DU Journal of Undergraduate Research and Innovation
Volume 4, Issue 1, pp 14-25

Growth Stimulation in Wheat and Brinjal by Impact of Water-Soluble Carbon Nanotubes

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ABSTRACT

Nano-biotechnology came as a hybrid discipline, a combination of biotechnology and nano-science. Carbon nanotubes (CNTs) have wide range of applications because of their unique properties. Seeds of wheat (Var.3043) and brinjal (Var.PPL) were exposed to different concentrations of –OH functionalized carbon nanotubes either directly or indirectly through the nutrient media. Factors such as seed germination, root elongation, plant biomass (fresh and dry), root length, shoot length, number of leaves, etc. were evaluated. No oxidative stress or change in photosynthetic activity was observed. The observations were taken at least thrice a week and the day of germination was also observed. It was seen that a treatment with MWCNTs led to an early germination in the treated samples vis-à-vis control. The treated seedlings showed improved growth parameters in terms of their root length, shoot length, fresh weight and dry weight. An improvement in the percent seed germination was also observed. Our findings suggest a possibility of the use of carbon nanotubes as growth stimulating additive when used in low doses. A detailed understanding of the molecular mechanisms would pave the way for the use of these materials in agriculture, where they could soon emerge as a novel technology.

Keywords: Carbon nanotubes, growth parameters, MWCNTs, nanomaterial SWCNTs.

INTRODUCTION

Nano-biotechnology, bio-nanotechnology, and nano-biology are terms that refer to the intersection of nanotechnology and biology. New discoveries in nanotechnology provided knowledge and technological platforms for a number of applications in medical science, aerospace, electronics and defense industries. Nanoparticles are particles with size of the order 10^{-9} m. Their small size results in a variation in their physical, chemical and biological properties that were otherwise unknown in their bulk form. The high surface area, simple functionalization, conductivity, magnetic properties and catalytic activity of CNTs have resulted into the applications of these materials into a wide range of areas, including plant growth and development. In the last many decades, a significant advancement in yield, quality, diseases and insect-pest resistance of crop plants has occurred due to improvement in production technology, protection measures and the genetic engineering. The role of

MWCNT (Multi-walled Carbon Nanotube) and SWCNT (Single Walled Carbon Nanotube) on the physiology of certain plants has been studied. Reports suggest an increase in fresh and dried biomass in the plants treated with SWCNT and MWCNT. CNT induced growth enhancement in tobacco has been reported. They are shown to enhance plant growth and increase fruit yield in tomato and tobacco (1), (2), (3). Application of nanotubes can help hasten seed germination and consequently results in faster growth and maturation of plants. It is thus important to understand the possible impact of CNTs on the physiology and development of plant systems, since there is little understanding about their plant interactions. The ability of carbon nanotubes to interact with the plant cells and the seed coats has been known.

Two agronomic plant species *Brassica juncae* and *Phaseolus mungo* were evaluated for phytotoxicity of MWCNTs. Both of these species showed 100% seed germination indicating non-hazardous nature of MWCNTs (4). When mesophyll cells of *Arabidopsis thaliana* were exposed to single walled carbon nanotubes, they showed dual phase regulation. At low concentrations (50 µg/ ml), SWCNTs stimulated plant cell to grow out trichome clusters on their surface, while at concentrations higher than 50µg/ml, toxic effects were observed (5). Similar effects were reported in a pilot study on root elongation of selected crop plants (6) (15). Despite the beneficial effects of CNTs on plant growth, some reports have suggested toxicity at higher concentrations (7), (8), (9), (10). Recent studies have also focused on the uptake of the MWCNTs in terrestrial higher plants (11). There is an increased need to develop an understanding of the complex interactions of the nanomaterials with biological systems. Our study is an attempt to understand this facet of material sciences in interaction with plant life.

