The properties of Natural Organic Matter (NOM) affect the impact of Multi-walled Carbon Nanotubes (MWCNTs) on Tomato Plants (SOLANUM LYCOPERSICUM)

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Abstract-- The adsorption of natural organic matter (NOM) or surfactants onto raw multi-walled carbon nanotubes (MWCNTs) was shown to effectively enhance the dispersibility and stabilization of MWCNTs. The two kinds of dispersants used were humic acid and peptone. Based on the dynamic light scattering (DLS) analyses, the use of surfactants increased the steric hindrance as well as the charge repulsion between adjacent CNT particles, thereby enhancing their suspension. In addition, TEM images agreed with the DSL analysis that HA-stabilized MWCNTs were well-dispersed compared to pep-stabilized MWCNTs. Decreases in the growth rate, water uptake, dry weight, and root elongation rate along with a rise in mortality were detected as an indication of phytotoxicity in both the pep-MWCNT suspensions at 1000mg/L and the peptone control seedlings. This was an indicator for the presence of suspended MWCNTs as well as their unstable dispersion in the water column. However, the interaction between the HA-CNTs and the plants improved development in terms of water uptake, dry weight and root elongation rate due to their well-dispersed stability in water. Overall, our results suggest that the nature of the dispersant agent itself plays an active role in the toxicity of MWCNTs on tomatoes.

Index Term-- Multi-walled carbon nanotubes, phytotoxicity, humic acid and peptone.

1. INTRODUCTION
Nanotechnology has recently been used in many different sectors, enhancing products such as cosmetics and recreational equipment along with industrial and medical applications. An example of nanoparticles is carbon nanotubes which exhibit outstanding electronic characteristics and strong molecular structure, which makes them suitable for many industrial and commercial applications. There are two types of CNTs: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). SWCNTs and MWCNTs vary by diameter, length, and chirality. Both have large amounts of surface area in relation to their mass, giving CNTs a great deal of space for bonding and reaction.

There are virtually several researches into the ecotoxicology or the potential negative impacts of nanomaterials on the environment. While some research has been conducted, there are still significant gaps in knowledge on nanoparticles impacts on environment. Some nanoparticles such as silver and copper have shown their toxicity to the environment. Removal of nanoparticles from the environment is difficult owing to their small size. Nanoparticles may conceivably be absorbed by plants from the soil or transported over great distances in the air or suspended in water. For instance, CNTs can be released into the environment at various stages of their life cycles through use or their accidental or incidental disposal (Lee et al., 2010). Due to CNTs’ potential mobility, they can have impacts in and across air, water, soil and biota. Thereby, they could have unforeseen environmental effects on humans and ecosystems when released into the environment.

The investigation on the influence CNTs on plants has increased recently in both in vivo and in vitro toxicity studies. Khodakovskaya et al. (2009) demonstrated that exposing tomato seeds to CNTs enhanced the germination rate of tomato seeds and growth of seedlings and no adverse effect was observed on root development and root elongation of tomato seedlings. The authors contribute the enhanced growth
of tomato to the penetration of tomato seed coat by CNTs and subsequent increase of water uptake for seeds during the germination stage (Khodakovskaya et al., 2009). Another study showed that non-functionalized MWCNTs were toxic to Arabidopsis T87 cells based on the size of agglomerates (Lin et al., 2009a). Rice cells cultured in MWNTs suspension displayed lower cell viability and higher reactive oxygen species (ROS). Both ROS content and cell viability returned to the baseline levels when dispersant agent was added to the culture medium (Tan et al., 2009).

Generally, MWCNTs toxicity may depend on their physicochemical properties and their preparation solution chemistry for analyzing the possible impacts of CNTs in ecosystems. Likewise, the effects of CNTs on plant growth and development depend on upon the plant species, the applied concentrations, the circumstances of the experiment, surface properties of CNTs, and their dispersion approaches (Khodakovskaya et al., 2009).

The initial studies of CNTs demonstrated that these nanotubes are bioavailable to a variety of organisms. Previous studies assessing both the toxicity and the bioavailability of CNTs to plants have shown positive, negative and neutral effects on seedling growth and seed germination. The inconsistency may arise from differences in CNT dimensions, degrees of surface functionalization, and catalyst impurities used in different studies. Thus, this research expected to clarify the use of natural organic matter (NOM) (both humic acid and peptone as surfactant) for dispersion MWCNTs in medium and studying their toxicity to tomato plant under controlled environments. In other words, this research examines how multi-walled carbon nanotubes (MWCNTs) dispersed using different types of NOM affect the impact of MWCNTs on plants through their life development.

2. Review of Literature

The chemical, physical, and electronics properties of Carbon nanotubes make them potentially useful in nanotechnology, optics, and many other fields of material science. Subsequently, increase of inadvertent exposure of CNTs to human beings and ecosystems will be occurred with the increasing use of CNTs in both basic science and applied technology.

