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RESEARCH ARTICLE

PHYTOTOXICITY OF NANOPARTICLES TO SEED GERMINATION OF PLANTS

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Abstract

Phytotoxicity is an important consideration to understand the potential environmental impacts of manufactured nanoparticles, which ultimately accumulated in soil. In the present study, our aim was to assess the effects of two metal oxide nanoparticles (NPs), nano-ZnO, nano-Fe_2O_3 and one carbon based (fullerene) NPs, on seed germination, root and shoot elongation of wheat, rice, green gram and cucumber plants. These parameters were quantified following exposure to each type of NPs at different concentrations, from 10 mg/L to 1000 mg/L. Inhibition of root growth varied greatly among NPs and plant types. Among NPs, nano-ZnO found to be most phytotoxic, followed by nano-Fe_2O_3 and fullerene. Reduction in root and shoot growth found to be a more sensitive parameter than germination percentage. Results also indicated that NPs at low concentrations can be useful for seedling growth. Fifty percent inhibitory concentrate ions (IC_{50}) varied with type of NPs and plant species. IC_{50} value of nano-ZnO was about 50 mg/L for cucumber and 100 mg/L for other tested plant species. Overall, this study has shown that direct exposure of germinating seeds to nanoparticles may cause phytotoxicity and underscores the need for eco-responsible disposal of wastes and sludge containing NPs which ultimately comes into soil system.

INTRODUCTION

Nanotechnology is a rapidly growing industry that is expected to reach a market size of approximately 2.6 trillion dollars ($) by 2015 (Holman 2007). Increasing numbers of commercial nano products, from cosmetics to medicine, incorporate these nanomaterials that can be accidentally or incidentally released to the environment and ultimately soil ecosystem (Colvin 2003; Service 2008). Concern over the potentially undesirable effects of such nanoparticles (NPs) has stimulated the advent of nanotoxicology as a unique and significant research discipline (Brumfield 2003; Service 2003; Wiesner et al. 2006; Lin and Xing 2007). However, the majority of the published nanotoxicology articles has focussed on mammalian cytotoxicity or impacts to animals and bacteria, and only a few studies have considered the toxicity of these NPs to plants system. Developmental phytotoxicity of manufactured nanoparticles (MNPs) is a critical knowledge gap because NPs entering wastewater streams may predominantly be incorporated into sewage sludge and reached to agricultural fields.

The impact of MNPs on different plant species can vary greatly, and there are conflicting reports of both types, positive and negative effects. Among positive effects, the addition of nano-TiO_2 at 2.5 - 40 g/kg of soil promoted the growth of spinach, likely by protecting the chloroplasts from aging during long-term illumination (Zheng et al. 2005). Lin and Xing (2007), reported significant inhibition of ryegrass germination by 2,000 mg/L nano-Zn.

Similarly, 2,000 mg/L of nano-Al_2O_3 inhibited corn germination, whereas no inhibition was observed for 2,000 mg/L of multiwalled carbon nanotubes (MWCNT). In contrast, Canas et al. (2008) showed that non-functionalized nanotubes inhibited root elongation of tomato and enhanced root elongation of onion and cucumber, while the functionalized nanotubes inhibited root elongation in lettuce. Recently, Barrena et al. (2009) evaluated the toxicity of three types of NPs: Au (10 nm), Ag (2 nm), and Fe_3O_4 (7 nm) using germination tests with cucumber and lettuce, but detected no or only low toxicity at the assayed concentrations (62, 100, and 116 mg/L, respectively). Temsah and Joner (2010) also pointed out inhibitory effect of germination on ryegrass, barley, and flax by aqueous suspensions of zero-valent Fe NPs at 250 mg/L and 10 mg/L for Ag NPs suspension. Overall, the current phytotoxicity profile of nanomaterials is highly empirical and preliminary, and the effects of NPs elemental composition, size, and stability are poorly understood.

There are thus still many unresolved issues and challenges concerning the biological effects of NPs on terrestrial organisms, and particularly higher plants. Thus the present study, was designed to assess the developmental phytotoxicity exerted by two metal oxide NPs—nano-ZnO, nano-Fe_2O_3 and a carbon based (C_{60}) NPs. We selected higher plant species, wheat, rice, green gram and cucumber since these are widely grown and cover a large cultivated area. This study was undertaken to generate information about phytotoxicology of NPs by investigating the effect of three types of MNPs on seed germination, root and shoot growth of four higher plant species.

1. MATERIAL AND METHODS

1.1. Nanoparticles

Commercially produced metal oxide NPs namely iron (III) oxide zinc oxide and carbon based NPs fullerene (C_{60}) were obtained from Sigma Aldrich Co., USA. The detailed properties of MNPs in Table 1.