METHODOLOGY

A) Plant samples and type of carbon nanotubes

Wheat (Var. 3043) and Brinjal seeds (Var. PPL) were collected from IARI, Pusa, New Dehi. MWCNTs non-functionalized as well as –OH functionalized were purchased from SRL. Multi-walled carbon nanotubes were functionalized on the outer surface with different groups like OH, COOH, NH_2, F etc., thus promoting its dispersion in variety of different solvents. In the current study, –OH functionalized tubes were used

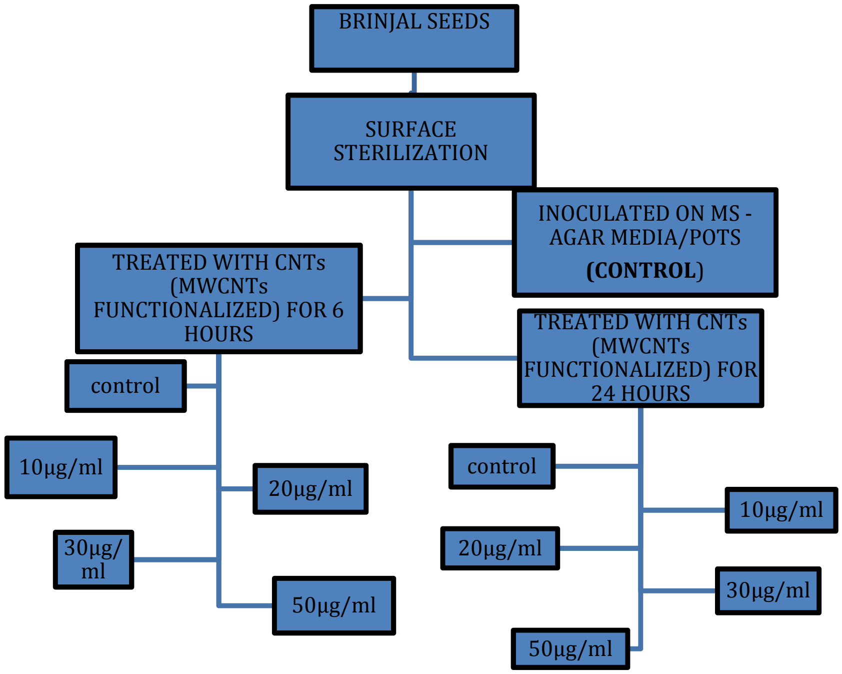
B) Seed sterilization

Brinjal seeds were washed with Tween-20 and sterilized with 0.1% (v/v) mercuric chloride solution for 45 seconds. The seeds were then washed with double distilled water. Wheat seeds were washed in Tween-20 and sterilized with 70% (v/v) ethanol for one minute, followed by treatment with 0.1 % (v/v) Mercuric chloride for six minutes. Seed sterilization was done in laminar air flow.

C) CNT treatment, seed germination and early seedling growth

i) Brinjal − Surface sterilized seeds were germinated in controlled conditions on ½ MS-Agar as well as in pots with autoclaved soil:vermiculite (1:1). Routine observations were noted for root and shoot lengths and on the time of germination, in each case. Fresh weights and dry weights of each sapling were evaluated. The surface sterilized seeds were treated with different concentrations of MWCNTs (0 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml and 50 µg/ml) for 6 hours and 24 hours. All the experiments were performed with at least 30 replicates.

FigureI:



Schematic representation of methodology used in Brinjal

ii) Wheat − Standard MS medium was supplemented with different concentrations (0 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml, and 500 µg/ml) of non-functionalized and –OH functionalized MWCNTs. The MS medium without nanotubes was used as an internal control. All seeds were germinated under control conditions. The –OH functionalized MWCNTs were dispersed in water at various concentrations (0, 50 µg/ml, 100 µg/ml, 250 µg/ml, and 500 µg/ml) for 15 hours and 24 hours. For comparison control seeds were dipped in water for 15 and 24 hours. Observations were taken daily.