In vivo toxicology of CNTs has been investigated recently in substantial depth and the previous research showed that CNTs induce toxic responses in biological systems. Skin contact, a likely route of exposure may cause inflammatory response via dermal toxicity. One study dealt with animal toxicity suggested that exposure of mice to raw CNTs induced oxidative stress and led to the depletion of glutathione, increase of dermal cell number, and localized alopecia and skin thickening (Koyama et al., 2009; Murray et al., 2009). The observed toxicity was partially attributed to the metals contained in CNT structures which interact with skin and initiate oxidative stress thereby lead to inflammation. The conclusion was supported by the subsequent observation that mice exposure to highly pure and clean tubes did not cause any skin hair loss, indicating that purification of CNT may develop the biocompatibility of CNTs (Zhao & Liu, 2012).

Inhalation is another possible route of exposure of CNTs to lungs. Inhalation studies are precise model deposition and pathologic responses to “real world” exposure scenarios since airborne CNTs can reach to distal region of lung, more dispersed and less agglomerated in aqueous liquid compare to instilled CNTs (Zhao & Liu, 2012). Inhalation of CNTs may cause lung pathologies related to CNTs characteristics in which they have high aspect ratio length to width. This characteristic led CNTs to cause asbestos fiber (Crouzier et al., 2010; Elgrably et al., 2008; Tantra & Cumpson, 2007). But the inhalation of MWCNTs may cause focal sub-pleural fibrosis and mononuclear cell aggregation (Ryman-Rasmussen et al., 2009) while asbestos fiber may cause both pleural inflammation and diffuse pleural fibrosis (Choe et al., 1997; Kane, 2006).

In addition, the toxicity of CNTs can be exposed to aquatic environment. For instance, choline coated SWCNTs were released to water, D. magna fish were able to swallow the tubes and used lysophosphatidyl choline as food source. It was observed that there was accumulation of SWCNTs on the external surface of D. magna and that was at the concentration of 1mg/L of SWCNTs (Roberts et al., 2007).
2.1 Toxicity of Carbon Nanotubes to Plants

Some researchers indicated that surface functionalization affects the toxicity of CNTs (Canas et al., 2008). Khodakovskaya et al. (2009) demonstrated that exposing tomato seeds to CNTs enhanced the germination rate of tomato seeds and growth of seedlings and no adverse effect was observed on root development and root elongation of tomato seedlings. The authors contribute the enhanced growth of tomato to the penetration of tomato seed coat by CNTs and subsequent increase of water uptake for seeds during the germination stage (Khodakovskaya et al., 2009). Another study showed that non-functionalized MWCNTs were toxic to Arabidopsis T87 cells based on the size of agglomerates (Lin et al., 2009a). Rice cells cultured in MWNTs suspension displayed lower cell viability and higher reactive oxygen species (ROS). Both ROS content and cell viability returned to the baseline levels when dispersant agent was added to the culture medium (Tan et al., 2009).

Although several studies on CNT toxicity have been done, research is still needed in order to investigate how different preparation methods such as the dispersing methods affect the toxicity of CNTs. Moreover, the negative effects of CNTs are dependent on time exposure and dose. The functionalization of CNTs depends on how tubes interact with the body, cells and the environment. It seems that interactions between plants and CNTs show either positive and negative effects on plant growth and development in some species, with neutral or no-effects at early stages of development.

Therefore, CNTs in term of their impact depend upon the methods used for dispersion CNTs in medium, properties of surfactants or antioxidant, surface properties of CNTs, plant species, certain concentration, and plant growing conditions. This research expected to clarify the use of NOM (both humic acid and peptone as surfactant) for dispersion MWCNTs in medium and studying their toxicity to tomato plant under controlled environments.

3. MATERIALS AND METHODS

3.1. Tomato Seeds

This Tomato is one of the recommended plant species for chemical testing by the US Environmental Protection Agency (USEPA, 1996). In this study, tomato plants were grown hydroponically. The tomato seeds (Solanum lycopersicum) were purchased from Johnny’s Selected Seeds (NY). Before use, the seeds were soaked in a 15% Clorox treatment for 15 minutes and immediately washed three times with deionized water to prevent fungal contamination. The 15% (v/v) Clorox solution prepared by adding 15 mL of concentrated Clorox to 85 mL of deionized or sterile water in a 100 mL flask.

3.2. Seed Germination and Root Elongation (germination Assay of Tomato Seeds)

Sterile polystyrene petri dishes sized 150 mm X 15 mm were used to germinate tomato seeds. Filter paper soaked with twenty or thirty mL of deionized water was placed in the petri dish and upon which thirty tomato seeds were placed. The seeds were approximately 15 mm apart. These plates were distributed randomly in a temperature-controlled growth chamber at 16h/8h, 25 co/18 co. Approximately sixty-five percent of the control seeds germinated after seven days, and developed roots at least 20 mm long. Root elongation was measured twice: at the beginning, before the seeds were exposed to MWCNTs, and at the end, after these seeds had been exposed to CNTs.

3.3. Carbon Nanotubes

The MWCNTs (>95%, OD: < 7nm) were purchased from US Research Nanoparticles, Inc., (Houston, TX). The MWCNTS used in this experiment were created using the chemical vapor deposition method. The non-functionalized MWCNTs have the diameter range (<7nm) with an inner diameter of 2-5 nm.

