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THE EFFECT OF FUNCTIONALIZED MULTI-WALLED CARBON NANOTUBES (F-MWCNTS) ON TOMATO PLANTS (SOLANUM LYCOPERSICUM)

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Abstract:

Surfactants increased the steric hindrance as well as the charge repulsion between nearby CNT particles, which improved their suspension, according to dynamic light scattering (DLS) investigations. In addition, hydroxyl alteration of MWCNTs resulted in stable dispersions in water containing HA at 10 and 1000 mg/L, but COOH-MWCNT suspensions only showed stable dispersion with decreased negative surface charges at 100 mg/L. Agglomerates were significantly reduced in the f-MWCNT kinds. The surface characteristics of f-MWCNTs were found to have an important role in the possible hazardous consequences of CNTs. Due to their well-distributed stability in water, the interaction between the HA-CNTs and the plants increased development in terms of water intake, growth rate, chlorophyll index, dry weight, and root elongation rate. Furthermore, there were no variations in chlorophyll concentration between the f-MWCNT and HA plant groups. The f-MWCNTs dramatically improved the plants' growth, water transpiration, and dry shoot and root weight. Exposure to OH-MWCNTs appeared to benefit tomato development in terms of water uptake, root elongation rate, and growth rate, but exposure to COOH-MWCNTs appeared to be hazardous in terms of root leakage and growth rate. Overall, our findings imply that the surface features of CNTs, as well as their dispersion stability, determine their impact on tomato plant growth. According to our findings, there is a strong link between the toxicity of f-MWCNTs and the toxicity of the dispersing agent. Thus, MWCNTs with hydroxyl modifications were found to be stable.

Keywords: Functionalized Multi-Walled Carbon Nanotubes, Phytotoxicity, Humic Acid, OH-Mwcnts And COOH-Mwcnts.

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1. Introduction

Nanotechnology is defined by the National Nanotechnology Initiative (NNI) as "the science, engineering, and the technology conducted at the nanoscale, which is 1 to 100 nanometers."

The concepts and ideas behind nanotechnology were first stated by the physicist Richard Feynman at a California Institute of Technology (CalTech) lecture on December 29, 1959 entitled "There's Plenty of Room at the Bottom." [7].

At very small scales, Feynman observed fluctuations in the size of numerous physical processes. Gravity, for example, would be overshadowed by surface tension and Van der Waals attraction. Dr. K. Eric Drexler, who advanced the technological relevance of nano-scale phenomena and technologies through his many speeches and books, examined this notion of nanotechnology in much greater depth in the 1980s. Engines of Creation: The Coming Era of Nanotechnology (1986) [16] was his first book on the subject of nanotechnology. The birth of cluster science and the creation of the scanning tunneling microscope (STM) [15] marked the beginning of modern nanotechnology.

Quantum phenomena occurs naturally when matter is systematized at nanoscales and materials at nanoscale exhibit new properties such as electrical conductivity, elasticity, greater strength, different color and greater chemical reactivity [6]. Other properties, such as melting point, fluorescence, and magnetic permeability will also change as a function of the size of particles at nanoscale.

CNTs have unique properties due to their small size and very large aspect ratio. Due to their large specific surface area, their chemical and thermal stability morphologies make CNTs an attractive absorbent in wastewater treatment. It is hypothesized that functionalized and non-functionalized multi-walled carbon nanotubes could be used in such treatment by dispersing them using various kinds of natural organic matter, though allowing them to interact with the surrounding environment over the long term might have either positive or negative effects on plant species in the environment. Some studies have determined the interaction between CNTs and ecosystems in the laboratory.

This study examines the effects of f-MWCNTs on plants throughout their lives and how varied surface features of MCNTs affect flora through an in-depth examination of tomato plant growth and development. Previous studies on the toxicity and bioavailability of MWCNTs to plants have found that they have positive, negative, and neutral impacts on seedling growth.

In Miralles et al.'s study, the phytotoxicity of industrial grade multi-walled CNTs to two crop species, wheat and alfalfa were investigated. Root elongation in the presence of up to 2650 mg/L of MWNTs and 640 mg/L of catalyst impurities was enhanced for both species. In this study, Al2O3 was the catalyst used to synthesize CNTs. Both Raman mapping and TEM showed that the alfalfa and wheat were not any damaged even though these plants endured high concentrations of MWCNTs and their catalytic impurities. Furthermore, no MWCNT uptake was observed in the alfalfa roots, but MWCNTs were observed in the wheat "epidermal" root cell. Raman mapping showed that the CNTs were absorbed into the alfalfa and wheat roots, but without changing the plants' development or the morphology of the root tissues [13].

Moreover, functionalized MWCNTs used to investigate how different functional group on the surface of MWCNTs could affect the toxicity to tomato plant.

2. Materials and Methods

2.1. Tomato Seeds

The US Environmental Protection Agency [14] recommends this tomato as one of the plant species for chemical testing. Tomato plants were grown hydroponically in this study. Johnny's Selected Seeds provided the tomato seeds (Solanum lycopersicum) (NY). To avoid fungal contamination, the seeds were steeped in a 15 percent Clorox solution for 15 minutes before being washed three times with deionized water. In a 100 mL flask, make a 15 percent (v/v) Clorox solution by mixing 15 mL concentrated Clorox with 85 mL deionized or sterile water [1].