1.2. Seeds

The certified seeds were obtained from the National Seed Corporation, New Delhi. The protocol for seed germination toxicity tests was followed according to the United State Environmental Protection Agency (EPA, 1996; Yang and Watts, 2005). The selection of plant species was based on two criteria. First, they represent both monocotyledonous and dicotyledonous plants. Second, the two monocotyledonous species have very different seed sizes, and thus may be affected differently by the NPs.

1.3. Preparation of nanoparticles suspensions

Nanoparticles stock suspensions (1000 mg/L), was prepared by mixing pre-weighed nano-ZnO and nano-Fe_2O_3 in double distilled water (DDW), fullerene NPs in ultra-pure ethanol. The dispersed NPs were ultrasonicated for 30 minutes. To further stabilize this nanoparticle suspension, 10% (v/v) polyethylene glycol (PEG-400), a dispersant was added as described by Zhang et al. (2007). The suspensions were sonicated again for at least 1 min before use.

1.4. Seed germination and exposure

Seeds were immersed in a 10% sodium hypochlorite solution for 10 min and then rinsed three times with DDW to ensure surface sterility. Then the seeds were soaked in DDW (control) or different NPs suspension solution (10, 20, 50, 100, 200 and 500 mg/L) for about 2 h. One piece of filter paper was put into each 100 mm x 15 mm Petri dish, and 5 ml of a test medium (10, 20, 50, 100, 200 and 500 mg/L) was added. Seeds were then transferred onto the filter paper, with 10 seeds per dish and 1 cm or larger distance between each seed (Yang and Watts, 2005). Petri dishes were covered and placed in the dark, in a growth chamber at 25 °C. After 5-12 days (because different types of seed geminate at different dates) as per type of seed, the germination of all the treatments were halted, and seedling root and shoot length was measured.

Germination tests in soil used the same plant species and seed densities, placing seeds in petri dishes and covering them with 50 g of air dry soil that was subsequently maintained to 75% of their respective water holding capacities using water or aqueous NPs suspensions to provide final concentrations of 1000 mg/kg of soil. The physicochemical properties of the soil used for germination purpose are in Table 2. Petri dishes with seeds and soil were placed in an incubation chamber with 12/12 h light/dark cycle. After 5-12 days the percent seed germination was recorded.

1.5. Statistical analysis

Each treatment was replicated thrice, and the results were presented as mean values with respective standard deviations. Phytotoxicity endpoints for all treatments were compared to those of unexposed controls using the Student's t-test paired two samples for means. The statistical significance of differences between treatments was determined at the 95% confidence level ($P<0.05$).

2. RESULTS AND DISCUSSION

Seed germination and seedling growth are being widely used to test phytotoxicity of chemical species such as engineered nanomaterials which may be released into the environment. This is because seed germination, shoot and root elongation measurements are quite rapid for use on acute phytotoxicity tests with several advantages: sensitivity, simplicity, low cost, and suitability for reactive chemicals and contaminated soil samples (Munzuroglu and Geckil, 2002). The present study successfully demonstrated the feasibility of using seed germination tests, both in water and in soils, as acute toxicity tests for readily suspended NPs.

2.1. Effect of nanoparticles suspensions on seed germination

Effects of NPs at 1000 mg/L on seed germination are shown in Figure 1. The minimum germination percentage was observed with nano-ZnO suspension in all the plant species except rice. Cucumber recorded only 71% germination rate in nano-ZnO, which was significantly ($P < 0.05$) lower than fullerene NPs treated seed. The inhibitory effect of nano-ZnO might be due to some compound formation with this particular NPs that check germination of seed of a cucumber. Because seed coats have pores that exhibit selective permeability, the interaction between solid or particulate constituents and the plant may be limited until the radicles emerge and come into direct contact with the growth medium. However, intracellular spaces (<10 mm) in the seed coat parenchyma may be filled with aqueous media facilitating the transport of soluble nutrients as well as small particles to the embryo (Van Dongen et al. 2003). Phytotoxicity of nano-ZnO to seed germination indicating that, the elemental composition as well as the seed coat type may play a significant role in developmental phytotoxicity. Seed coat plays a very important role in protecting the embryo from harmful external factors as seed coats can have selective permeability.

2.2. Effect of nanoparticles suspensions on seedling growth

The effects of NPs suspensions at 1000 mg/L on seedling growth varied with the types of NPs and plant species, as shown in Figure 2 to 5. For example, root elongation of wheat (Figure 2) in nano-ZnO and C_{60} NPs suspension was reduced by 35% and 27% respectively as compared to control, whereas nano-Fe_2O_3 had no significant effect. The shoot elongation of wheat was significantly inhibited by all the three NPs suspensions and reduction was maximum in C_{60} (31%) followed by nano-ZnO (30%) and nano-Fe_2O_3 (17%) with respect to control. The seedling growth of rice (Figure 3) was not retarded by NPs, but shoot tip yellowing was observed. Root length of green gram (Figure 4) showed more negative response to nano-Fe_2O_3 and nano-ZnO NPs, but this effect did not occur in the case of C_{60} NPs. The root length of cucumber seedlings exposed to different NPs suspensions were found, 3.4 cm (26% of control), 7.2 cm (75% of control), and 7.3 cm (76% of control) under, nano-ZnO, nano-Fe_2O_3 and fullerene NPs, respectively (Figure. 5). However, reduction intensity of shoot growth was recorded 25%, 24% and 18% than control in nano-ZnO, nano-Fe_2O_3, and C_{60} NPs respectively.