3.4. Natural Organic Matter (NOM)

The two kinds of NOM were also investigated, dissolved in tap water as surfactant-CNTs. The humic acid was purchased from Alfa Aesar, Johnson Matthey Company and the peptone, an enzymatic hydro-lysate derived from animal tissues with a total nitrogen content of approximately 12.2% and an amino nitrogen content of 4.8% was purchased from Sigma Company. Moreover, peptone is a typical fresh natural organic matter defined as polymers of amino acid monomers that are
hooked by peptide bonds and it is considered to be good natural sources of amino acid, peptides, and proteins in growth media. The concentrations of the MWCNTs at 10, 100, 1000 mg/l used in this study.

3.5. Statistical Analysis
Each treatment of non-functionalized MWCNTs stabilized in HA and peptone surfactants had ten replicates. All reported data represent the means values for each treatment and the error bars represent the standard deviation (± SE). All statistical analysis of experimental data was conducted with SPSS 2.1 software using one-way analysis of variance (ANOVA) and two-way ANOVA (factorial ANOVA) followed by the Duncan test if the means’ values were significantly different and the statistical significance was specifically determined (p<0.05) by the post-hoc test (Duncan’s Multiple Range Test, or MRT).

4. RESULTS AND DISCUSSION
4.1. Dynamic Light Scattering (DLS) Analyses (Zeta Potential)
4.1.1. Non-Functionalized MWCNTs Solutions
The zeta potential (ZP) is measured for non-functionalized MWCNTs to evaluate their stability in water. The zeta potentials of peptone-stabilized MWCNT dispersions shown in the table 1 are -0.051333 mV, -12.0667 mV and -0.0628 mV for 10, 100, and 1000 mg/l peptone-stabilized MWCNTs respectively. As a standard interpretation for ZP, particles with a ZP above or below 30 and -30 mV respectively may be stable in relation to electrostatic repulsion interactions (Cheng et al., 2011). These values indicate that peptone can be adsorbed onto the surface of MWCNTs and that they have negative surface charges, but the peptone stabilized MWCNTs were not stable at varying concentrations.

Additionally, the data shows that the zeta potential for HA-stabilized MWCNTs is a negative surface charge for both 10 and 1000 mg/l HA-stabilized MWCNTs (-33.2333 and -21.96667 mV, respectively) reflecting that molecules of HA might adsorb onto the surfaces of MWCNTs. There is a positive surface charge (0.0523 mV) for 100 mg/l HA-stabilized MWCNTs, a value below the 30 mV standard value for the zeta potential, meaning that it cannot remain stable in water. Based on the zeta potential, only HA-stabilized MWCNTs at 10 mg/L was stable.

In summary, it appears that MWCNTs suspensions were not stable even with high concentrations of peptone or humic acid. The only exception was the suspension containing 10 mg/L of MWCNTs and 200 mg/L of HA. Comparing these two organic matters, the HA appeared to be more effective than peptone to disperse and stabilize MWCNTs. The results might be attributed to the highly negatively charged surface of an HA. It is also possible that the surface oxygen content increases as a result of HA sorption, leading to interactions among large numbers of O-containing moieties with water molecules.

Table I
The Zeta potential of non-functionalized MWCNTs.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>ZP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWCNTs+peptone (10mg/L)</td>
<td>-0.051333333</td>
</tr>
<tr>
<td>MWCNTs+peptone (100mg/L)</td>
<td>-12.06666667</td>
</tr>
<tr>
<td>MWCNTs+pep(1000mg/l)</td>
<td>-0.0628</td>
</tr>
<tr>
<td>MWCNTs+HA (10mg/l)</td>
<td>-33.23333333</td>
</tr>
<tr>
<td>MWCNTs+HA (100mg/l)</td>
<td>0.052333333</td>
</tr>
<tr>
<td>MWCNTs+HA (1000mg/l)</td>
<td>-21.96666667</td>
</tr>
</tbody>
</table>

4.2. TEM Observations and Characterization of Non-functionalized and functionalized MWCNTs
4.2.1. Non-functionalized MWCNTs
TEM was used to characterize the morphology as well as the individual size of MWCNTs. One parameter that can be identified is the length distribution for carbon nanotubes dispersed in water. It is clear from the images that the length distribution for HA-stabilized MWCNTs and pep-stabilized MWCNTs are different. Figures 1 and 2 show non-functionalized MWCNTs at a high magnification on a scale of 100nm and at a lower magnification of 500nm.