2.2. Carbon Nanotubes

Chemical vapor deposition was employed to make the MWCNTS utilised in this work. US Research Nanoparticles, Inc. provided the carboxyl and hydroxyl functionalized MWCNTs with the properties (>95 percent, OD: 7nm, -OH) and (>95 percent, OD: 7nm, –COOH), respectively (Houston, TX). With an inner diameter of 2-5 nm, both types have the same diameter range (7nm).

2.3. Seed Germination and Root Elongation

To grow tomato seedlings, sterile polystyrene petri plates of 150 mm X 15 mm were utilized. In the petri dish, twenty tomato seeds were placed on filter paper that had been steeped in twenty or thirty mL of deionized water. The seeds were spaced around 15 millimeters apart. After one week, the seed germinated and formed roots. The length of the roots was measured twice: first before the seeds were treated to f-MWCNTs and again after they were exposed to CNTs [12].

2.4. Plant Growth

After the seeds germinated and their roots reached a length of approximately 5 to 7 cm, the seedlings were transferred to a 50 mL plastic tube. These tubes were filled with a half-strength (1/2) Hoagland solution. The half- strength solution was prepared with 0.815 grams of Hoagland solution diluted in 1L of medium. The Hoagland solution was then replaced with the solutions containing the varying concentrations of f-MWCNTs dispersed with humic acid.

The tomato plants were grown in a greenhouse room at room temperature (23 degrees Celsius) with a 16-hour/8-hour light/dark light cycle [2]. The functionalized f-MWCNTs in two different functional groups (-OH and –COOH) containing a surfactant (humic acid) at varying concentrations were also investigated. The tomato plants were exposed to these various treatments for four weeks with the solutions being refilled every other day throughout the experimental period, and several analyses on plant physiological health such as water transpiration, biomass weight, shoot and root length, and chlorophyll fluorescence were monitored.

Additionally, the control treatments included contained two types: Tap water and a 0.2 g humic acid solution as a positive control. There were ten replicates for each treatment.

At the end of experiment, the fresh and dry weights of the shoot and root systems along with root leakage and membrane integrity were measured [3].

2.5. Statistical Analysis

There were 10 replicates of each treatment of functionalized MWCNTs stabilized with HA. The error bars show the standard deviation (SE), and all presented data represent the mean values for each treatment. All statistical analysis of experimental data was done with

SPSS 2.1 software, and one-way and two-way ANOVA (factorial ANOVA) were used, followed by the Duncan test if the means' values were significantly different, and the statistical significance was determined ($p < 0.05$) by the post-hoc test (Duncan's Multiple Range Test, or MRT).

3. Results and Discussion

Carbon nanotubes bind strongly due to their large specific surface area and aspect ratio (length to diameter ratio, or L/D). This is due to the van der Waals interactions that exist between tubes. As a result, they clump together and settle in water, forming enormous conglomerates. Humic acid was utilized as the dispersant. To test the effect of surface property on CNT biological interactions, two functionalized MWCNTs (-OH and –COOH functional groups) were disseminated in humic acid (HA). The negative effect of CNTs has been noticed. MWCNT was shown to be toxic to red spinach. After 15 days of hydroponic exposure, cell death and growth inhibition of spinach were observed when red spinach was exposed to solutions containing 0-1000 mg/L CNTs.

In addition, the root and leaf morphology, leaf number, and plant growth, were also affected and the effect was concentration dependent. After the addition of ascorbic acid to the growth medium, the CNT toxicity was reduced. The results suggest that the generation of Reactive Oxygen Species (ROS) and the induction of oxidative stress were deemed as the primary mechanisms for CNT toxicity as primary mechanisms [14].

Serag et al. (2011) state that MWCNTs can penetrate the cell membranes of plant protoplasts. TEM techniques and confocal imaging identified the capability of MWCNTs to traverse cell walls through mechanisms of direct penetration or internalization rather than endocytosis. An evaluation of trafficking through protoplast membranes was also done using functionalized MWCNTs (f-MWCNTs) to observe subcellular distribution. The results show that the endosome-escaping uptake mode of MWCNTs is recognized by plant protoplasts, indicating that direct penetration presents a slow endocytosis rate in a CNT medium, enhancing the CNTs' ability to escape from endosomes. Besides, at low concentrations of MWCNTs (20 mg/L), most cells display variations in different morphology. For example, chromatin levels begin to be reduced inside the cytoplasm resulting in cell death. After five days of exposure the cells showed further features of apoptosis such as the separation of the plasma membrane from the cell wall while the cells shrank in overall size. Moreover, at higher concentrations (80 mg/L), cytoplasm leakage and membrane disturbance appeared, meaning that the cells were undergoing necrosis. In comparison, untreated cells presented cytoplasm containing a nucleus and nucleolus along with normal organelles and vacuoles. This indicates that both apoptotic and necrotic processes occur when rice cells in suspension are exposed to both S-MWCNTs and A-MWCNTs [11].