A significant negative influence on root elongation was observed at tested concentrations of nano-ZnO in all tested plant species except rice. Radicles, after penetrating the seed coats, could contact the NPs directly. Since roots are the first target tissue to confront with excess concentrations of pollutants, toxic symptoms seem to appear more in roots rather than in shoots. In rice most probably radicals are stronger than the other tested plant which led to somewhat less negative impact of NPs on their growth. This indicates the role of genetic diversity for differential responses to environmental factors, such as exposure to NPs.

2.3. Dose–response relationship

To further understanding of the phytotoxicity of engineered NPs the IC_{50} was determined for the seedling growth of experimental plant species. Nano-ZnO and nano-Fe_2O_3 promoted root and shoot elongation of wheat at low concentration (up to 50 & 20 mg/L respectively), then a drastic decrease occurred up to 100 mg/L NPs concentration, and thereafter the growth was restricted (Figure. 6A, B). Fullerene NPs didn't significant effects root and shoot growth of wheat and rice (Figure 6C and 7C). Root and shoot length of germinating seed of rice was responding in a very less sensitive way to almost all the NPs treatments (Figure 7). Nano-ZnO reduced root and shoot elongation of green gram (Figure 8A) up to 100 mg/L and for cucumber (Figure 9A) reduction was limited

only up to 50 mg/L of NPs suspension. Then, as the concentration of NPs increased root and shoot growth of green gram and cucumber were restricted. The root and shoot length of green gram and cucumber increased slightly up to 20 mg/L and 50 mg/L of nano-Fe_2O_3 concentration respectively. Thereafter, elongation of root and shoot of green gram and cucumber shortened under the treatment of nano-Fe_2O_3 at the highest concentration (above 50 mg/L). Weak inhibition of fullerene NPs of root and shoot elongation of green gram and cucumber were observed for all the concentration tested. Root elongation of wheat, rice and cucumber were higher than respective shoot elongation under all the concentration of the testing NPs suspension. Root and shoot elongation of cucumber affected differently under nano-Fe_2O_3 suspension, initially up to 50mg/L of NPs enhances root elongation and further increase in the concentration of NPs suspension drastically decreased their influence on root growth, but this trend was not observed with the shoot elongation.

The dose response curve of our study reveals IC_{50} value of plant species under studied NPs varies between the range of 50 to 100 mg/L of NPs suspension. Paschke et al. (2006) also reveals that the IC_{50} of nano-Zn and nano-ZnO on the three test plant species (less than 50 mg/L) was lower than that of Zn^{+2} on some forb species (65 to 156 mg/L) indicating their remarkable phytotoxicity. Mechanism of nanotoxicity remains unknown; though it has been attributed to two different actions (1) a chemical toxicity based on the chemical composition, e.g., the release of (toxic) ions; and (2) stress or stimuli caused by the surface, size and/or shape of the particles. Brunner et al. (2006) also showed that the solubility of oxide NPs greatly affected the cell culture response.

Few toxicity tests have been made with fullerene NPs and terrestrial species, and to our knowledge, none with plants especially wheat, rice and other tested species. Our results indicated a certain impact of aqueous suspensions of C_{60} NPs at 100 mg/L for wheat and at 200 mg/L for other crops, but higher concentration did not consistently lead to further reduction in seedling growth. There are some studies performed by some researchers that report no associated toxicity or beneficial and protective effects of C_{60} NPs (Gharbi et al. 2005; Mori et al. 2006). Within the scientific community, opinions are mixed regarding the toxicity and safety of C_{60} NPs. This suggests that, there is a need for further research study for determining the critical threshold value of C_{60} NPs of various crops as most of the report are related to animal and human being.

The root length of tested plant species showed a detrimental effect under the all NPs treatment (except C_{60} NPs in cucumber) at the higher concentration (100 mg/L), but the root diameter was not changed in a uniform manner. Diameter of rice shoot was swollen approximately two times as thick as compared to the control. The root diameter of green gram and cucumber was swollen approximately two to three times as thick as the control, along with shortness and twist. The typical root symptoms, characterized by formation of brownish lesions and irregular curving, were also observed on green gram, cucumber and wheat.