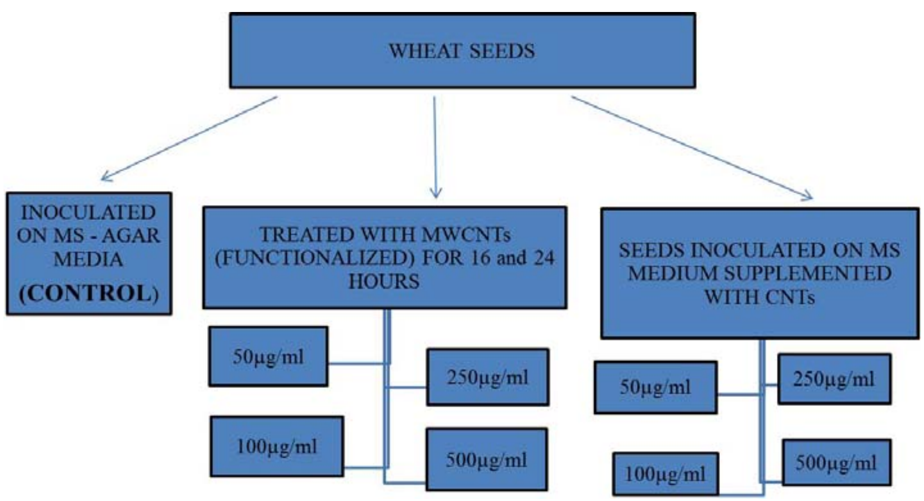


Figure II: Schematic representation of methodology used in Wheat

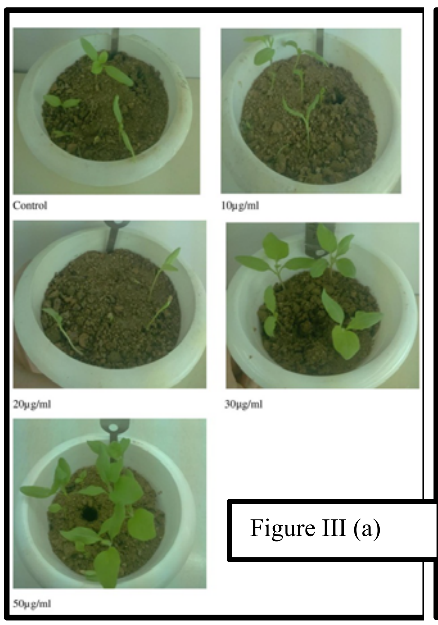
D) Statistical Analysis

All experiments were carried out with at least 30 replicates for each treatment. All figures are represented as Mean ± Standard Error. All data was analyzed using ANOVA.

RESULTS

A. Brinjal − Treatment with different concentrations of –OH functionalized MWCNTs (0, 5, 50,100, 250 μg/ml) for 6 hours and 24 hours:

Solanum melongena was observed to be a slow growing plant. The seeds showed very slow germination when grown on MS or ½ MS–Agar medium with varying concentrations of sucrose (Table I and II). The seeds grew better when sown in autoclaved soil and vermiculite at a ratio of 1:1. The seeds were then treated with varying concentrations of functionalized MWCNTs for 6 hours and 24 hours. The observations were taken at least thrice a week and the day of germination was also observed. It was seen that a treatment with MWCNTs led to an early germination in the treated samples vis-à-vis control. The treated seedling showed improved growth parameters in terms of their root length, shoot length, fresh weight and dry weight. An improvement in the percent seed germination was also observed. However, concentrations higher than 50 μg/ml proved toxic in Brinjal. (Figure IIIa and IIIb)



