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Application of multiwalled carbon nanotubes on the germination and seedling growth of wheat (*Triticum aestivum* L.)

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Abstract
Nanotechnology has been found to revolutionize the agriculture with new techniques to enhance the supply of nutrients to the plants. The uses of nano particles as modern agricultural practice favor the growth of plants. Carbon Nano Tubes (CNTs) have the potential to increase the germination and growth of some plants, extending the applicability of the new field of nano-biotechnology to enhance productivity. CNTs can be used for boosting up the early germination process as well as disease resistance by providing desired molecules and reducing the lifecycle of the crop. In present experiment attempts were made to find out the response of Multiwalled Carbon Nanotubes (MWCNTs) upon the vegetative growth of *Triticum aestivum* L. at experimental farm of Dayalbagh, Agra. Total four treatments i.e., 20 (T_1), 30 (T_2), 40 (T_3) and 50 (T_4) µgm/ml along with control (T_0) were applied in triplicates. Germination percentage, plant height, no of tillers /unit area were observed at 30, 60, 90 Days after sowing. Data was analyzed and interpreted. The study concluded that MWCNTs played significant role in germination and vegetative growth of *Triticum aestivum* L. More significant responses were observed in T_3 and T_4 than T_1 and control respectively.

Keywords: Multiwalled carbon nanotube, germination/seedling growth, response, wheat crop

1. Introduction
Nanotechnology deals with creation and uses of materials devices and systems through the control of the physical and chemical properties of the matter at the nano-metric scale. Development of targeted research to understand, manipulate and measure of the materials with atomic, molecular and super molecular dimensions is called nanotechnology. It is a wide field and applicable recently in most of the fields of agricultural and allied science. Nanotechnology can revolutionize the agriculture with new tools to enhance the capability of plants to absorb the available nutrients.

Nano particles interact with the living cells at molecular level and nano agriculture involves the employment of nano particles in agriculture. These particles impose beneficial effects to the crop productivity. Recently main emphasis is given to apply nano technology for enhancement of crop production and the control of plant diseases.

Carbon nano tubes (CNTs) are tubular form of Buckminster fullerene was discovered by Iijima in 1991. They are straight segments of carbon hexagonal units. MWCNTs are formed by several graphene sheets wrapped around the tube core [9]. Interaction between plants and CNTs needs to be investigated from the cellular to the organism level to understand its multifaceted complexity. This will provide a way to develop a new area of biotechnology called as 'Nanoagriculture' that holds promise for acceleration of growth and productivity of crop plants [10].

Many researchers have reported the dramatic effects of multi walled carbon nano tubes (MWCNTs) on seed germination and plant growth. MWCNTs have been shown to penetrate the seed coat and stimulate the growth of tomato [3, 12] and mustard seeds [6]. Water soluble MWCNTs have been shown to exhibit similar dramatic improvement of the growth of gram plant [11].

Keeping in view the application and importance of Multiwalled Carbon Nanotube in farming, an attempt is made to find out the response of Multiwalled Carbon Nanotubes upon

germination and seedling growth of Wheat (*Triticum aestivum* L.) in this paper.

2. Materials and Methods

2.1 Nano Particle Processing: MWCNTs were purchased from Arry International, Germany with 60% purity and having a diameter of 30 nm. MWCNTs were activated by following the technique of [8] with the following modification. At first, MWCNTs were refluxed with 2 M nitric acid at 130 ^{0}C for 16 hrs and then sonicated for 3 hrs in the same acid. Resulting material was collected by filtration and washed in water and ethanol successively until the pH of solution became 6. The resulting MWCNTs are now in oxidized form and well dispersed in water owing to presence of hydrophilic carboxyl (–COOH), hydroxyl (–OH) and carbonyl groups (C=O) along with their side walls. Finally, the oxidized MWCNTs were dried in hot air oven at 80 ^{0}C for 6 hrs.

2.2 Seed Collection: Good quality certified seeds of Wheat cultivar (HD 2967) were procured from seed stores and the R.S.S. Agricultural farm Dayalbagh, Agra for present study.

2.3 Pretreatment: Seeds were initially surface sterilized with 0.1% $Hgcl_2$ for 1 minute. One lot of seeds was immersed in distilled water and another lot in MWCNTs solution for 6 hrs. Seeds were stirred frequently and excess solution was decanted. After completion of the treatment the seeds were separately surface dried with blotting paper and dried back to original weight under sunlight. Another lot of seeds which was not soaked in any solution but sun dried, taken as control. After pretreatment the seeds were stored in normal laboratory condition in perforated paper bags and used after 15 days for experimental purpose.

2.4 Preparation of Nano Particle Solution: The Multiwalled Carbon Nanotubes were suspended directly in double distilled water by sonication in an ultrasonic bath. MWCNTs solution was prepared at different concentrations i.e., T_0 (Control), T_1 (20 µg/ml), T_2 (30 µg/ml), T_3 (40 µg/ml) and T_4 (50 µg/ml).