Individual MWCNTs were observed using TEM. For HA-stabilized MWCNTs at the lowest concentrations (100mg/l), the outer diameter (OD) was 38.79 nm and the inner diameter (ID) was 15.14nm. In comparison, at their highest concentration (1000mg/l), the MWCNTs’ outer diameter was 19.73nm and the inner diameter was 11.62nm. This means that even the 1000mg/l concentration of HA-stabilized MWCNTs dispersed more fully in water than the lower 100mg/l concentration of HA-stabilized MWCNTs. The aggregation
of carbon nanotubes still seemed obviously to exist at both HA-MWCNTs which were characterized by large bundles. The HA layer was adsorbed onto the MWCNT surface with a thickness of 2 to 4 nm according to the TEM image. When peptone was used as a natural surfactant for CNTs, large bundles with a diameter above 20 nm were found. The OD and the ID were 23.04 nm and 12.78 nm respectively in the pep-stabilized MWCNT solution at 100 mg/l. At the highest concentration of 1000 mg/l pep-stabilized MWCNTs, the OD and the ID were 21.88 nm and 12.95 nm respectively, slightly smaller than that of the 100 mg/l concentration of pep-stabilized MWCNTs dispersed in water. So, it is obvious that pep-stabilized MWCNTs disentangle to form huge densely packed bundles which are typically observed in both 100 mg/l and 1000 mg/l concentrations of pep-stabilized MWCNTs. The 1000 mg/l pep-stabilized MWCNTs contained individually dispersed MWCNTs. The agglomeration of 100 mg/l pep-stabilized MWCNTs is evidence for a less than superior dispersion, even when the dispersions had remained stable for two months.

The TEM imaging indicates that the majority of the tubes are double-walled or multi-walled with some large-diameter tubes in non-functionalized MWCNTs. There was bundling in the as-received MWCNTs dispersed in both HA and peptone and clusters of tubes, but they were shortened at the highest concentrations of MWCNTs in both surfactants. In a word, HA surfactant is indeed active to disperse and stabilize MWCNTs as compared to peptone dispersant based on TEM observation.

Fig. 1. The TEM for pep-stabilized MWCNTs, A for 10 mg/L concentration, B (1-3) the pep+MWCNTs (100 mg/L) while C (1-4) at 1000 mg/L concentration at 100 nm and 500 nm scale.

Fig. 2. The TEM images for HA-stabilized MWCNTs at lower and higher magnifications 100 nm and 500 nm scale. The pictures of D (1-2) represent the 100 mg/L concentration while the pictures of E (1-5) represent the MWCNTs at 1000 mg/L.
4.3. The Effect of Non-functionalized MWCNTs on Weight of Biomass

Figure 3 exhibits the growth rate of vegetative biomass, representing an essential investigation into the effects of CNTs on the growth and development of tomato plants. No significant difference was observed between HA and water control groups. It is obvious that there was a significant and dramatic increase in the growth rate of the biomass of seedlings grown on the medium supplemented with HA+ MWCNTs at 10 and 100mg/L concentrations as compared with plants treated with HA solution, while at 1000mg/L it was similar to that of the HA group. Seedlings grown in HA-stabilized MWCNT media in concentrations of 100mg/l and 10mg/l exhibited approximately 2.5% higher growth rate as compared to seedlings grown in HA. Plants exposed to pep-MWCNTs at a concentration of 100mg/L grew better than those exposed to MWCNTs at 10 and 1000mg/L which exhibit a diminishment in growth of biomass. However, these decreases in growth weight were observed to be similar to that of seedlings grown in peptone media. There was a significant decrease in biomass of plants grown in a medium supplemented with pep+ MWCNTs as compared to HA-stabilized MWCNT groups with the exception of the 100mg/L pep+ MWCNTs medium, which indicated a growth rate different to that of the peptone control.

Therefore, MWCNTs enhanced plant growth at 10 and 100 mg/L in HA stabilized solutions but the stimulative effect disappeared at 1000 mg/L. In peptone stabilized solutions, MWNTs displayed similar effect at 100 mg/L, MWCNTs enhanced plant growth compared with peptone solution alone but such benefit was not observed at 10 and 1000mg/L.

4.3.1. Morphological Observation of Leaves

Plants exposed to peptone-stabilized MWCNTs presented several symptoms such as changes in leaf color, which was more greatly affected by peptone surfactants than by the HA-MWCNT treatment (Figure 4). The amount of green pigment was reduced in plants exposed to HA-MWCNT treatments as well as at high concentrations of MWCNTs at the end of experiment as compared to those seedlings exposed to HA-MWCNTs at the beginning of experiment. Leaves and leaf blades turned yellow while presenting an appearance of wilting in plants exposed to pep-MWCNTs and peptone as compared to those exposed to HA-MWCNTs, their leaves became brawn/light green with increasing CNTs level. As suggested by previous studies, the manifestation of such senescence could be a result of the promotion of gene expression in plants through an increase reactive oxygen species. In general, more leaf number, area, and length of stem was observed in plants treated with HA-MWCNTs as
compared to seedlings treated with pep-MWCNTs expressed a reduction in leaf number with short stem length.

4.4. The Effect of Non-functionalized MWCNTs on the Dry Weights of Shoots and Roots

Figure 5 below show the dry weight of tomatoes treated with non-functionalized MWCNTs dispersed in both peptone and HA. It was found that the HA-stabilized MWCNT treatments expressed a concentration-dependent enhancement in both shoot and root dry weight. For instance, plants exposed to a 1000mg/L medium had the greatest dry weight for both shoots and roots while other dosages of MWCNTs resulted in a dry weight not significantly different from the positive control (HA). The water control was not significantly different from plants treated with HA solutions in terms of their dry shoots and roots.