Table 1. Characteristics of different nanoparticles used in this study

Properties	**Zinc Oxide (ZnO)**	**Hematite (Fe_2O_3)**	**Fullerene (C_{60})**
Appearance	Milky White	Red to Brown	Black
Assay	97%	96%	98%
Molecular Weight (g/mol)	81.39	159.69	720.64
Particle Size (TEM)	≤ 50.0 nm	≤ 50.0 nm	-----
Surface area (BET) (m^2/g)	10.8	50-245	-----

Table 2. Initial soil properties of the experimental soil

pH	EC	CEC	OC (%)	Available Nitrogen	Available Phosphorus	Available Potassium	Zinc	Iron
				(kg/ha)				
8.39	0.38	10.1	0.51	435	41.2	346	5.1	9.7

pH (1:2.5), EC (1:2.5), electrical conductivity (dS m^{-1});

CEC, cation exchange capacity [cmol(p+) kg^{-1}]

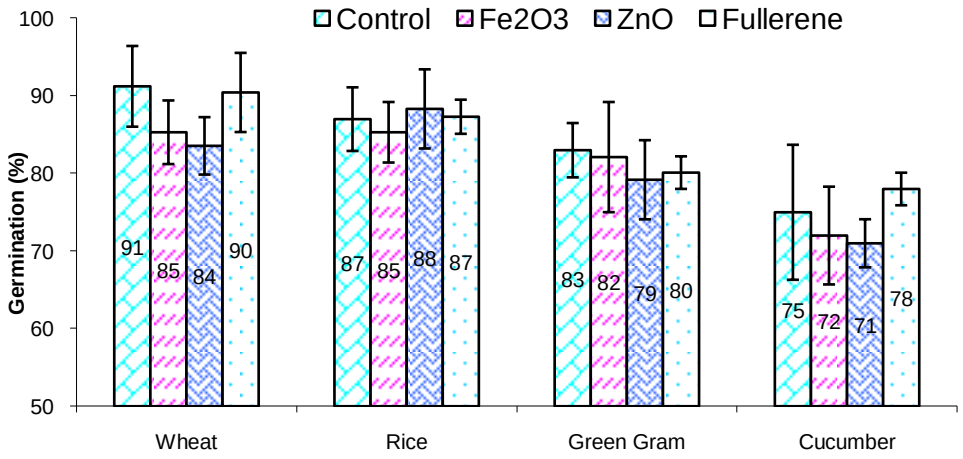

Figure 1 Effect of different nanoparticles on seed germination (%) of wheat, rice, green gram and cucumber. Error bars indicate the standard error of mean (±SEM) (n=10).

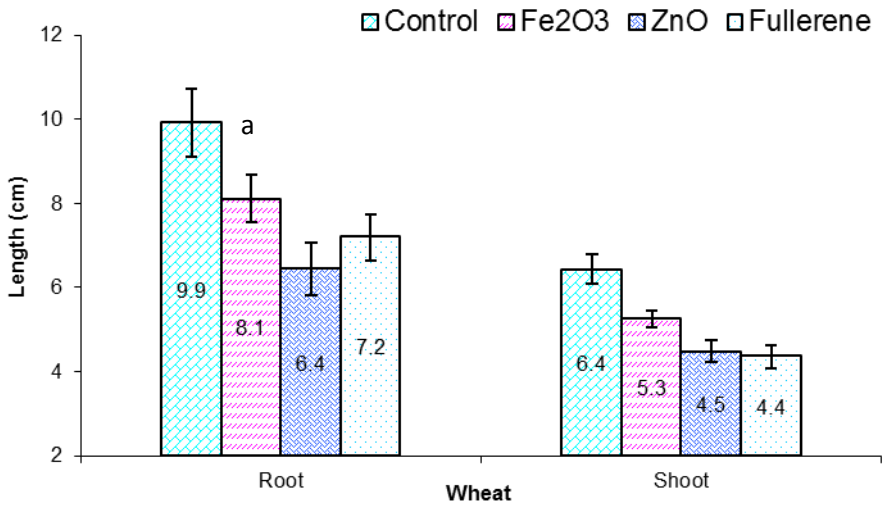

Figure 2 Effect of different nanoparticles on root and shoot elongation of wheat. Error bars indicate ±SEM. Bars within the same bar cluster labelled by the same letter do not differ at P < 0.05.