In Wang et al.'s study (2012); varying concentrations of oxidized multi-walled carbon nanotubes (o-MWCNTs) were added in suspension (10, 20, 40, 80, and 160 lg/mL). The roots were observed to be longer in the seedlings grown in the o-MWCNT suspensions in comparison to the control group on the fourth and seventh days. On the third day, the relative root lengths of seeds were enhanced by 50% as well as the root growth rate as measured by root length over time. The total vegetative biomass including the stems, leaves, and roots of seedlings of wheat germinated and grown on o-MWCNT media demonstrated a significant enhancement in biomass due to the fact that CNTs create new pores that increase the rate of water uptake by the seeds, acting like molecular channels to increase the amount of water uptake by 30 to 40 percent in comparison to seedlings geminated in water only. O-MWCNTs did not affect seed germination despite the observation at 24 h and 48 h of CNTs exposure. Finally, o-MWCNTs can enhance cell elongation in root systems and induce root dehydrogenase activity which can promote water uptake by seedlings, leading to faster root growth and higher biomass production. Molecular mobility such as dehydrogenase mobility could by moderately promoted due to the resulting higher water content of plants. Another possibility is that dehydrogenase activity can be increased by the oxygen reduction reaction process occurring when CNTs absorb dehydrogenase into the sidewall of CNTs, producing a higher local electron density leading to charge transfer [13].

The genotoxicity of MWCNTs on plant tissues have also been studied. In *Allium cepa*, DNA damage, strand breakage and fragmentation along with chromosomal aberrations have been observed in the root cell which is associated with the internalization of CNTs into plant cells [8]. The DNA was studied in the absence of any cellular enzymes in order to determine whether the damage was arbitrated by the MWCNTs through direct interaction or metabolic activation. The clastogenicity of the MWCNTs was observed in the resulting chromosome breaks and formations of micronuclei at interphase. Chromosome aberrations such as fragmentation and chromosome losses can cause micro-nucleation in cells, an important parameter for detecting cytological damage. The interactions between MWCNTs and DNA are strengthened with increasing dosages and durations of exposure. For instance, the DNA crosslinking potency of MWCNTs can be measured at 24 hours as compared to three hours: 24 hours' exposure reveals an increase in DNA migration, the form of crosslink being MWCNTs-DNA or DNA-DNA. An initial dose can cause an increase in DNA damage in a short time (3hrs) followed by a decrease over a longer time (24hrs), and the opposite is also true. Moreover, MWCNT- treated cells show large numbers of "block dots" distributed in the cytoplasm and a large number of vacuoles, suggesting that CNTs penetrate subcellular structures such as the mitochondria and nucleus [8].

3.1. Zeta Potential

Zeta potential measurements tabulated in the table 1 showed that both of the two types of f-MWNTs had negative surface charges but different zeta potential values. For OH-MWCNTs, the zeta potential is -28.9 mV for 10mg/l HA-stabilized OH-MWCNTs, much lower than the 100mg/l HA-stabilized OH-MWCNTs (-0.274 mV) but similar to the 1000 mg/L HA-stabilized MWCNTs (-24.5667 mV). This is an indication that both functionalization treatments at 10 and 1000mg/L might be stable and increase the dispersion stability of MWCNTs in water. Thus, the stability of the dispersions might be occurred at the lowest and highest concentrations of HA-stabilized OH-MWCNTs. Whereas, at 100mg/L OH-MWCNTs suspensions the zeta potential had a high negative surface charge indicating that the suspension was not stable, aggregations might have occurred due to van der Waals inter-particle attractions through electrostatic repulsion.

The zeta potentials of HA-stabilized COOH-MWCNTs show varying values at different concentrations of COOH-MWCNTs but all have a negative surface charge. The zeta potential were -0.143466667 and -0.061233333 mV for HA-stabilized COOH-MWCNTs at 10 and 1000 mg/L and -28.0667 mV for the 100mg/l HA-stabilized COOH-MWCNTs. The dispersion stability of COOH-MWCNTs (100mg/L) in water might be occurred and the HA may adsorbed onto the nanotubes' surfaces. Both 10 and 100mg/L COOH-MWCNTs suspensions were not stable in water.

In summary, the zeta potential was affected by functionalized MWCNTs at varying concentrations. With reduced negative surface charges, the OH-MWCNTs may display stable dispersions in water at 10 and 1000 mg/L. While COOH-MWCNT suspensions at 100g/L showed steady dispersion and lower negative surface charges than those at other concentrations, those at other concentrations did not. This is implying that functional groups on the surface of MWCNTs may affect the zeta potential and therefore the stability of their dispersion in water.

Table 1. The zeta potential of functionalized MWCNTs dispersed using HA surfactant.

Sample Name	ZP (mV)
OH-MWCNTs+HA (10 mg/l)	-28.9
OH-MWCNTs+HA (100 mg/l)	-0.274
OH-MWCNTs+HA (1000mg/l)	-24.56666667
COOH-MWCNTs+HA (10 mg/l)	-0.143466667
COOH-MWCNTs+HA (100 mg/l)	-28.06666667
COOH-MWCNTs+HA (1000 mg/l)	-0.061233333

3.2. The Effect of F-MWCNTs on The Growth of Tomato Plants

The growth rate of plants exposed to MWCNTs and f-MWCNTs is shown in Figure 1. Growth rate is essential common indicator of plant health. There were no significant variations in tomato vegetative biomass after exposure to MWCNTs and f-MWCNTs at a concentration of 1000 mg/L. The growth rate of the HA control decreased by 5.5% compared to the plants that were exposed to water. plant biomass exposed to HA alone was significantly smaller than plants exposed solutions containing CNTs. Seedlings grown in a medium treated with100mg/L of unfunctionalized MWCNTs had much smaller growth rate than those exposed solutions containing OH-and COOH-MWCNT, difference between the two functionalized CNTs was insignificant. Though exposure to a concentration of 10mg/L of COOH-MWCNTs resulted in the lowest growth rate as compared to OH-MWCNTs and unfunctionalized MWCNTs at 100mg/L dose, both grew better than plants exposed to COOH-MWCNTs, indicating that, overall, this growth rate was not dose-dependent. Overall, the capability of MWCNTs to enhance tomato growth at a range of dosages follows the order: OH-MWCNTs>COOH-MWCNTs>MWCNTs.