In contrast, the seedlings exposed to pep+ MWCNTs showed no significant differences in root dry weight as compared to the peptone control, with both exhibited a reduction in dry root weight when compared to plants exposed to HA-stabilized MWCNTs. It was observed that the pep-stabilized MWCNTs did not differ significantly from the peptone group in terms of dry shoot weight. Nevertheless, exposure to pep+ MWCNTs (100mg/L) and (1000mg/L) resulted in a significant increase in plant growth compared with plants in peptone solution alone, but the shoot dry weight was significantly different from that of the water control. Therefore, peptone appeared to be toxic to plants and the addition of MWCNTs has reversed some of the damage caused by peptone.

**Figure 5** The shoot and root dry weight of tomato seedlings in media supplemented with HA- and pep-MWCNTs at concentration of 10, 100, and 1000mg/L respectively.

4.5. Morphological Observation of Roots Using Light Microscopy

Light microscopy was used to investigate the root morphology to understand whether MWCNTs have affected the root structure. There was significant distinction in the thickness of root tissues as it was shown in Figure 6. There was a change in color of root tissue as compared to control groups. The peptone control plants had roots with significant damage in root caps and intact epidermis. In HA control, it seems there is no damage in root caps. No root hair was noticed in control groups. There were not root hairs developed at low concentrations of both HA- and pep-MWCNTs exposure. However, root hairs were developed at higher concentrations for both HA- and pep-MWCNTs treatments. The number and length of prolific root hairs was enhanced at 1000mg/L HA-MWCNTs exposure. Whereas at 1000mg/l pep-MWCNTs reductions were detected in both number, length root hairs and deformation of root caps as an evidence for pep-MWCNTs toxicity. There was clear damage in root caps and intact epidermis at 1000mg/L pep-MWCNTs exposure while it was not existed in HA surfactants with CNTs. MWCNTs adsorbed to root plants at low and high concentrations. At high dose of MWCNTs in HA solutions, the formation of lateral root was observed with impaired in thickening of root caps. In comparisons to pep-MWCNTs no lateral root formation was indicated and damage in root caps existed at high dose.

There could be damaged in root cell walls in pep-MWCTs. The MWCNTs accumulation presented on the root
caps might cause damage. There could be a possibility of increasing in root diameters due to presence of MWCNTs in root zone as a result of water uptake by MWCNTs.

Based on morphological observations of root, it was clear that the roots of plants treated with pep-MWCNTs had solely main (primary) root with few branches of secondary root which decreased with increasing concentration of CNTs. In comparison, plant treated with HA-MWCNTs had primary and secondary roots and their development was enhanced with increasing dose of CNTs.

![Image](image_url)

**Fig. 6.** Light microscopic picture of 30 day-old roots (10x) for plants treated with peptone solution and pep-MWCNTs at concentrations of 10, 100, and 1000mg/L respectively.

### 4.6. The Effect of Non-functionalized MWCNTs on Water Uptake

Figure 7 shows the water uptake of MWCNTs for HA surfactant. It was found that the seedlings exposed to HA+MWCNTs had a significantly higher level of water usage as compared with the seedlings that were not treated with MWCNTs (HA solution). As indicated in Figure 7, exposure to HA+MWCNTs at concentrations of 10, 100, and 1000mg/L significantly increased water uptake. The rate of accumulated water transpiration for seedlings exposed to higher concentration of MWCNTs was approximately 43mL greater than that of those unexposed to MWCNTs including the HA and water controls. The HA control showed a significantly lower rate of water uptake as compared to the seedlings exposed to MWCNTs, suggesting that HA-stabilized MWCNTs significantly enhances water uptake inside plant cells.

However, exposure to pep+MWCNTs resulted in a significant decrease in water uptake. The water control had a significantly higher rate of water transpiration as compared with peptone treatment as indicated in Figure 7. Seedlings exposed to a concentration of 1000mg/L of both pep+MWCNTs and peptone as a positive control showed the lowest level of water usage as compared to plants exposed to concentrations of 10 and 100mg/L, higher than that of those exposed to higher concentrations of CNTs. The rate of accumulated water transpiration in the plants treated with pep-MWCNTs at 100mg/L was approximately 21mL greater than that of those exposed to 1000mg/L of pep-stabilized MWCNTs. This clarification suggests that pep-stabilized MWCNTs can significantly improve water uptake inside the cells of tomato plants only at 10 and 100mg/L while at high dose and peptone control they reduced the water uptake which affect the vegetative biomass production.
4.7. The Effect of Non-functionalized MWCNTs on Chlorophyll Content

As chlorophyll is essential to the production of carbohydrates by photosynthesis, it is important to measure the amount of this green pigment to determine whether it is influenced by the presence of MWCNTs. Healthy plants will have a greater amount of chlorophyll than less healthy ones; this relative chlorophyll content is measured as an indicator of the overall condition of the plants. As shown in Figure 8, the plants exposed to the HA control exhibited a significant reduction of chlorophyll while the plants exposed to 1000mg/L HA+MWCNTs showed a significant increase in chlorophyll content. Moreover, the plants grown in 100mg/L of HA+MWCNTs indicated a significant enhancement in
chlorophyll content as compared to those exposed to 10mg/L HA+MWCNTs, which is not significantly different from that of the HA and water control seedlings.