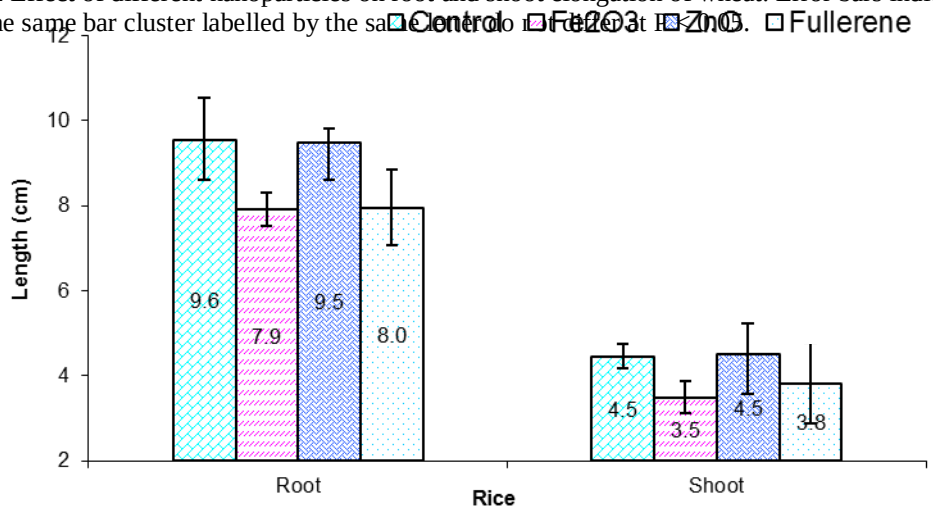

Figure 3 Effect of different nanoparticles on root and shoot elongation of rice. Error bars indicate ±SEM. Bars within the same bar cluster labelled by the same letter do not differ at P < 0.05.

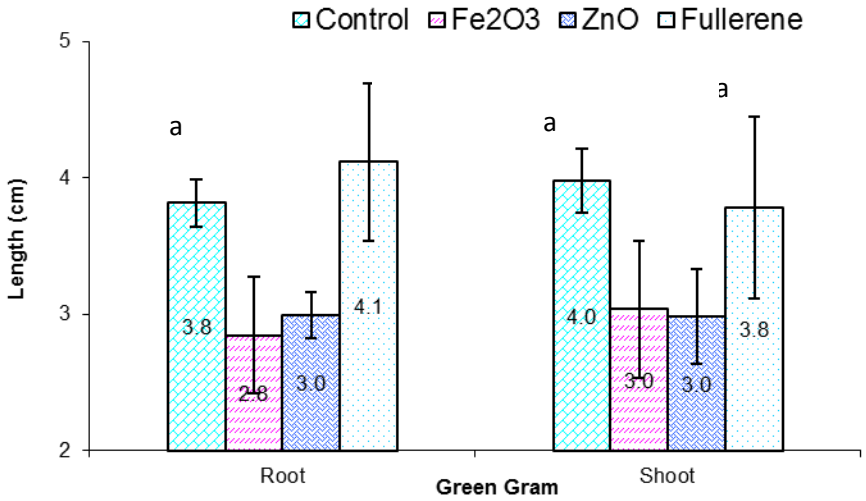

Figure 4 Effect of different nanoparticles on root and shoot elongation of green gram. Error bars indicate ±SEM. Bars within the same bar cluster labeled by the same letter do not differ at P < 0.05.

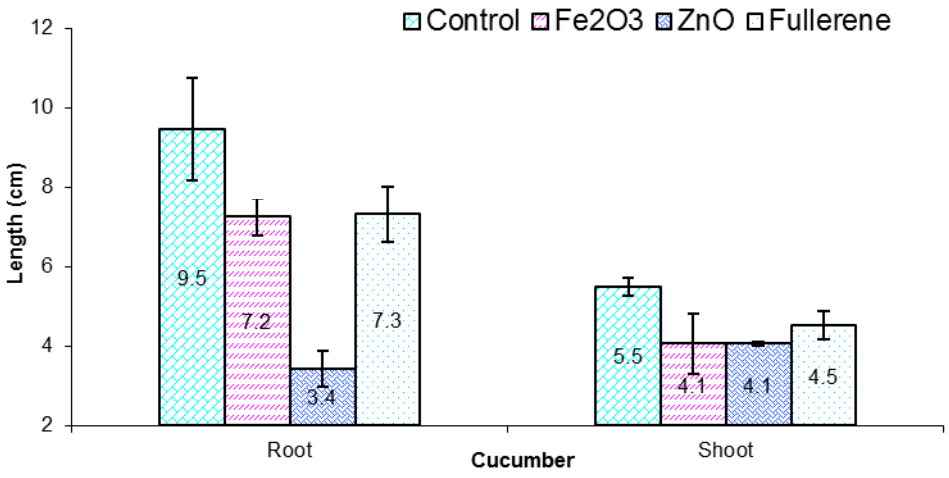

Figure 5 Effect of different nanoparticles on root and shoot elongation of cucumber. Error bars indicate ±SEM. Bars within the same bar cluster labeled by the same letter do not differ at $P < 0.05$.