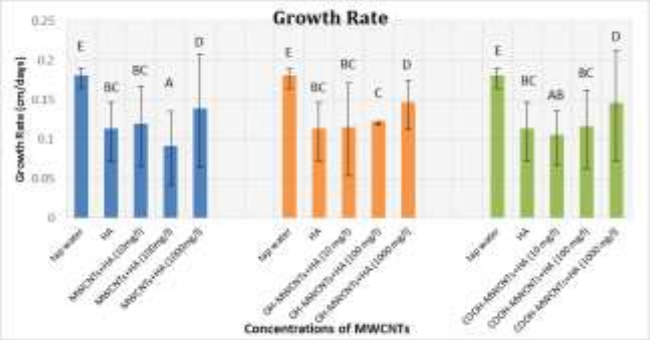

Fig. 1. Growth rate of tomato plants with OH-MWCNTs and COOH-MWCNTs at various concentrations. Different letters within a bar indicate significant differences at P <=0.05 by Duncan's multiple range test.

Besides, a slight hindering of plant growth in terms of number of leaves and shoot size was observed in plant, those who were exposed to MWCNTs were shown to be healthier than those who were exposed to f-MWCNTs. A change in leaf color was observed in plants exposed to the f-MWCNT treatments. Yellowing of leaves or leaf blades was observed at HA control and functionalized MWCNTs. However, no senescence or wilting was observed in any of the treatments.

3.3. The Effect of F-MWCNTs on Dry Shoots, and Dry Roots

Figure 2 show that seedlings hydroponically grown in 1000 mg/L of OH-MWCNTs had the highest dry root weight. In comparison, both control groups had similar dry root weight. Similarly, COOH-MWCNTs at high dosages also resulted in an increase in dry root weight. Remarkably, the plants' dry weight was less at lower concentrations of f-MWCNTs. Exposure to high concentrations of F-MWCNTs increased dry root weight in comparison to the lowest dosage. Therefore, f-MWCNTs improve root growth in contrast to HA control.

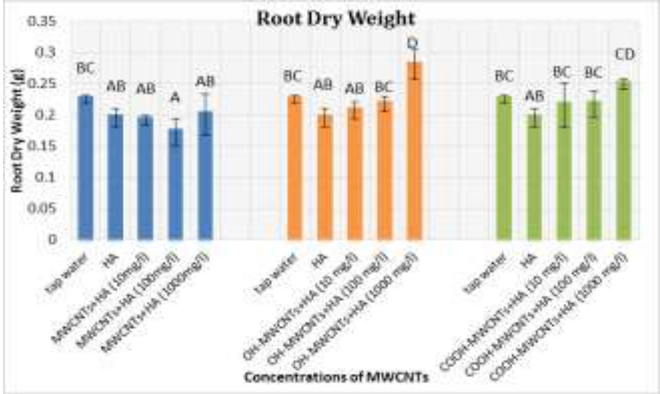

Fig. 2. The root dry weight of tomato seedlings grown on media supplemented with OH- and COOH-MWCNTs at concentrations of 10, 100, and 1000mg/L respectively.

Figure 3 shows that shoot dry weight varied with different treatments. OH- and HA-MWCNT- treated plants displayed a significant increase in shoot dry weight at 1000mg/L while the COOH-MWCNT- treated plants had similar dry shoot weight as the HA control plants. Remarkably, plants grown in a medium treated with 100mg/L of HA-MWCNTs showed a reduction in shoot growth as compared to those grown with dosages of 10 and 1000mg/L; these plants grew better, similar to the growth exhibited by the HA and water control plants. The shoot growth of F-MWCNTs seedlings improved with increasing concentrations of CNTs. However, OH-exposed plants showed greater improvement in their shoot dry weight at high and low dosages than the COOH-exposed seedlings. In comparison to HA-MWCNTs, f-MWCNTs enhance both root and shoot dry weight. In particular, OH-MWCNT- treated plants demonstrate better development in shoot and root weight, as with those exposed to the carboxyl treatment.

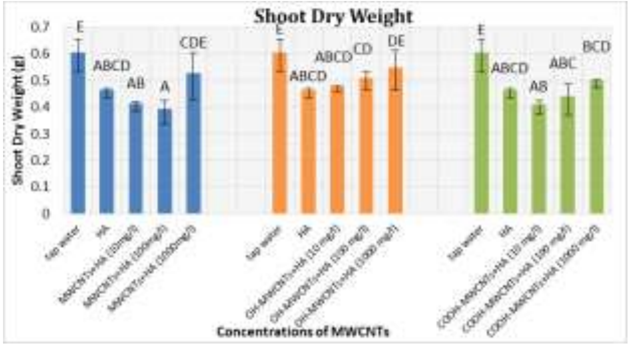

Fig.3. The shoot dry weight for OH- and COOH-MWCNTs at different concentrations.