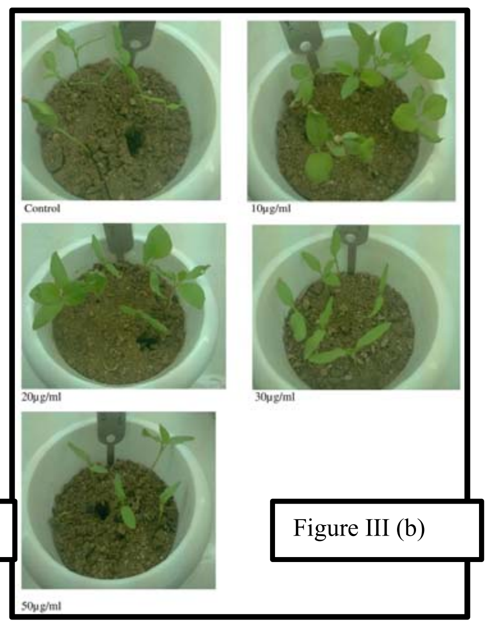


Figure III (a): -OH Functionalized MWCNT treatment for 6 hours affecting seeds germination and early seedling growth

Figure III (b): -OH Functionalized MWCNT treatment for 24 hours affecting seeds germination and early seedling growth

Table-I: -OH Functionalized MWCNT treatment for 6 hours affecting seed germination and early seedling growth parameters

Sl. No.	Conc. (μg/ml)	Seed germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)

1	control	60	2.58 ± 2.61	2.23 ± 1.95	0.036 ± 0.048	0.003 ± 0.002
2	10	60	3.89 ± 1.72	2.96 ± 1.32	0.04 ± 0.048	0.003 ± 0.002
3	20	70	1.83 ± 2.09	2.31 ± 2.08	0.028 ± 0.023	0.004 ± 0.001
4	30	70	4.66 ± 2.26	3.02 ± 1.66	0.089 ± 0.055	0.007 ±0.003
5	**50**	**85**	**4.95 ± 1.41**	**3.53 ± 0.47**	**0.137 ± 0.014**	**0.0109 ± 0.003**

Table-II: -OH Functionalized MWCNT treatment for 24 hours affecting seed germination and early seedling growth parameters

B.

Sl. No.	Conc. (µg/ml)	Seed germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)
1	control	60	4.54 ± 1.49	3.19 ± 0.80	0.084 ± 0.04	0.005 ± 0.003
2	10	70	3.18 ± 0.71	3.42 ± 0.40	0.126 ± 0.05	0.010 ± 0.004
3	20	70	4.14 ± 1.86	2.79 ± 0.44	0.066 ± 0.026	0.005 ± 0.003
4	**30**	**85**	**5.2 ± 1.25**	**2.88 ± 0.63**	**0.125 ± 0.048**	**0.012 ± 0.004**
5	50	75	4.28 ± 2.15	2.24 ± 1.064	0.086 ± 0.062	0.08 ± 0.004

Wheat − Treatment of wheat seeds with different concentrations of –OH functionalized MWCNTs for 15 hours and 24 hours:

In order to test whether functionalized MWCNTs can affect germination and seedling growth, different concentrations of –OH functionalized MWCNTs (0 µg/ml, 5 µg/ml, 50 µg/ml, 100 µg/ml and 250 µg/ml) were used. Seeds were surface sterilized and treated with MWCNT solution for 15 hours and 24 hours. They were then inoculated on ½ MS medium. The observations were made on a daily basis so that the day of germination could be recorded. It was observed that the treated seeds germinated faster and had a better percent seed germination also. It was also observed that seedlings growing on medium supplemented with MWCNTs had better developed leaves than the control seeds. Significantly improved growth parameters like: shoot length, root length, shoot fresh weight, root fresh weight and seedling dry weight were observed at higher concentrations of MWCNTs (Table III & IV Figure IV & V).

However, concentrations higher than 100 µg/ml proved deleterious for the growth of the plant material. Treatment of seeds for 24 hours with the functionalized MWCNT solutions gave better results in terms of growth parameters than the 15 hours treatment with the same concentrations. Although all the concentrations of MWCNTs gave improved results than the control, best results were obtained at 25 and 50 µg/ml where the shoot length was almost twice the control. The shoots appeared healthier with greener leaves and broader leaf lamina.