Fig. 8. The chlorophyll content of tomato grown in media supplemented with pep- MWCNTs and HA-MWCNTs respectively at 10, 100, and 1000mg/L concentrations respectively.

4.8. Discussion On the Phyto-toxicity of Non-functionalized MWCNTs on Tomato Plants

4.8.1. Growth Rate and Dry Weight

Seedlings grown in pep-MWCNTs at 1000mg/L exhibited a significant decrease in growth rate and water uptake as well as the peptone used as a positive control. In comparisons, other concentrations of MWCNTs resulted in a significant increase in plant growth and water transpiration. Both peptone and pep-MWCNTs had the same root dry weight but peptone treatment displayed diminishment in term of shoot dry weight and root elongation rate. Therefore, peptone appeared to be toxic to plants and the addition of MWCNTs at high dose has reversed some of the damage caused by peptone.

In contrast, HA-MWCNTs treatment enhanced the root and shoot dry weights at the lowest concentrations due to increased water uptake by CNTs, while at their highest concentration their growth rate were similar to the HA control. Hence, MWCNTs dispersed in HA solutions at high dosage have been found to positively stimulate the root and dry weight, root elongation rate as well as increased the water uptake inside plants cell as much as other doses and HA control did. But, HA indicated a similar positive effect on plants as it was shown in those exposed to 10 and 100mg/L HA-stabilized MWCNTs.

Basically, both negative and positive effects of MWCNTs on plant physiology have been reported. Lin and Xing (2007) have recently reported that the effects of nanoparticles on plant development and growth depend upon the concentration and the type of nanoparticles, the plant species, the uptake mechanism of the nanoparticles into the plants and the conditions of the experiment. In our experiment, it was found that the toxicity of MWCNTs depends on the type of surfactant or dispersant used, which could exhibit either negative or positive impacts on plants. The aggregation of nanoparticles plays a role, as toxicity depends on whether a CNT treatment was more or less fully dispersed. In a word, the preparation method of MWCNTs may play an important role in their phytotoxicity. Both toxic and non-toxic effects of MWCNTs were indicated in terms of root development, root
elongation, dry weight, fresh weight, and total vegetative biomass of tomato plants in the concentration range under study.

Regarding to HA control, our findings agreed with the recent studies that humic substances (HS) can modify root growth, morphology and architecture of plants. Predominately, HA treatment resulted in a greater percent of marketable onion bulbs after four and a half months of controlled atmosphere storage. HA treatment had an effect on onion growth and its size as compared to untreated one as it was reported by Boyhan and Torrance (2008).

It was indicated in our findings that there was better development in root systems obtained from plants treated with HA. Khaled and Fawy (2011) reported that corn seedlings treated with HA had significant effect on the uptake of nutrients. At 0.1% dose of HA, the dry weight of seedlings increased while at 0.2% dose of HA a significant reduction was detected. Consequently, HA improved the salinity of soil, enhanced uptake of some nutrients and reduced uptake of toxic elements. This is related to the surface activity of HA in both hydroponic and hydrophilic sites. HA interacts with phospholipid structures of the cell membrane and carries nutrients through them (Khaled&Fawy, 2011).

Another research conducted by Turkmen et al. (2004) showed the highest contents of N, P, S, Mn, K, Ca, Mg and Zn in shoot and root exposed to 1000 mg/Kg of HA. Enhancement in growth rate was found in treatment of 1000 mg/Kg as compared to 2000mg/Kg which was exhibited a case of decrease. The concentration-related effect of HA is mainly due to hormones like activities of HA through their involvement in cell respiration, protein synthesis, photosynthesis, antioxidant and other enzymatic reactions. In other word, HA was known to stimulate plant growth by induce plant hormones, but no studies yet has been indicated that HA contains hormone like components (Turkmen et al., 2004).

Based on our findings, it was clear that HA at 200mg/L at higher dose of CNTs enhanced root growth and increased the uptake of nutrients over the control. This might be due to the HA ability for permeability of cell membranes. Thereby, it may act like hormone activity as an approach to increase nutrients uptake instead of using high nutrients concentration. HA is utilized as a promising compound for improving nutrients uptake (Nikbakht et al., 2008).

Regarding to peptone treatment, some researchers have studied oxygenated peptone on different crops; it was found to improve the germinations of tomato, brinjal and chilli.

Thakare et al (2011) studied the influence of oxygenated peptone on chick pea plants. They found that 50 ppm and 1% aqueous solution of oxygenated peptone affect germination and seedling establishment on chick pea (Cicerarietinum.L. cv. Vijay) in a positive manner. Peptone treatment showed an increase in root length, shoot/root ratio, biomass and vigour index. In this case, peptone treatment showed an increase in the total carbohydrates and soluble protein content (Thakare et al., 2011).