The shoot-root ratio (S:R) shown in Figure 4 shows that the decrease in this ratio was a result of greater root size and not of a decrease in shoot dry weight. The results suggest that the plants exposed to f-MWCNTs were healthier in terms of their increases in both root and shoot dry weights. Both the HA and water control plants showed a high S: R ratio, an indication of increases in shoot dry weight and decreases in root dry weight. The reduction in dry root weight leading to an increase in the S: R ratio may be an indication of an unhealthy root system, since roots have the ability to absorb water and nutrients from the medium allowing plants to grow.

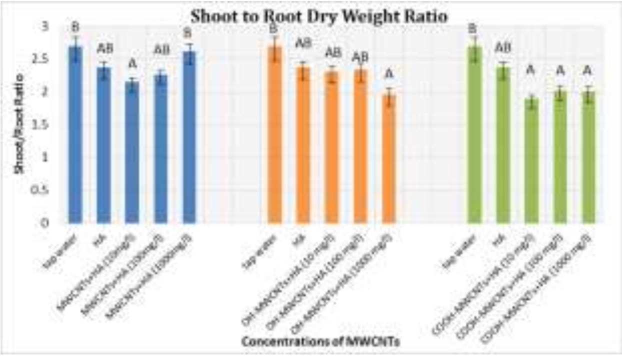

Fig. 4. The shoot-to-root ratio for dry weight of for OH- and COOH-MWCNTs.

3.4. The Effect of F-MWCNTs on Root length Rate

Figure 5 shows that seedlings exposed to a 10 mg/L concentration of HA-MWCNTs had considerably longer roots than those exposed to control groups (HA and water). The root elongation rate per day for OH-MWCNTs at 1000mg/L and HA-MWCNTs at 1000mg/L increased significantly, whereas the root growth rate of plants exposed to 100mg/L of HA- and OH-MWCNTs decreased dramatically. Plants exposed to HA also had a reduced rate of root elongation. When compared with OH-MWCNTs, root growth rate remained higher only for seedlings exposed to 10mg/L of COOH-MWCNTs while lower doses (100 and 1000mg/L) showed a statistically significant lessening in root growth rate. OH-MWCNTs enhanced root elongation rates at higher concentrations while plants exposed to COOH-MWCNTs showed a substantial diminution with increasing doses of CNTs. COOH- and HA-MWCNTs stimulated root growth rate considerably at low dosages with only the hydroxyl group showing a decrease at the lowest concentration.

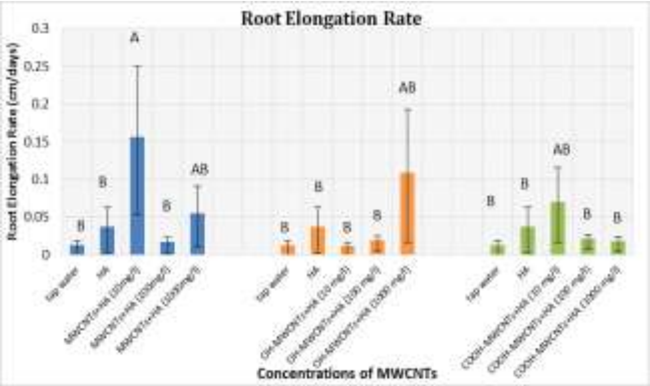

Fig. 5. The root elongation growth rate for OH- and COOH-MWCNTs.

3.5. The Effect of F-MWCNTs on Water Uptake

Figures 6 and 7 show how hydroxyl and carboxyl groups alter the water intake of f-MWCNTs. When compared to the HA control seedlings, the plants treated with MWCNTs exhibited a considerably higher amount of water uptake.

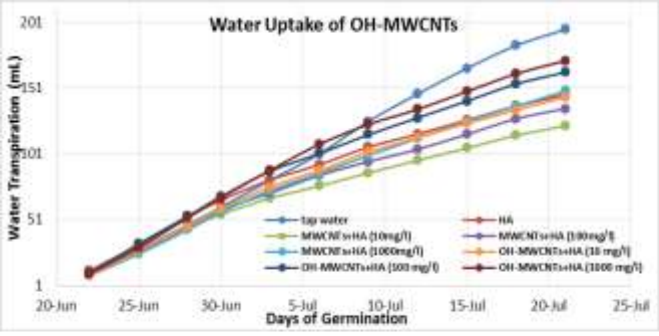

Fig. 6. The accumulative water uptake for OH- MWCNTs.

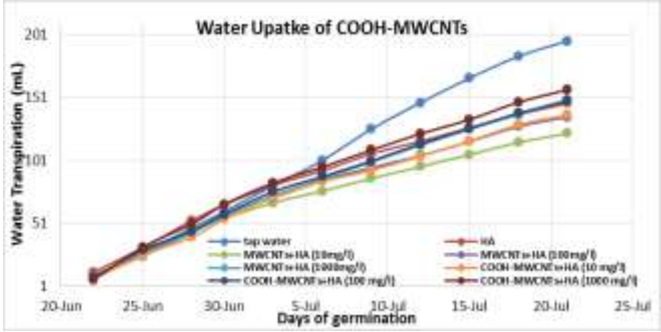

Fig. 7. The accumulative water uptake for COOH-MWCNTs at various concentrations.

