ENHANCEMENT OF SEED GERMINATION AND PLANT GROWTH OF WHEAT, MAIZE, PEANUT AND GARLIC USING MULTIWALLED CARBON NANOTUBES

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The uptake of carbon nanomaterials by plants has shown a very recent field of nano-agriculture. This work investigated about the beneficial effects of functionalized multiwalled carbon nanotubes on wheat, maize, peanut and garlic. Here in, we explore the potential influence of 0-50 μg mL^{-1}, the MWCNTs on these different seeds at different concentration. The effects of MWCNTs on root and shoot growth, biomass, number of leaves were investigated. It was hypothesized that MWCNTs were able to penetrate the seed coat by creating new pores; thereby enhancing water uptake. The results of the combined morphological and physiological analysis indicate that after about 5-10 days exposure under our experimental conditions MWCNTs significantly enhances plant growth and biomass compared to control. The number and size of leaves of the MWCNTs treated plants had shown positive effects in a dose dependent manner. Nanotubes exposed seeds sprouted up to three to four times faster than controlled. TEM images of peanut root shows the presence of carbon nanotubes that could explain the enhanced water delivery. Overall after investigation we conclude that low dose MWCNT have seen to be beneficial, improving water absorption, found to accelerate the process of germination by shortening the germination time and higher biomass production.

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Introduction

Carbon nanotubes are well ordered, high aspect ratio allotropes of carbon having unique physico-chemical characteristics. It has acquired an important status in medicinal and biological application such as gene and drug delivery, tissue engineering, biosensing as well as in diagnostic area.[1-3] Therefore the probability of plant exposure to carbon nanotubes has increased to a greater extent with the rapid growth of research, increasing production and use of nanomaterials in different area.[4]

Changes in agricultural technology have been a major factor shaping modern agriculture, Carbon nanotubes are among the most widely used Carbon based nanomaterial which can easily penetrate membrane like cell wall of plants. As a result CNTs have enormous potential for use in agriculture as directed delivery systems for pesticides, fertilizer and other chemicals. Plant cell wall acts as a barrier for easy entry of any external agent including nanoparticles into plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 10 nm.[5] Hence only nanoparticles with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasma membrane.[6] There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which inturn enhance nanoparticle uptake. Therefore the study on the effects of nanoparticles in plant science is a newly emerging area of research.[7,8] Recently confocal fluorescence image studies have revealed the capacity of single walled carbon nanotubes (SWCNTs) to traverse across both the plant cell wall and cell membrane. Growing interests in applying nanoparticles to plants for agriculture and horticulture[9,10] have shown both positive and negative effects of nanomaterials on the growth and development of seedlings.[11] It is mainly dependent on concentration of nanomaterials delivered to plants. Plants and plant cells showed high tendencies to accumulate CNTs, making plants as an important link in the pathway by which CNTs enter the food chain and biological cycles.[12] Torney and coworkers demonstrated that CNTs can assist the delivery of biological molecules into plant cells.[13] Current literature revealed that the uptake, translocation and accumulation of nanoparticles depend on the species of plant and the size, type, chemical composition, fictionalization and stability of nanoparticles. At the whole plant level a number of researches have reported the dramatic effects on seed germination and plant growth by multi walled carbon nanotubes.[14] More efficient water uptake induced by CNT has been implied as the growth stimulator.[15] On the other hand there exist some contradictory reports that show the toxicity of CNTs to the growth of a number of different plants.[16]

Experimental

The carboxylic acid functionalized multi-walled Carbon nanotubes were purchased from SISCO Research Laboratories Pvt. Ltd. Mumbai. The as-received MWCNTs were powder with 95 % purity, OD 10-20nm and length between 10-30 μm.

Seeds of wheat (*Triticum aestivum*), maize (*Zea mays*), peanut (*Arachis hypogaea*) and garlic bulb (*Allium sativum*) were purchased from local market. These all were washed with deionized water. $MWCNT_S$–COOH in different concentration 20 μg mL^{-1}, 50 μg mL^{-1} were prepared directly in deionized water and dispersed by ultrasonic vibration for one hour. Seeds of all food plant species were soaked in nanoparticle solution for overnight in the dark. Garlic cloves had also kept in solution for 30 minutes. There garlic cloves were planted into soil about 2 inches deep. In the same way soaked seeds of wheat, maize and peanut were sown, one inch deep in soil. There all were exposed for equal amount of sunlight and equal amount of water was sprinkled on top of soil time to time.

The experiment for wheat seeds was conducted to determine the effects at 20 μg mL^{-1} and 50 μg mL^{-1} and for maize, peanut, garlic at only 50 μg mL^{-1} solution. The controlled sets for germinations were also carried out at the same time along with treated seeds. Every treatment, conducted triplicates each of which 8-10 seeds were maintained and examined for root and shoot development. These results were presented as mean ± SD (Standard deviation). Each of the experimental values was compared to its corresponding control.

Result and Discussion

A significant positive influence on root and shoot elongation was observed for all seeds in compared to those of unexposed control germination. Time of germination, germination percentage, vegetative biomass have shown encouraging results using low concentration of oxidized MWCNTs treated seeds as compared untreated. The effects of the CNTs on growth and development of the germinated sprouts were studied. Root and shoot systems were well recognized as they were fully germination in treated experiments in less time as compared to control.

Growth enhancement of CNTs-treated plants may have been due to the major changes of morphological and well developed root system. Wheat seeds were examined with two different concentration (20 and 50 μg mL^{-1}) of multi-walled Carbon nanotubes and also without MWCNTs. The treated and untreated seeds germinated at 3rd day but percentage of germination was significantly higher in tested medium in comparison to control. Wheat plant grown in 50 μg mL^{-1} possessed well developed long stems compared to the control (Figure 1).