Nhut et al. (2008) reported that seedlings treated with 1.5% and 3.0% of peptone showed development in shoots formation uniformly to the highest average length, while with 2.0% dose seedlings observed a significant diminishment in shoots regeneration. It was concluded that peptone can promote the growth of explants at an efficient concentration and it was used as carbon nitrogen sources for plant tissue culture. Hence, 2.0% peptone with MS medium was most sufficient for shoot regeneration of avocado compared to other concentrations displayed negative effects in the formation of the shoot system.

To sum up, peptone displayed both positive and negative effect on plant growth. The net effect depends on the concentrations that are utilized. Peptone promotes the growth process at lower concentrations but shows negative impacts on plants’ growth at higher concentrations. Regarding to our investigation, tomato plants treated with 2000mg/L of peptone showed negative effects in both shoot and root systems. The media that was used to expose peptone to plants could be another factor affecting their toxicity. Such studies exposed peptone to soil or foliar spraying for a short period while in
our studies the exposure of peptone to seedlings was performed hydroponically by dispersing peptone in water which might increase their toxicity to seedlings.

4.8.2. Root Elongation Rate

Based on analyses of root elongation and total weight, it can be concluded that after a long-term exposure (4 weeks) MWCNTs enhanced biomass weight and increased root length using HA as a dispersant, but that they did not induce any significant change in the height of shoot systems as compared with the HA control group. However, tomato seedlings exposed to peptone-MWCNTs medium did induce a significant change in terms of in vegetative biomass, dry and fresh weights of shoots and roots and root length. Our results confirmed previous findings indicating positive impacts of MWCNTs on root growth, development, and increased growth rate of tomato seedlings (Shweta et al., 2011 and Khodakovskaya et al., 2009).

Furthermore, root elongation increased in the seedlings exposed to HA-stabilized MWCNTs, suggesting that additional physiological analysis is needed to detect changes in morphology such as cell length in the root zone. One explanation for the enhancement of root elongation could be that HA-stabilized MWCNTs promote cell elongation which is considered to be an important primary growth process responsible for root growth. Secondly, it could be deduced that HA+MWCNTs taken up by roots may play an active role in water uptake and dehydrogenase activity. The dehydrogenase activity, the inclusive valuation index, reveals that the increased metabolic activity and the roots’ water and nutrient absorption capability could be responsible for increases in water uptake and production of vegetative biomass (Taniguchi et al., 2008; Wang et al., 2012). Further investigation is needed at the cellular level in order to identify whether there is promotion of cell elongation in the elongation zone in plants exposed to HA+MWCNTs. Wang et al.’s (2012) statement that enhancement in root elongation is found in plants exposed to HA+MWCNTs is consistent with our results.

However, the mechanism by which HA+MWCNTs promote cell length is not obvious yet. Liu et al. (2010) stated that it is possible that MWCNTs affect hormone distribution or microtubule organization. Another possibility is that HA+MWCNTs improve dehydrogenase activity in tomato plants. MWCNTs may improve the dehydrogenase electron-transfer reaction (Wang et al., 2012). This could be related to the large surface area created by humic acid; acidic sites would absorb a range of active molecules such as protein, amino acids, nucleic acids and enzymes such as lysozyme, horseradish peroxidase and glucose oxidase, which would become more biocompatible with active molecules (Chen et al., 2001; Yu et al., 2003; Merli et al., 2011; Rafeeqi & Kaul, 2011; Guiseppi-Elie et al., 2002). It could be that their large surface areas and efficient electron-transfer rate cause a stable electrocatalytic response towards both glucose and NADH. Modification of the MWCNTs’ surfaces may increase their electrical conductivity as has been previously reported (Baby et al., 2011; Musameh et al., 2002; Ye et al., 2011; Lee et al., 2011).

On the one hand, it could be that higher water uptake by roots indicates a comparatively higher water content in the plants which may promote molecular mobility as does dehydrogenase in root cells (Zeng et al., 2010). On the other hand, the promotion of dehydrogenase activity may come from the adsorption of dehydrogenase by nanotubes, bringing it to the sidewall and thereby providing higher local electron density which would increase the charge transfer in an oxygen reduction reaction (Zeng et al., 2010). Further investigation is needed to study the mechanism of electron transfer in nanotubes.

4.8.3. Water Uptake

HA+MWCNTs were found to enhance water uptake inside plant cells at highest and lowest concentration while HA did not improve water transpiration. However, peptone control displayed negative effect in terms of water uptake as well CNTs with peptone at 1000mg/L while those at low doses (10 and 100mg/L) resulted in higher water transpiration. Previous studies have indicated that CNTs are able to penetrate both the roots systems of developed plants and seeds, dramatically affecting their biological activity by
promoting the amount of water penetrating seeds during germination or absorbed by the roots of more developed plants (Khodakovskaya et al., 2009). It is possible that MWCNTs create new pores for water permeation through the penetration of root cells. It is possible that MWCNTs are able to control gating for extant water channels within the tissues of plant cells. This water channel gating may be delimited by diverse stresses such as anoxia, salinity, and pH along with the presence of heavy metals and high osmotic pressure. Another clarification is that CNTs act like channels beside the xylem and phloem tubes and are able to adjust the amounts of existent water in the plant cells (Khodakovskaya et al., 2009).

