (a, b & c): There is no significant difference ($P > 0.05$) between any two means within the same column (cultivar); those with the same superscript letter are not significantly different.

For the uppercase letters A, B & C, there is no significant difference ($P > 0.05$) between any two means within the same row (concentration).

These data agree with an investigation demonstrated an increase in fresh weight to 0.28±0.006g and in dry weight to 0.13±0.003g per plant at a 20 ppm Cu_2O NPs concentration (Ananda et al., 2019).

In contrast, (Pandya et al., 2024) showed that the biomass of wheat seedlings, increased gradually with increasing doses of ZnO nanoparticles. Moreover, the deleterious impact of elevated NPs doses in both individual and combined regimens on root length, branch length, and seedling fresh weight (Singh and Kumar, 2019). The toxic interaction between CuO and ZnO NPs, found to be antagonistic, was responsible for the toxic effect. Both copper and zinc were observed to accumulate in seedlings following a 7-day exposure period. In comparison to ZnO NPs, CuO NPs exhibited a greater propensity to adhere to the surface of the seedlings. In contrast, ZnO NPs were absorbed more than CuO NPs.

The carbon nanotubes could penetrate seed coats and enhance water uptake, thereby accelerating germination. These nano materials when applied at optimal concentrations, improved root growth and seedling development in tomato by facilitating nutrient uptake and stimulating cell division (Lahiani *et al.*, 2013). However, the phytotoxicity observed at higher CNT concentrations in some studies is reasonable in the light of oxidative stress induced by excessive exposure to nanomaterials, which can impair cellular function and reduce growth (Lin and Xing, 2007).

3.4.6. Seedling vigor in response to the different treatments

Germination vigor is a key parameter in assessing seed quality, reflecting the ability of seeds to germinate quickly, uniformly, and effectively under varying environmental conditions (Priyanka et al., 201**9)**. In this study, the CuO/MWCNTs treatment revealed that the lowest concentration of 0.5 cm³/L was the most effective, achieving the highest germination vigor values of 722 and 694 for Gana F1 and Alissa F1 control plants, respectively, compared with 659 and 648 for the Gana F1 and Alissa F1 control plants, respectively. This trend indicated a stimulating effect at mild levels. However, increasing the concentration led to a gradual decline in the germination vigor, likely due to nanoparticle-induced stress or toxicity.

Seeds treated with ZnO/MWCNTs showed a different pattern, with the 1.5 cm³/L concentration yielding the highest germination vigor values of 826±31.94 and 770±23.52 for Gana F1 and Alissa F1, respectively, suggesting enhanced metabolic and physiological activity at this level. Besides, the mixed treatment of CuO/MWCNTs and ZnO/MWCNTs (V/V) verified 1.0 cm³/L CuO/MWCNTs + ZnO/MWCNTs (V/V) as the optimum concentration