Table III: -OH Functionalized MWCNT treatment for 15 hours affecting seed germination and early seedling growth parameters

S.No.	Concentration (µg/ml)	Root length (cm)	Shoot length (cm)	Germination (%)
1	**Control**	**6.15±0.141**	**11.5±0.495**	**100%**
2	50 µg/mL	2.55±0.848	8.8±0.495	100%
3	**100 µg/mL**	**10.25±0.282**	**18±0.991**	**90%**
4	250 µg/mL	3.00±0.00	4.3±4.172	40%

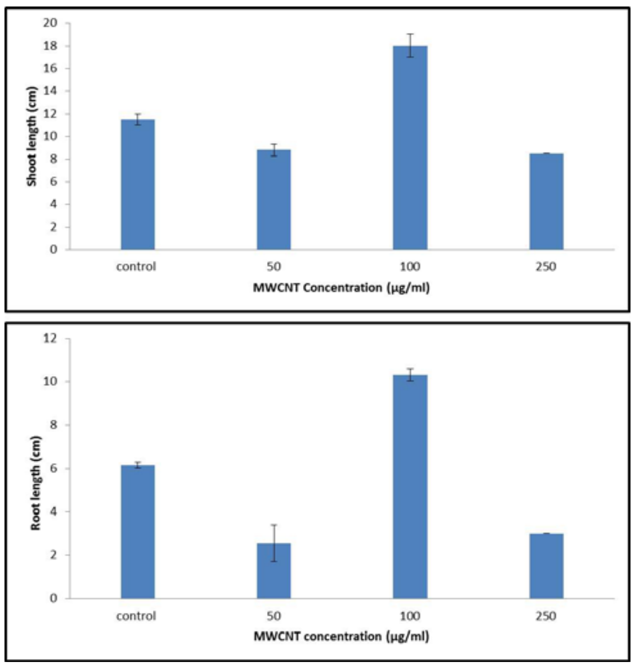


Figure IV: -OH Functionalized MWCNT treatment for 15 hours

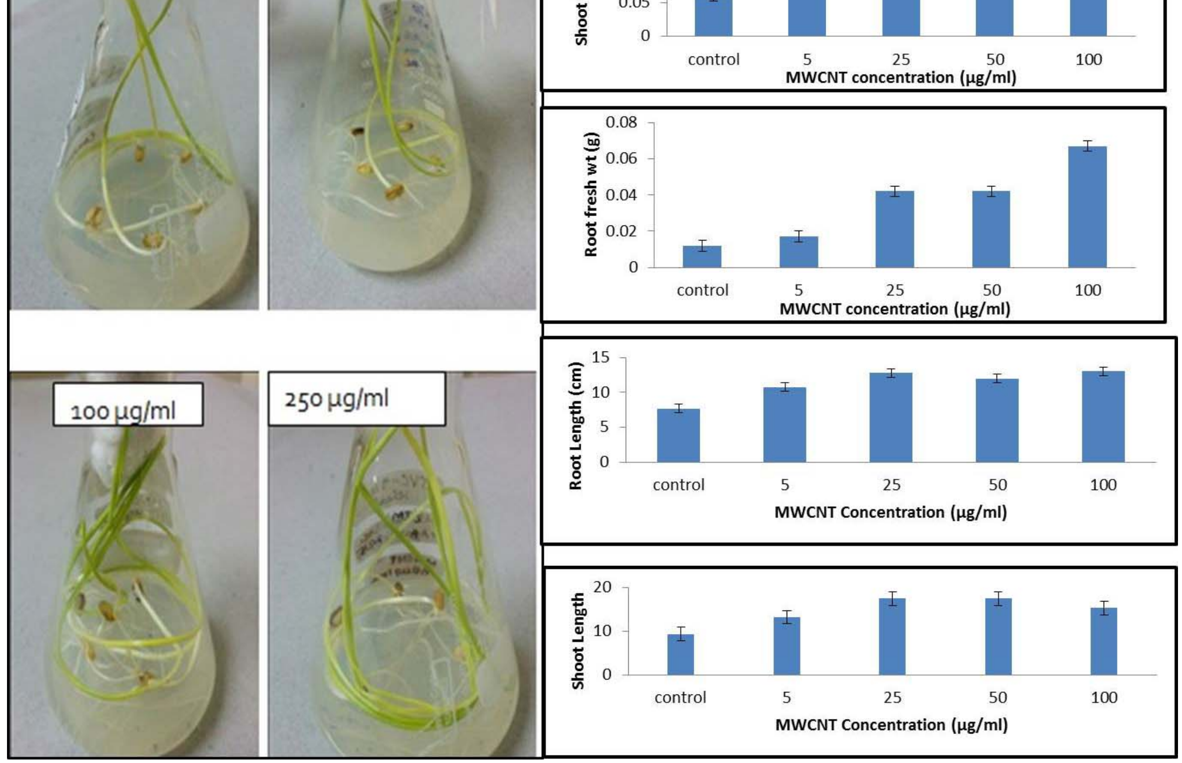


Figure V: -OH Functionalized MWCNT treatment for 24 hours affecting seed germination and early seedling growth parameters

Table IV: -OH Functionalized MWCNT treatment for 24 hours affecting seed germination and early seedling growth parameters

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (mg)	Fresh weight of root (mg)
Control	9.3 ± 4.012	7.7 ± 2.852	0.059 ± 0.003	0.012 ± 0.002
5	13.13 ± 1.18	10.76 ± 0.63	0.077 ± 0.006	0.017 ± 0.003
25	**17.45 ± 2.65**	**12.75 ± 0.65**	**0.15 ± 0.019**	**0.042 ± 0.008**
50	**17.4 ± 2.59**	**11.98 ± 1.18**	**0.11 ± 0.015**	**0.042 ± 0.006**
100	15.24 ± 1.54	13 ± 0.86	0.116 ± 0.011	0.067 ± 0.008