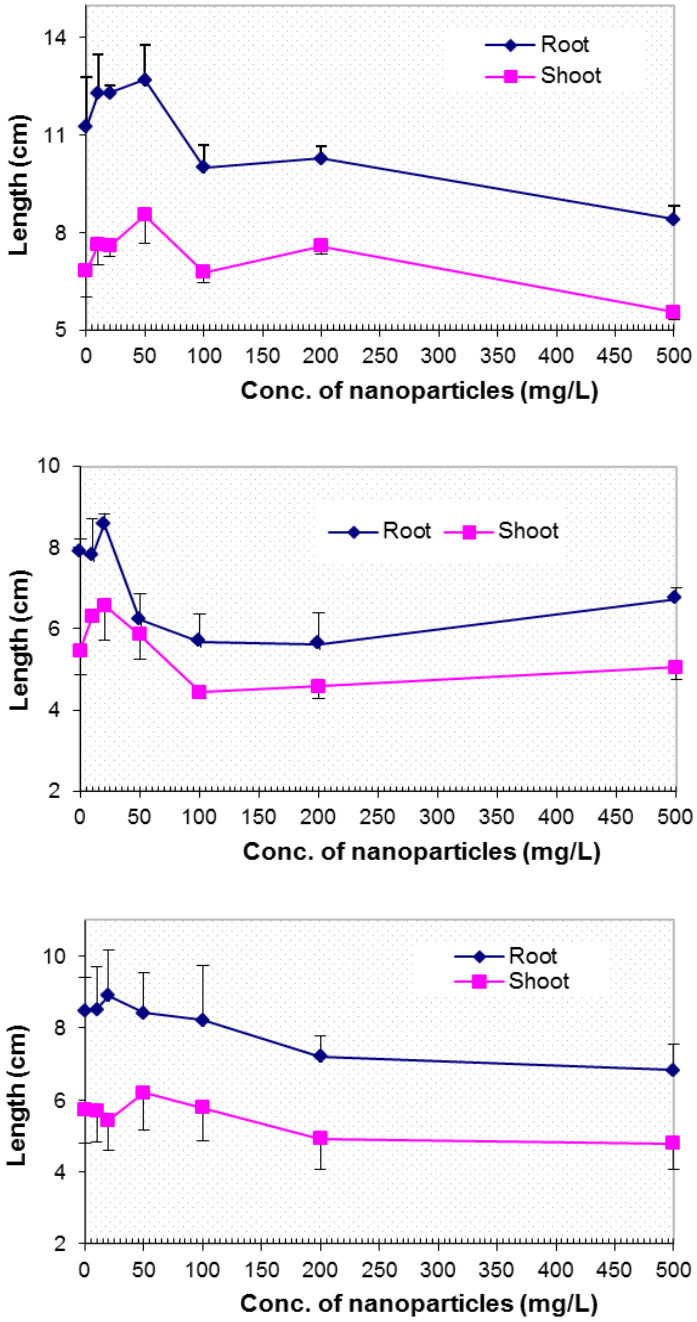

Figure 6 Dose-response curves of different nanoparticles on root and shoot growth of wheat (A) ZnO NPs, (B) Fe_2O_3 NPs and (C) Fullerene NPs. Error bars indicate ±SEM.