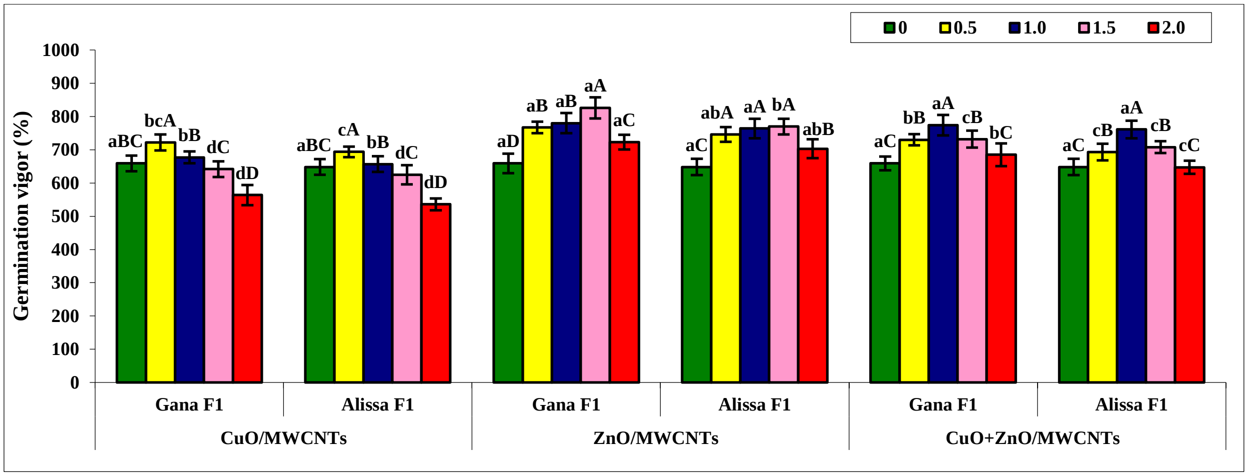


Figure 11: The Germination vigor of Gana F1 and Alisa F1 cultivars sprayed with Cu/MWCNTs and Zn/MWCNTs either separately or in combination with different concentrations (0.5, 1, 1.5, 2 cm^3/L)
The small case letters (a, b & c): There is no significant difference ($P > 0.05$) between any two means within the same column (cultivar); those with the same superscript letter are not significantly different.

For the uppercase letters A, B & C, there is no significant difference ($P > 0.05$) between any two means within the same row (concentration), and the same superscript letter is used.

that attained the highest germination vigor (774 and 761) for Gana F1 and Alissa F1, respectively (Fig.11).

Individually, copper oxide nanoparticles (CuO-NPs) and zinc oxide nanoparticles (ZnO-NPs) have been shown to increase seedling weight and vigor by improving nutrient uptake, root development, and photosynthetic efficiency. Moreover, combining metal oxide nanoparticles with MWCNTs greatly enhanced plant growth compared with individual metal oxide nanoparticles (Priyanka *et al.*, 2019). Although limited, studies on combined applications indicate that synergistic interactions can amplify these beneficial effects. For example, nanoparticle combinations can modulate oxidative stress responses and enhance biomass accumulation, depending on concentration and treatment duration (Tee et al., 2016). These results suggest that a combination of CuO-MWCNTs and ZnO-MWCNTs may significantly increase tomato seedling weight compared to individual treatments, highlighting the potential for optimized nano-enabled agricultural interventions (Priyanka et al., 2019). Furthermore, the differential response observed between the investigated cultivars, Gana F1 and Alissa F1 cultivars was attributed to genotypic variation in seed coat permeability, metabolic activity, or sensitivity to nanomaterials (Ratnikova et al., 2015).

Overall, these findings emphasize that the effectiveness of nanomaterials on germination vigor depends on both nanomaterial type and concentration, underscoring the need for careful optimization to maximize seedling vigor and early plant establishment (García-Locascio et al., 2024). Essential factors should also be considered, such as the method of nanomaterial application and the duration of exposure.

4. Conclusion

The present study represented a novel endeavor to employ MWCNTs as a smart vehicle for the delivery of Cu and Zn (acting as nano-fertilizers) to tomato seeds of two cultivars (Gana F1 and Alissa F1). Irrigation with different concentrations (0, 0.5, 1, 1.5, and 2 cm^3/L) of CuO/CNTs and/or ZnO/CNTs had a noticeable impact on the germination and early seedling establishment of both tomato cultivars. Perceiving the germination parameters, revealing stimulation or even inhibition among the different treatments, concentrations, and this effect was confirmed to be sometimes cultivar-dependent. The low to moderate concentrations (0.5, 1, and 1.5 cm^3/L) of both MOCNTs (CuO-MWCNTs and/or ZnO-MWCNTs) appeared to enhance germination percentage, radical and plumule elongation, seedling length and fresh weight, and seedling vigor, while the higher concentrations (2 cm^3/L) generally exerted inhibitory or toxic effects. The differential response between the two cultivars was attributed to genotypic variation in seed coat permeability, metabolic activity, or sensitivity to nanomaterials. Overall, the results suggest that while carbon nanotubes can be beneficial to seed germination and seedling vigor when applied at appropriate concentrations, careful optimization is crucial to avoid adverse effects. This careful case-by-case manipulation to optimize the nanomaterials applications protocols is an effective tool towards sustainable agriculture.

Author contributions:

Amany A. Hamed: Conceptualization, experimental design, data collection, manuscript drafting, and revision;

Eman Tawfik: Methodology support, supervision, and manuscript review;

Ashraf B. Abdel-Razik: Methodology support, supervision, Characterization examination and interpretation;

Samir H. Abdel-Aziz: Supervision, contributed to the conception of the study;

Aziza Nagah: Project administration, ethical approvals and final manuscript writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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