The results in Figure 6 indicate that OH-MWCNTs significantly improved water uptake. In comparison to plants exposed to MWCNTs at concentrations of 100 and 1000 mg/L and HA, there was a considerable increase in water uptake inside plant cells. Seedlings exposed to greater concentrations of OH-MWCNTs accumulated roughly 24 percent more water transpiration than seedlings exposed to MWCNTs and HA. Seedlings exposed to HA-MWCNTs showed a significant reduction in water transpiration compared to those exposed to f-MWCNTs, but had similar water uptake as HA. Those exposed to a 10mg/L concentration of MWCNTs had a significantly lower level of water uptake than seedlings exposed to f-MWCNTs at the same concentration, and HA.

Figure 7 showed that COOH-MWCNTs at high dose also resulted in a significant increase in water uptake when compared to the HA. However, no statistically significant difference was detected between those exposed to COOH- and HA-MWCNTs at a concentration of 1000mg/L. At low concentrations seedlings grown in media with COOH-CNTs used more water than those exposed to MWCNTs. Even the plants exposed to 10mg/l of HA-CNTs displayed reduction in water transpiration than those exposed to other treatment. All of the plants subjected to COOH-MWCNTs, as well as those exposed to HA-MWCNTs at high dosage and the HA control, showed a considerable improvement in water uptake by the roots.

3.6. The Effect of F-MWCNTs on Shoot Height

The shoot height all substantially increased, in particular for those in the OH- and COOH-MWCNT group. The HA and water control displayed similarity in shoot height. Shoot was longer in plants exposed to f-MWCNTs at a range of dosages in comparison with non-functionalized MWCNTs, indicating that these height increases were affected by surface property.

3.7. The Effect of F-MWCNTs on Root Electrolyte Leakage

Figure 8 represents the root membrane percentage of electrolyte leakage. A higher rate of electrolyte leakage represents more damage to the root membrane. Plants exposed to 100g/L of MWCNTs and 100mg/L of COOH-MWCNTs showed a critical increase in root leakage, while those exposed to 100mg/L of OH-MWCNTs exhibited significantly lower rates of leakage. On the one hand, plants exposed to 10mg/L of MWCNTs, OH-MWCNTs and COOH-MWCNTs displayed no significant difference in electrolyte leakage. On the other hand, it was importantly different from seedlings exposed to the HA which exhibited greater damage in root electrolyte leakage. Seedlings grown in a medium treated with10 mg/L of HA-MWCNTs had the lowest rate of electrolyte leakage. At 1000mg/L, it was observed that plants grown in solutions with COOH-MWCNTs showed the highest percentage of root leakage while those exposed to OH-and HA-MWCNTs exhibited no significant difference compared to the HA control, but a critical difference was detected when compared to the water control.

In summary, OH-MWCNT treatment resulted in significantly lower rates of leakage than did the other treatments (HA- and COOH-MWCNTs), suggesting that the surface properties of nanotubes play a key role in the toxicity of MWCNTs in tomato plants.

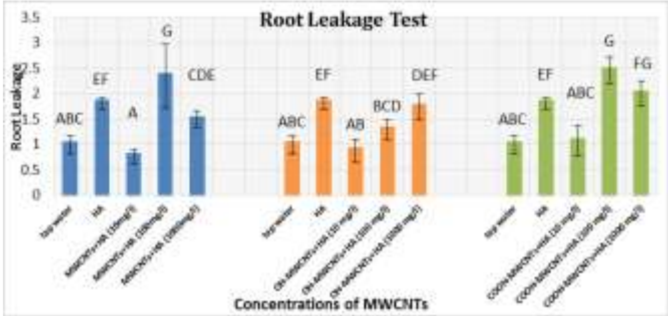

Fig.8. The root electrolyte leakage test for OH-and COOH-MWCNTs at varying concentrations.

3.8. The Effect of F-MWCNTs on Chlorophyll Content

Chlorophyll is necessary for the production of carbohydrates by photosynthesis. The relative chlorophyll contents of plants exposed to OH-MWCNTs is shown in Figure 9. It is obvious that there was no significant difference between the OH-MWCNT and the HA-stabilized MWCNT treatments, indicating that all of the treatments produced healthy plants. Subsequently, there were significant differences between these two types of MWCNTs and the HA control. HA seedlings showed the lowest value of chlorophyll content compared to 1000mg/l HA-MWCNTs. However, there was no significant difference between the MWCNT- and OH-MWCNT-treated plants.

Based on Duncan test in Figure 10, the seedlings exposed to 1000mg/L of MWCNTs and 1000mg/L of COOH-MWCNTs had a significant increase in chlorophyll content as compared to HA control. No differences were observed between different concentrations. The COOH-MWCNT and HA-stabilized MWCNT groups did not display any decrease in chlorophyll content.