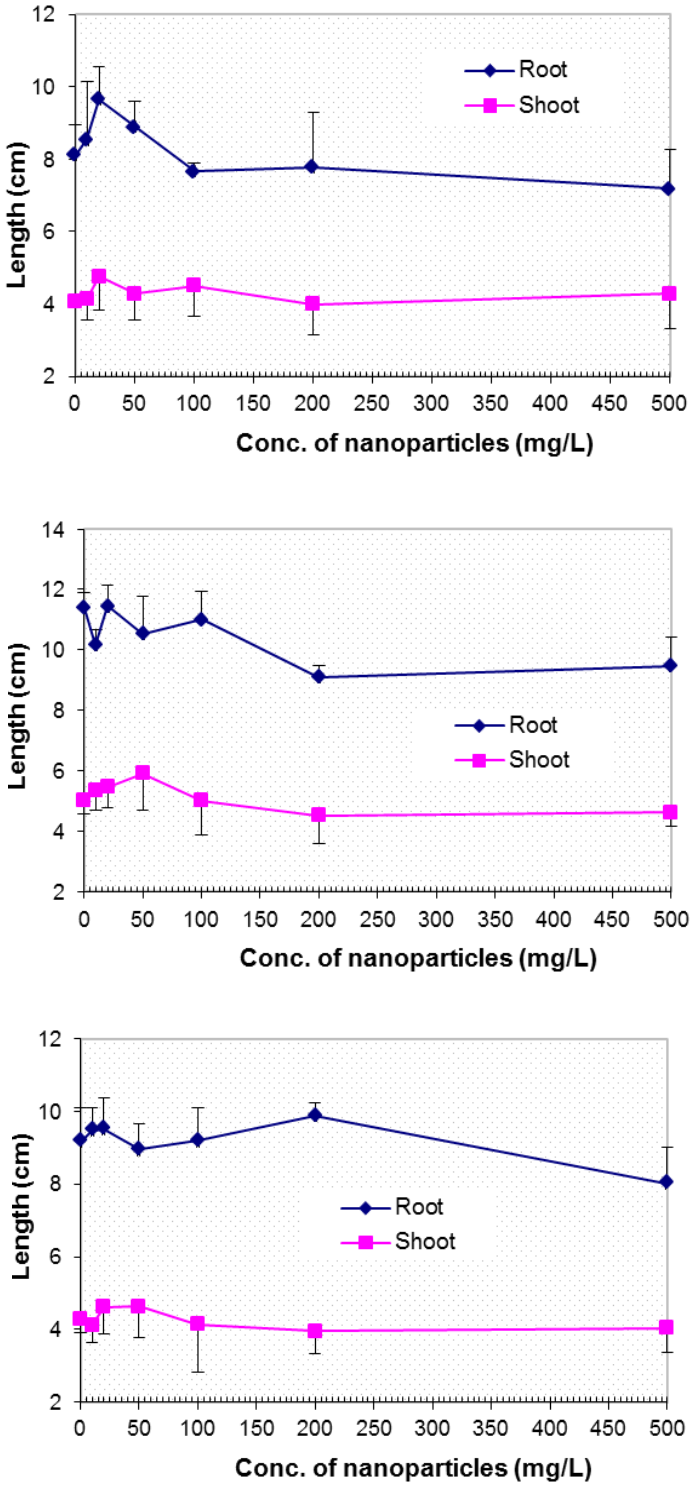

Figure 7 Dose-response curves of different nanoparticles on root and shoot growth of rice (A) ZnO NPs, (B) Fe_2O_3 NPs and (C) Fullerene NPs. Error bars indicate ±SE

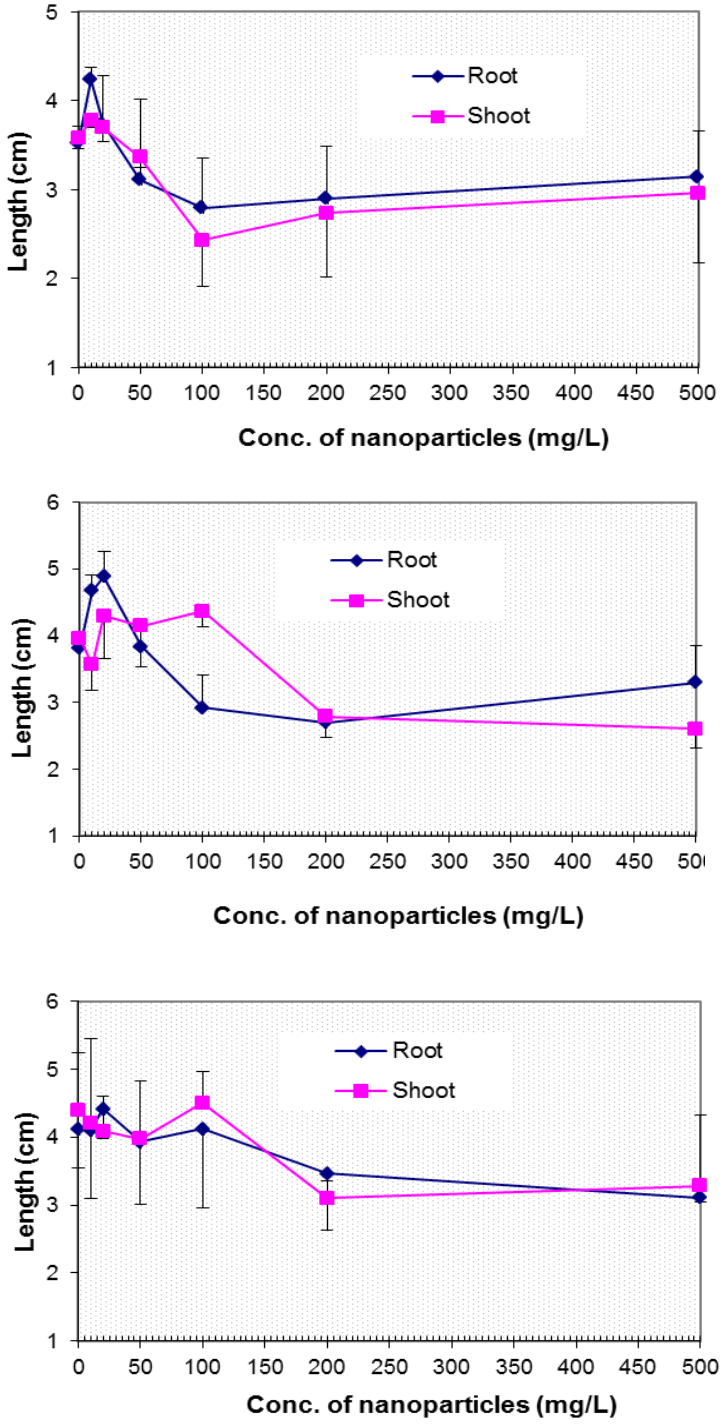

Figure 8 Dose-response curves of different nanoparticles on root and shoot growth of green gram (A) ZnO NPs, (B) Fe_2O_3 NPs and (C) Fullerene NPs. Error bars indicate ±SEM.