Figure 1. Effect of water-soluble multiwalled carbon nanotubes on the growth of wheat seedling after 5 days

Effects of MWCNTs at different concentration on root and shoot length are shown in Figure 2a and 2b. The influence of MWCNTs solution at 50 μg mL^{-1} on root and shoot growth of maize plantlet varied with exposure time as shown in Figure 3a and 3b.

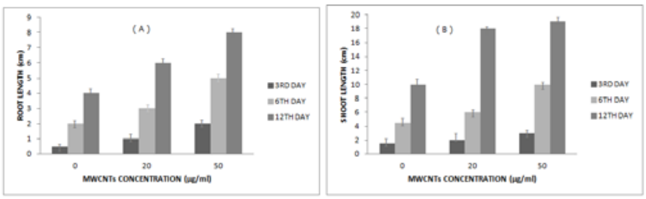

Figure 2. Effect of water-soluble multiwalled carbon nanotubes on wheat seedling. The values are given as mean +/SD (standard deviation) of triplicate samples with 10 seeds each.

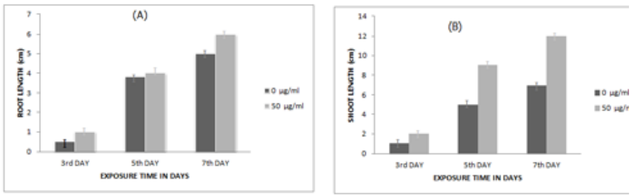

Figure 3. Effect of water-soluble multiwalled carbon nanotubes on germination of maize. The values are given as mean +/SD (standard deviation) of triplicate samples with 8 seeds each.

The well developed long stem and root and an increase in biomass compared to control is shown in Figure 4.

Figure 4. Effect of multiwalled carbon nanotubes on the growth of maize seedling after 5 days

Seed germination and embryo growth was much faster in case of MWCNTs, supplemented peanut seed. On third day it had seen that controlled seed was not germinated while treated seed had showed embryonic growth. There was enhancement of growth rate and biomass production Figure 5a and 5b The root and shoot length enhancement with respect to days is represented in Figure 6a and 6b.

Figure 5. Effect of water soluble carbon nanotubes on germination an growth of peanut seedling. A-without MWCNTs; B-with MWCNTs.

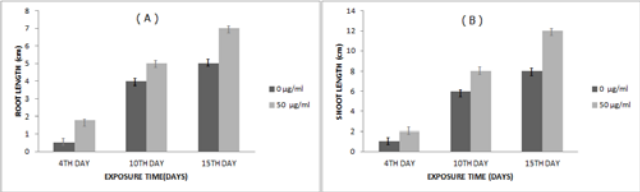

Figure 6. Effect of water soluble multiwalled carbon nanotubes on growth of peanut seedling. The values are given as mean +/SD (standard deviation) of triplicate samples with 8 seeds each.

Garlic cloves which were supplemented with MWCNTs sprouted 90 % till 12th day of sowing while untreated cloves have taken two weeks. There was much difference in root development Figure 7 in tested medium as compared to control. There is good correlation between root and shoot length of garlic plantlet with MWCNTs dose over control Figure 8a and 8b.

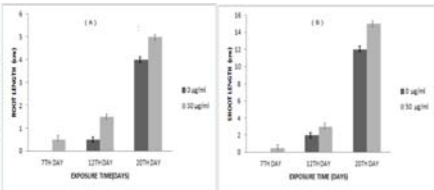

Figure 7. Phenotypes of 20 days old garlic plantlet growing on medium with and without CNTs

Figure 8. Effect of water-soluble multiwalled carbon nanotubes on garlic seedling. The values are given as mean +/SD (standard deviation) of triplicate samples with 10 seeds each.

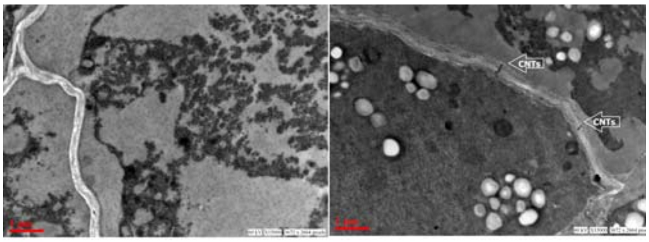

Figure 9. TEM images of the root system of 7 days old peanut seedlings growing on medium without CNTs and with CNTs

MWCNTs, internalization by plants was investigated by TEM (transmission electron microscopy). There is nanotubes accumulation in cell wall and inside the cell in TEM imaging of the root collected from peanut plantlet with and without exposure to MWCNTs. It can be seen in Figure 9 several CNT$_S$ which are completely missing in the images of the control sample. There studies indicate that the CNTs are able to penetrate in seedling as well as the root system and significantly affect their biological activity by enhancing the amount of water that penetrates inside the seed during the germination period.

Conclusions

Our results-demonstrated that all the wheat, maize peanut and garlic seedlings are affected by water soluble multi walled Carbon nanotubes in a concentration dependent manner. Carbon nanotubes can penetrate thick seed coat and support water uptake inside seeds. Lower concentrations are beneficial as they increases growth indices and water contents of the morphological parts most prominently for root which are directly in contact with the medium. The growth and higher biomass production for the plants that were exposed to 50 µg mL^{-1} MWCNTs-COOH, clearly indicate the positive effects of CNTs on seed germination which could have significant economic importance for agriculture.

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