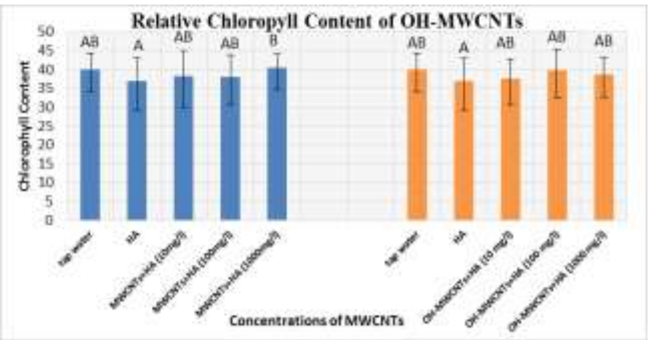

Fig.9. The chlorophyll content for OH-MWCNTs at various doses.

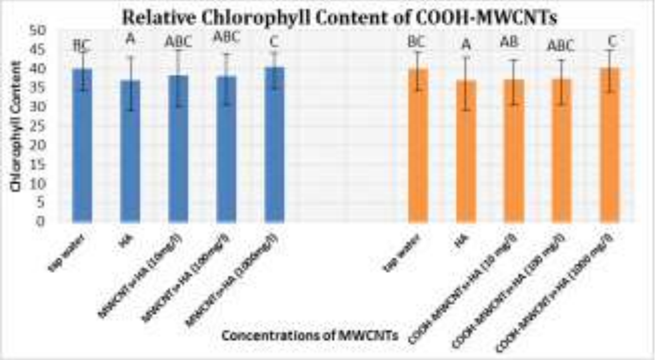

Fig.10. The chlorophyll content for COOH-MWCNTs various doses.

4. The Phto-Toxicity of F-MWCNTs on Tomato Plants

4.1. Growth Rate

A greater enhancement of growth in seedlings exposed to f-MWCNTs was observed as compared to those exposed to varying concentrations of non-functionalized MWCNTs. Overall, the HA control consistently showed a significant lower growth rate than those exposed to both MWCNTs and f-MWCNTs at high concentrations.

All plants had similar leaf numbers or morphology but change in leaf color was noticed. It was concluded that the effect of f-MWCNTs on plant growth is concentration-dependent. These results were not consistent with previous findings that HA-stabilized MWCNTs enhanced vegetative biomass production at low dose but inhibited it at high doses. Thus, f-MWCNTs enhanced growth rate at high doses and exhibited similar vegetative biomass production to HA seedlings at low doses. Thereby, plants respond to MWCNT toxicity differently based on their surface properties.

4.2. Root Electrolyte Leakage

The root electrolyte leakage was an indicator of root membrane injury, as has been reported previously by Aroca et al. (2005) [1]. The 10mg/L of HA-, OH- and COOH-MWCNTs did not cause much damage to root membrane. The rate of leakage gradually increased at a dosage of 100mg/L of MWCNTs and COOH-CNTs. However, at their greatest concentrations COOH-MWCNTs caused higher rates of leakage than those caused by HA- and OH-MWCNTs. These results suggest that surface characteristics of the MWCNTs affected their biological effects. Carboxyl group caused a high rate of electrolyte leakage compared with OH functionalized MWCNTs. The underlying mechanisms are not known, but it has established that membrane leakage is associated with reactive oxygen species (ROS) accumulation in roots.

Begum et al. (2012) report that oxidative stress and ROS generation are reasons for MWCNT-induced plant toxicity. Membrane damage in tomato seedlings was observed after

22 days of exposure to only COOH-MWCNTs at dosages of 100 and 1000mg/L and 100mg/l HA-MWCNTs, suggesting that MWCNTs may induce ROS formation, enhancing electrolyte leakage in the roots which may cause cell death. This is evidence that plants respond differently to MWCNTs and f-MWCNTs, indicating that the functionalization process and the chemicals used for it result in a change in their toxicity for tomato plants [3].

Furthermore, *Arabidopsis* T87 suspension cell were exposed to media containing purified MWCNTs with hydrochloric acid. The pristine and purified MWCNTs exhibited toxicities, causing changes in cellular organ structures and disintegration of cell membranes with an increase in the ROS and catalase content and decreases in glutathione and mitochondrial membrane potential along with a depletion of antioxidant enzyme activities. Membrane lipid peroxidation was observed in cells exposed to MWCNTs.

4.3. Root Elongation Rate

It was indicated that the hydroxyl group caused a statistically significant increase in root elongation rate compared to the carboxyl and HA-MWCNT groups at high dosages. Furthermore, the absorption of the MWCNTs aggregating on the root surfaces that was observed might have changed the vital biochemical processes necessary for plant growth.

Our results may disagree with prior studies reporting negative effects of MWCNTs on rice and Arabidopsis cells due to the increase in ROS and a diminishment of cell viability [16], [11], [12]. Lin reported that MWCNTs do not exhibit any significant toxicity in terms of seed germination or root growth. As for the positive impact of MWCNTs on plants, Khodakovskaya et al. (2009) state that MWCNTs enhance the growth of tomato plants. Thus, the impacts of MWCNTs on plants are dependent upon their concentration and surface properties [9].

