IMPACT OF ZINC OXIDE NANO PARTICLES ON SEED GERMINATION AND ROOT GROWTH

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ABSTRACT In this study, the phytotoxicity of metal oxide nano particle (NP) of zinc oxide (nano ZnO) and their counter parts (bulk ZnO) was assessed on seed germination and root growth of eight agriculturally worldwide used food crop plants (Rice, Soybean, Wheat, Cucumber, Sunflower, Lettuce, Maize and Tomato): The seed germination percentage and root length at different concentrations (0, 2, 10, 20, 50, 200 and1000 mg/L) of the nano ZnO and bulk ZnO was studied. The results showed that the inhibition on root length and root growth was observed, but there are no effects on the seed germination of all crops. The metal oxides (nano ZnO and bulk ZnO) Inhibition on root growth varied greatly among the varieties of plants studied. Suspensions of 1000 mg/L nano ZnO practically terminated root elongation of all the tested plant species. Fifty percent root growth inhibitory concentrations (IC50) of nano ZnO was estimated to near 50 mg/L for lettuce, Cucumber, Sunflower, Soybean, and Tomato and near 100 mg/L for Maize, Wheat, and Rice. The inhibition occurred for the duration of the seed incubation process rather than soaking process. From the above study reveals that the ZnO nano particles show toxic effects to the plants than their counter parts. So, some care needs to be taken for the disposal of different products containing nano particles into the environment and ecosystem.

KEYWORDS : Metal Oxide Nanoparticles, Phytotoxicity, Root growth, Inhibition, Seed germination

Introduction

The demand for nanotechnology-based products has been increasing in recent years, particularly in areas directly related to humans. Nano particles a size below 100 nm fall in the transition zone between individual molecules and the corresponding bulk materials, which generate both positive and negative biological effects in the living cells[1,2].

The effect of low concentration of alumina nano particles was found on the corn, cucumber carrot, cabbage, and soybean. They concluded that alumina nano particles had shown the phytotoxic effect on all the plants which were they tested. Similarly, [3] also found that the exposure to higher concentrations of aluminum, alumina, multi-walled carbon nanotube, and zinc and zinc oxide nano particles on root development and seed germination has a phytotoxic effect on the tested species of different. On the other side, the effect of the phytotoxicity of functionalized and non functionalized single-walled carbon tubes on the root growth and development of cabbage, carrot, cucumber, onion, lettuce, and tomato were investigated [4].The quantity of research has been increasing now a days on the biological effects of nano particles on higher plants [5].The use of nano particles in the growth of plants and for the control of plant diseases is a recent practice [6,7]. Among various metals, zinc (Zn) plays the vital role in biochemical, physiological and anatomical responses but below to threshold level. Zinc oxide (ZnO) NPs were widely used in paints, coating materials, medical and personal care products, and many more. ZnO NPs were also used as UV protector and absorber material. Besides their potential use, it has increased environmental and health risks due to their interaction with biological and chemical materials[8]. There are limited studies reported on phytotoxicity of ZnO, the presence of ZnO NPs in surrounding environment affects the plant architecture, physiology, and biochemistry. The toxicity was considered due to internalization of NPs, accumulation in root tissue and root surface, dissolution of zinc ions from NPs along with other physiochemical properties [9]. Some plant species, i.e., rape, corn, lettuce, radish, ryegrass, cucumber [3, 10], garden cress, and broad bean [11], and wheat [12] are sensitive towards ZnO NPs. Increased use in industrial products has led their release in the environment, i.e., from cosmetic products, sunscreens and others [13]. The research conducted on rye grass established that the accumulation and adsorption of the ZnO nano particles on to the surfaces of roots [14]. The cross-sectional transverse electron microscopy images of rye grass root clearly showed the presence of nano particles in the apoplast, cytoplasm, and nuclei of the endodermal cells and the vascular system. Later, [15] observed the uptake and accumulation of ZnO NPs on soybean seedlings with the concentration of 500 mg/L. They were reported that high concentrations of nano particles increased the agglomerate formations which may restrict the route of nano particles through the cell pore and resulted in to the reduce uptake and accumulation of nano particles inside the plant tissues.[16] Observed that the elongation and growth of rice roots were negatively affected by ZnO and found that ZnO NPs led to detrimental root growth termination of test plants. Furthermore, the exposures of soybean plants to ZnO NPs led to a decrease in above ground and root mass and altered the soil bacterial communities

The purpose to conduct this research is to determine the impact of ZnO nanoparticles on seed germination and root length on eight types of crop seeds and plants such as Rice (*Oryza Sativa L*), Soybean (*Glycine max*), Wheat (*Triticumaestivum*), Cucumber (*Cucumis sativa*), Sunflower (*Helianthus annus*), Lettuce (*LettuciaEdibelia*), Maize (*Zeamays*) and Tomato (*Solanumlycopersicum*).

Materials and methods

Nanoparticles

Nano- ZnO of purity 99.9% was purchased from Sigma-Aldrich USA, The size of the nanoparticles was determined by SEM, TEM Analysis. The Average size of the Nano ZnO was 50-70 diameters (nm). Bulk ZnO of purity 99% was purchased from RFCL, Ltd.

Seeds

Seeds of eight plant Species: Rice (*Oryzasativa L*), Soybean (*Glycine max*), Wheat (*Triticumaestivum*), Cucumber (*Cucumis sativa*), Sunflower (*Helianthus annus*), Lettuce (*LettuciaEdibelia*), Maize (*Zeamays*) and Tomato (*Solanumlycopersicum*) were purchased from Sriram Seeds, Kancheepuram, Lettuce Seeds were obtained from the USA.Three of the plant species (lettuce, maize, and Cucumber) are among the ten recommended species by USEPA[17] for the determination of ecological effects of pesticides and toxic substances, and other five plant species (Rice, Soybean, Wheat, Sunflower and Tomato) were world wise used food crops. The average germination rates of all plant seeds were greater than 90% as confirmed by a preliminary study. Before use, the seeds were kept in a dry place in the dark at room temperature.