Another possibility is that MWCNTs enhance water intake, thereby hindering the leaching of electrolytes and carbohydrates (basically, ions and salts) and improving the cell membrane. The presence of aquaporins in the cell membrane, which create channels that conduct water molecules into and out of the cells and prevent the passage of ions and other solutes and thereby enabling water transportation inside plants, increase the efficiency of this water absorption mechanism through the MWCNTs’ ability to control these water channels (Mondal et al, 2011).

Regarding CNTs’ promotion of penetration, Serag et al. (2011) stated that MWCNTs can penetrate the cell membranes of plant protoplasts (plant cells with empty cell walls obtained through enzymatic treatment). They found that MWCNTs are capable of facilitating transverse water movement through a mechanism of direct penetration or internalization rather than by endocytosis. HA+MWCNTs may penetrate the plants cells inside root tissues through direct penetration; a slow endocytosis rate in the CNT medium enables the CNTs to escape from the medium, promoting water uptake by creating multi-pipes beside the xylem and phloem channels in plants tissues, allowing greater water and nutrient absorption from the medium solution.

Taken together, seedlings that can tolerate exposure to high concentrations of HA-stabilized MWCNTs exhibit high chlorophyll content, they are producing protein and carbohydrates through photosynthesis in greater quantities indicating there would be no influence of CNTs. Whereas, plants treated with pep-MWCNTs revealed high chlorophyll compare to peptone. Both peptone and HA appeared to have negative effect to plants by reducing their chlorophyll pigment as an intimation of unhealthy plants. But overall, all treatments with both control groups displayed a value of chlorophyll index below the optimal range indicating they all are under stress and might cause toxicity to plants.

Therefore, tomato plants exposed to peptone exhibited toxic reactions such as reductions in total biomass, root development, chlorophyll content, dry weight of shoot and root systems and shoot height. In a word, both peptone and MWCNTs dispersed in peptone solutions at high dose produced toxic effects and had a negative influence on the tomato plants. While at low dose plants grew better in terms of growth rate, water uptake, and root length rate.

5. CONCLUSION

The natural organic matter is used as a dispersant to assist MWCNTs to disperse in water. This factor showed significant impact on the effect of CNTs to tomato plants. The physiological health of plants is indicated by a suite of parameters including the growth rates, water uptake, root length rate, height shoot and so on. This research exhibited that different methods of preparation of non-functionalized multi-walled carbon nanotubes (MWCNTs) dispersions affects their interactions with plants. Unfunctionalized MWCNTs with the outer diameter range (<7nm) and an inner diameter of 2-5 nm were used.

The HA-stabilized MWCNT treatments showed a concentration-dependent enhancement in both shoot and root dry weight. Meanwhile, plants exposed to a1000mg/L HA-MWCNTs medium had the greatest dry weight for both shoots and roots while other dosages of MWCNTs resulted in a dry weight not significantly different from either of the HA control or the 1000mg/L medium. The roots were clearly longer in the tomato plants treated with HA-stabilized MWCNTs. The root growth rates of seedlings exposed to HA+ MWCNTs at concentrations of 10, 100, and 1000mg/L were similar to each other. Seedlings that can tolerate
exposure to high concentrations of HA-stabilized MWCNTs exhibit high chlorophyll content. Seedlings exposed to MWCNTs dispersed with HA as a surfactant show an increase and enhancement in both shoot and root weights. While, pep-MWCNT-exposed tomato seedlings exhibited either a reduction in root weight or a similarity in shoot fresh weight when compared to the peptone control.

In general, exposure to MWCNTs affects tomato plants depending on the organic matter used to disperse them. Exposure to MWCNTs dispersed in HA enhanced growth rate and water uptake. In contrast, pep-stabilized MWCNTs exhibited a negative impact on growth rate and root development, the organs that were in direct contact with the MWCNTs.

The 10 and 100mg/L groups of pep-MWCNTs exhibited better water transpiration than those exposed to 1000mg/L of pep-MWCNTs and peptone solution, but in general all the plants exposed to pep-MWCNTs revealed a significant diminishment in water uptake by the roots as well as peptone solution compared with water control.

To summarize, the properties of NOM play key role MWCNTs dispersability and biological effects. The two approaches which were utilized in order to induce the deagglomeration of MWCNTs suspension were mechanical dispersion (by sonication bath) and the surfactant which was added to achieve the CNTs dispersion stability. In particular the HA and peptone were utilized for preparation stabilized CNTs dispersion. HA was shown to be a more efficient agent in CNTs dispersion. HA is particularly effective to prepare stable dispersion for functionalized CNTs as indicated by reduced-size agglomerates and increased numbers of individualized MWCNTs.

Further work should be conducted to assess the MWCNTs internalization by providing TEM observation of different organ sections of tomato such as roots, leaves, and stems. According to our observation no potential entry of CNTs was pointed out as an evidence for CNTs penetration inside plants’ cells. The mechanisms related to the interaction between MWCNTs and plants membrane in the presence of both dispersant agents are worth investigating for further research. Finally, further examination into morphology and cell length in the root zone, among other factors, is needed to test the effects of MWCNT activation on root elongation.

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