2.5 Bioassay Experimentation: Sterilization of glass wares was done before conducting an experiment in the autoclave at 120 ^{0}C, 15lb/cm^3 pressure for 15 min. and Wheat seeds were also Sterilized by $HgCl_2$ before starting of experimentation.

2.6 Germination Assay: Before starting germination all seeds were immersed in 10% Sodium hypochlorite for surface sterilization. To analyze percentage of seed germination 50 seeds were transferred to petridishes containing filter paper moistened with 10 ml of distilled water (control) and nano particle solutions (treatments). Germination data was recorded at every 24 hrs interval. Seeds were considered to be completely germinated when the radicle attained a length of 1 mm and plumule has just unfolded.

2.7 Field experimentation: Field experimentation was done in micro plots arranged in Randomize Block Design with three replicates. All the recommended package of practices were followed uniformly in all the plots except different treatments made by MWCNTs and untreated sown plots were considered as control.

2.8 Observation and Analysis: The frequent observations of the experimental plots were made at different stages during the experimentation and obtained data was analyzed statistically.

3. Results and Discussion

3.1 Germination: Germination in the case of control was 85%, which was least one. On the other hand Treatments T_1 & T_2 showed equal germination response (87%). Maximum germination (92%) was observed in the case of treatment T_4 followed by T_3 (90%) over the control and other treatments (Table 1). Germination per unit area gradually increases as increases the dose of carbon nanotubes. T4 showed higher value in comparison to others. Hence carbon nanotubes showed positive effect on the germination and growth of wheat. The stimulatory effects of MWCNTs have been reported for different crops such as improved germination rates were described for tomato (*Solanum lycopersicum*) [4, 1] and rice (*Oryza sativa*) [7], while germination speed was sufficiently enhanced in barley (*Hordeum vulgare*), soybean (*Glycine max*), maize (*Zea mays*) [5] and mustard seeds (*Brassica juncea*) [6].

The increase in water flow into the seeds has been associated with the ability of CNTs to penetrate the seed coat [3]. Later, the effect of MWCNTs with different concentration on the expression of aquaporin genes has been found for germinating seeds of soybean (*Glycine max*), barley (*Hordeum vulgare*) and maize (*Zea mays*) [5]. Due to a central role of aquaporins in germination, it has been concluded that the beneficial role of MWCNTs on water uptake in seeds and germination may be mediated by the described aquaporin effect in different crops including wheat.

3.2 Seedling Growth: The growth in control was found 17.2 cm which is least in comparison to all treatments. The higher growth was found in the treatment (T_4) i.e. 23 cm. The effect of CNTs on wheat seeds was positively significant at 30 DAS. The same trend was observed at 60 DAS and 90 DAS. The number of tillers per unit area also increases as the concentration of carbon nanotubes increases. Number of tiller per unit area was found maximum in the T_4 followed by T_3 as compared to control (table 2). These results are in favor of earlier reports that showed MWCNTs positively affects the root and shoot elongation in a range of plant species, such as wheat (*Triticum aestivum* L.) [13], tomato (*Solanum lycopersicum* L.) [4, 7, 5], soybean (*Glycine max* L.), maize (*Zea mays* L.) [5], black lentil (*Phaseolus mungo* L.), and mustard (*Brassica juncea* L.) [1]. On the other hand the effect of carbon nanotubes on the growth of *Triticum aestivum* L. was found positive effect as the dose of carbon nanotubes increases.

Table 1: Effect of Mwcnts on the germination of tested wheat seeds

Treatments	Germination (%)	Germination/m^2	(%) Change over Control
T_0	85	86.5	
T_1	87	102.1	18.03
T_2	87	107.8	23.70
T_3	90	122.7	41
T_4	92	124.8	44.27
S.Em±	-	1.5	-
CD (p=0.05)	-	3.4	-

Table 2: Effect of MWCNTs on the vegetative growth parameters of wheat

Treatments	Vegetative Growth Parameters				
	Plant height			Number of Tillers/m^2	
	30 DAS	60 DAS	90 DAS	60 DAS	90 DAS
T_0	17.2	47.7	89.3	572.6	313.0
T_1	19.3	50.8	101.6	703.6	332.3
T_2	20.3	52.9	102.0	744.0	347.3
T_3	22.0	54.3	103.0	747.0	361.0
T_4	23.0	59.5	106.0	773.0	380.0
S.Em±	0.55	2.11	1.37	10.78	6.50
CD (p=0.05)	1.27	4.85	3.20	24.80	14.9

CD- Critical difference, DAS- Days after sowing

4. Conclusion

In this study it is found that the germination of wheat seedlings is significantly affected by different concentrations of MWCNTs. 40 (T_3) and 50 µgm/ml (T_4) have shown the positive effects on germination and growth attributes of tested cultivar of wheat in comparison to other treatments. Most accepted theory explained that CNTs accelerate the water uptake capacity of seeds at the time of germination by enhancing activities of Aquaporin associated genes and has been demonstrated in barley (*Hordeum vulgare* L.), soybean (*Glycine max* L.) and maize (*Zea mays* L.) [5]. Hence effect of carbon nanotubes at every stage of plant growth was positively significant.

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