250	13.23 ± 1.16	11.2 ± 0.7	0.102 ± 0.02	0.034 ± 0.004

C. Wheat seeds inoculated on MS-Agar supplemented with different concentrations of functionalized MWCNTs (0, 50,100, 250 µg/ml)

As has already been seen, the direct exposure of seeds to the carbon nanotubes impacts their growth and development. Next, it was investigated whether the incorporation of MWCNTs into the growth medium can have any significant effect on the growth of seeds. The results have been tabulated in Table V. Although, 100 µg/ml concentrations showed improved response to the plant growth, but this effect was not as remarkable as that seen upon the direct exposure of seeds to the nanomaterial (Table V, Figure VI).

Table V: Growth parameters of seeds inoculated on MS-Agar Supplemented with different concentrations of Functionalized MWCNTs (0, 50,100, 250 µg/ml).

S.No	Concentration (µg/mL)	Shoot length (cm)	Root length (cm)	% germination
1	**Control**	**6.15±0.141**	**11.5±0.495**	**100%**
2	50	2.55±0.848	8.8±0.495	100%
3	**100**	**10.25±0.282**	**18±0.991**	**90%**
4	250	3.00±0.00	4.3±4.172	40%

Figure VI: Growth of seeds inoculated on MS-Agar supplemented with different concentrations