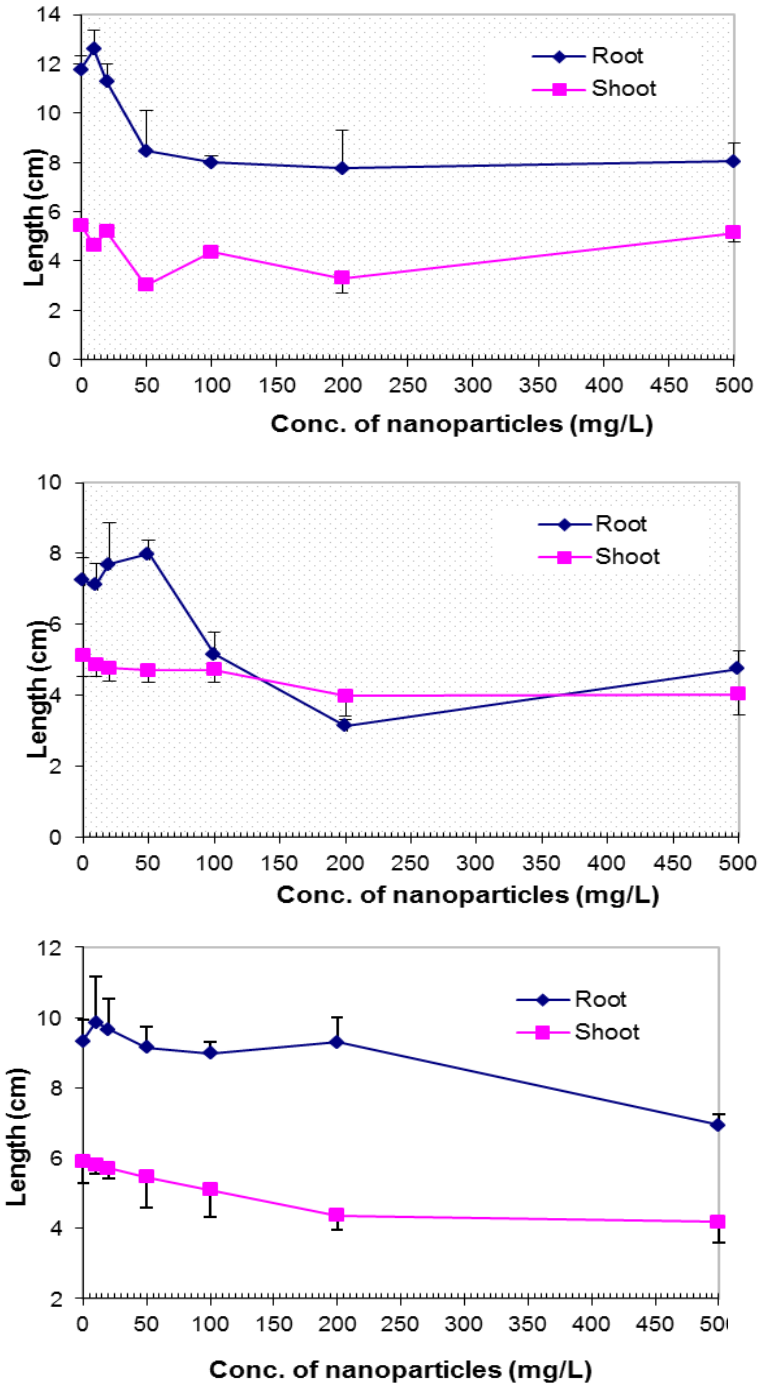

Figure 9 Dose-response curves of different nanoparticles on root and shoot growth of cucumber (A) ZnO NPs, (B) Fe_2O_3 NPs and (C) Fullerene NPs. Error bars indicate ±SEM

3. CONCLUSIONS

Nano-ZnO caused significant inhibition on seed germination (cucumber) as well as shoot and root growth wheat, green gram and cucumber but not significantly inhibit germination of rice. Nano-Fe_2O_3 and fullerene NPs did not affect germination of seed. Among all crops, cucumber found to be most vulnerable and rice least affected by the phototoxicity effects of all nanoparticles. The toxicity of NPs is species specific and also size dependent. Fullerene nanoparticles toxicity to seedling growth occurs at higher concentration (200 mg/L) and that of the nano-ZnO and nano-Fe_2O_3 toxicity appears at lower concentration (100 mg/L).

Overall, the study demonstrates the adverse effects of metal oxide and carbon based nanomaterials on plants, which underscores the necessity for taking remedial measure in the disposal of wastes and sludge containing these nanoparticles and calls for further research for assessing the potential impacts of manufactured nanoparticles on agricultural and environmental systems.

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