Regarding to root elongation rate, one possibility is that OH-MWCNTs and HA-MWCNTs may promote cell elongation, and dehydrogenase activity which can serve as an indication of root vitality related to more efficient water uptake and a faster production of biomass. It is possible that f-MWCNTs taken up by root tissues align with the xylem and phloem; enhancing plant water uptake. F-MWCNTs may therefore play an essential role in root elongation by controlling tomato seedlings' water uptake. To investigate the impact of OH-MWCNT activation on root elongation, more research into morphology and cell length in the root zone, among other parameters, is required. Another possibility is that OH- MWCNTs may improve the dehydrogenase electron-transfer reaction [19] which is related to the large surface area produced by hydroxyl- created acidic surface sites that absorb a range of active molecules such as proteins, amino acids, nucleic acids and enzymes [5]. Because the MWCNTs' surfaces have been modified, it's possible that the OH-MWCNTs' wide surface areas and high electron-transfer rate produce a stable electrocatalytic response to both glucose and NADH. In this way, hydroxyl may increase their electrical conductivity as reported by Baby et al., 2011 and Lee et al., 2011 [2], [10].

4.4. Water Uptake

The f-MWCNTs displayed a significant enhancement in water transpiration in comparison to HA control at a high dose. F-MWCNTs are effective to improve water uptake

Our findings are in agreement with Mondel et al. study [14]. They report that o-MWCNTs have positive effects on seed germination rate, growth rate and on the water absorption machinery of root tissues and that both f-MWCNTs can transport water through a plant's vascular tissues. One interpretation of our findings that OH-MWCNTs enhance water absorption and that is related to that CNTs penetrate root tissues and increase their ability to control the gating of aquaporins; thus allow water molecules to pass in and out of plant cells by inhibiting ions and solutes from passing through them. The presence of aquaporins in the cell membrane could be the cause of an increasing growth rate due to their ability to regulate the membrane's electrochemical potential [8]. Further investigation is needed to determine the effects of MWCNTs on aquaporins at the molecular level.

Additionally, the CNTs penetration into plants' cells could be depended upon their solubility in water. For example, f-MWCNTs were very well dispersed aiding them in penetrating plant cells.

4.5. Chlorophyll Content

Our observations led to the conclusion that f-MWCNTs did not caused any significant alteration in the chlorophyll content. Essentially, both revealed an increase in chlorophyll content as compared to the HA seedlings.

Overall, f-MWCNTs have a positive impact not only on shoot height but also on the root elongation rate, growth rate and water uptake. But different functional groups modified CNTs presented different influences on the plants. It appeared that hydroxyl groups resulted in better improvement and performance in term of development of tomato seedlings during their time exposure than carboxyl functional group.

5. Conclusion

The impact of surface properties of carbon nanotubes has been investigated. This variable has a major impact on CNTs' effect on tomato plants. A number of measures, such as growth rates, water uptake, root length rate, height shoot, and so on, show the physiological health of plants. This research exhibited that different properties of functionalized multi-walled carbon nanotubes (MWCNTs) affects their interactions with plants. To determine whether the surface properties of MWCNTs in HA solution affect plant development as compared to non-functionalized MWCNTs in an HA solution. Functionalized and unfunctionalized MWCNTs with same diameter range (<7nm) and an inner diameter of 2-5 nm were used.

For functionalized CNTs, the carboxyl group had the highest damage to root membrane, while hydroxyl had the lowest rate of damage. In addition, when compared to MWCNTs, the f-MWCNTs dramatically improved plant growth, water transpiration, and dry root and shoot weight. Therefore, surface properties affected the biological interactions of CNTs and COOH-CNTs appeared to be more harmful than the hydroxyl group.

It is recognized that there is no difference between f-MWCNTs and HA plant groups in terms of their chlorophyll content. The seedling biomass grown in HA media was smaller than that of seedlings grown in f-MWCNT medium. Despite the higher rates of root growth and greater total weight, plants exposed to f-MWCNTs presented similar chlorophyll content and quantum yield. However, there is still better performance detected in f-MWCNTs in accordance with HA control in terms of water uptake, and root leakage. Overall, HA control displayed a significant reduction in chlorophyll content and water uptake as compared to CNTs seedlings. In contrast, it showed better growth rate, root length rate, root and shoot dry weight.

Moreover, seedlings grown in an HA-MWCNT-treated medium and HA seedlings were significantly shorter than those grown in COOH-and OH-MWCNT-treated media. Although the untreated seedlings exhibited a lower level of water transpiration, those treated with f-MWCNTs showed an increase in water uptake as compared to the HA-MWCNTs and HA control, a sign that f-MWCNTs can promote water uptake.

No change in leaf color was displayed in seedlings exposed to COOH- and OH-MWCNTs at high doses. While green pigment was displayed in plants exposed to f-MWCNTs by the termination of the experiment. The attachment of MWCNTs to the tomato roots was observed though no wilting or reduction in the size of the leaves was noticed.

To summarize, HA was found to be a more effective dispersion agent for CNTs. Reduced-size agglomerates and higher quantities of personalized MWCNTs show that HA is particularly effective at preparing stable dispersion for functionalized CNTs.

In the case of exposure to f-MWCNTs, the enhancement in the tomato was observed. Different biological responses were obtained in tomato from exposure to OH-and COOH-MWCNTs. The exposure of OH-MWCNTs was found to improve the development of tomato, whereas COOH-MWCNTs proved to induce toxicity. Overall, the findings revealed that the surface features of CNTs, as well as their dispersion stability, determine CNTs' impact on tomato growth.

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