DISCUSSION

Lately, an increase in the application of nanotechnology in plant genetic engineering and agriculture has resulted due to the highly sensitive methods of delivery and visualization of the distribution and uptake of nanomaterial on plants. An exponential growth on nanotube research is taking place that aids the emergence of new technologies. As such some novel and exquisite applications of nanotechnology have been seen in the agricultural sector. There are ample examples of the same in improvement of nutritional value of food, assessment of nanoscale nutrient delivery systems, harvesting energy and conversion, livestock reproduction enhancement, sensing technology and enhancement of plant growth that primarily includes the increase in the root and shoot lengths of various agriculturally important crops. Positive impact on growth of plants has been previously reported in tomato, corn (12), (13), (14). Based on the two-delivery system for multi-walled carbon nanotubes (sterile agar medium and deposition on seed surface) in three important plant (barley, maize and soyabean) showed positive affect of MWCNTs, as early seed germination and activation of growth in exposed seedling was observed. For the first time, it has been shown that the expression of seed located water channel genes (aquaporins) can be increased significantly by using reverse transcription polymerase chain reaction (RT–PCR), thus supporting the hypothesis about the involvement of carbon nanotubes in low concentration in regulating the activity of water channels in plants exposed to MWCNTs (1). Two agronomic plant species *Brassica juncae* and *Phaseolus mungo* were evaluated for phytotoxicity of MWCNTs. Both of these species showed 100% seed germination, indicating non-hazardous nature of MWCNTs (4). Previously, carbon nanotubes were considered to have toxic effect on the environment. In an experiment involving exposure of red spinach to different concentration of MWCNTs (0-1000mg/L), CNT exposed plants showed growth inhibition, adverse effect on root and leaf morphology and death of the plant after 15 days. However, introduction of ascorbic acid was observed to reverse the toxic effects and this effect was attributed to reduction in oxidative stress (16). Earlier studies have, in fact, shown that the phytotoxic effect of MWCNTs is mainly related to reactive oxygen species. Canas et al. (17) described in their study that the species would respond differently to the nanomaterials, even under the identical experimental conditions. This differential toxicity tentatively suggests that agricultural use of MWNTs may not negatively affect all crop species. There are studies where positive effects of MWNTs have also been reported (18) (19).

This conclusion was further validated by an experiment involving the exposure of MWCNTs to lettuce seedling grown in Hoagland's media. Toxic effects were observed after two weeks, which involved inhibition of seed germination, plant growth and plant biomass. Further, *in situ* detection of hydrogen peroxide has also proposed the reactive oxygen species for mechanism of toxic effect of MWCNTs (20). Pathway of uptake and accumulation of

MWCNTs is a major area of study suggesting that MWCNTs penetrate the cell walls, accumulate in the plant tissue and cells, and are translocated from root to stem to leaves (21) (22).

CONCLUSIONS

The recent studies in the field of nanotechnology can benefit a number of fields in plant biology and agricultural sciences. In the current study, we have demonstrated that the application of MWCNTs to wheat and brinjal can improve seed germination and early plant growth. The current studies throw light on the possibility of use of the CNTs in improvement in yields and seed germination of recalcitrant crops and plants. A number of studies throw light on the possible mechanism of CNT transport and uptake (23), (24), (25). In the current study, we have observed:

- Improvement in the growth
- Early maturation
- Yield improvement

Given the present size of our population, it is a great challenge to meet the requirement of food for all. We need to find measures to enhance the yield of the various food crops that are being cultivated in the country. However, increased and persistent use of chemical fertilizers not only increases the toxicity of the crop but they also render the soil unfertile and increase the salinity of the soil, hence leaving it unsuitable for further agricultural purposes. The need of the hour is a new “Green Revolution” for a sustainable increase in the food production, sans the indiscriminate use of the chemical fertilizers (26). Therefore, the role of carbon nanotubes in this context is an exciting prospect that needs to be further explored given its potential to improve the plant growth parameters.

ACKNOWLEDGMENTS

The financial support given by University of Delhi under the Innovation Project Scheme is gratefully acknowledged. We are grateful to Prof. MV Rajam, Department of Genetics, Delhi University, South Campus for mentoring the project. We express our gratitude to Dr. P. Hemalatha Reddy, Principal, Sri Venkateswara College for providing the necessary infrastructure and facility.

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