Preparation of Test Solution

Chelated bulk $ZnSO_4$ was used as a reference Zn source. Because bulk ZnO will not dissolve in water and plants cannot absorb it, farmers are widely using chelated $ZnSO_4$. The materials (nano ZnO and bulk ZnO) were suspended directly in distilled water and dispersed by ultrasonic vibration (100 W and 40 KHz) for about 45 min. To avoid the aggregation of the particles in the suspension small magnetic bars were placed.

Germination

Seeds were immersed in a 10% sodium hypochlorite solution for 10 min to ensure surface sterility [17], then, they were soaked in DI-water for two hours, rinsed four times with distilled water; and then soaked in a series of prepared nanoZnO and bulk ZnO Suspensions for approximately 2 hours. In a Petri dish, a small piece of filter paper was placed, and 5 mL of test solution was added. To this filter paper, the larger distance between each seed and ten seeds per dish were transferred [2]. These dishes were incubated at room temperature with proper sealing. After ten days in the dark under room temperature, more than 90% of the control seed were germinated and developed roots that were at least 10 mm long. Then, the germination was stopped, the seedling root length was measured, and the seed germination rate was calculated.

Statistical analysis

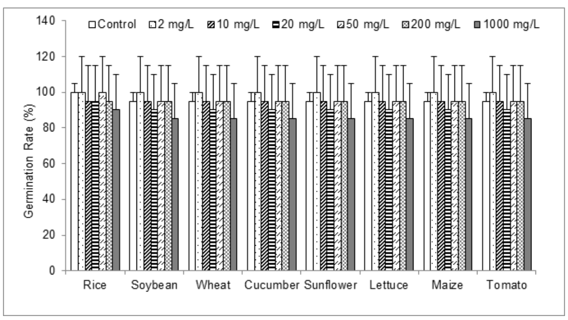
Each concentration (treatment) was conducted with three replicates, and the results were presented as mean ± SD (standard deviation). The data obtained from the various treatments were statistically analyzed using the t-test at a significance Level of 0.05. Each of the experimental values was compared to its corresponding control.

Results

Effect of nano zinc oxide and bulk zinc oxide suspension on seed germination and root growth

Different concentrations of nano ZnO and bulk ZnO were applied. The dosages were 0, 2, 10, 20, 50, 200 and 1000 mg/L to investigate phytotoxicity of the ZnO nano particles in this study. Effects of nano particles at applied dosages on seed germination and root growth are shown in Fig.1 and Fig. 2, respectively. Seed germinations rates were not affected by the nano ZnO and bulk ZnO in all types of seeds. The influence of metal oxides suspensions at 1000 mg/L on root growth varied with types of metal oxides and plant species. Bulk ZnO suspension did not show any significant difference from the deionized water (Control). Phytotoxicity of nano ZnO was evident. These suspensions significantly inhibited root growth and practically terminated root development of the all eight plant species. Nano ZnO was showing the greatest impact on all eight types of crops; hence nano ZnO was selected for further experiments.

(a)



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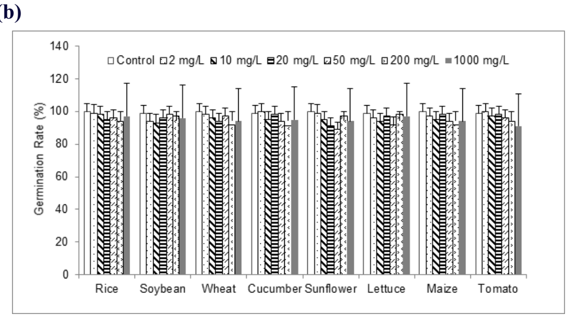
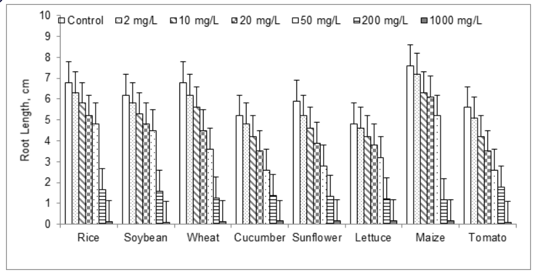


Fig.1. (a) The germination rates of seeds soaked and incubated with different concentrations of Nano ZnO, (b) Bulk ZnO particle suspensions. The values were given as mean ± SD (standard Deviation) of triplicate samples with 10 seeds each.

(a)



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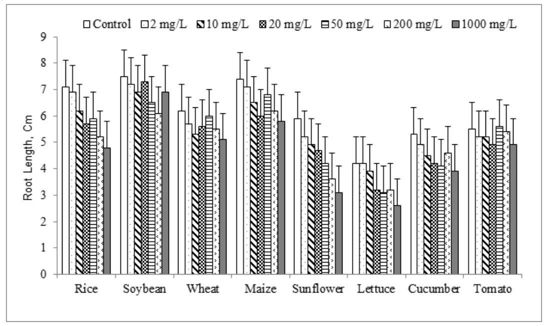

Fig. 2. (a) Root length of the seeds soaked and incubated with different concentrations of nano ZnO, (b) Bulk ZnO particle suspensions. The values were given as mean ± SD (Standard Deviation) of triplicate samples with 10 seeds each. The ZnO nano particles at 1000 mg /L almost terminated the root growth.

Effect of seed soaking on root growth in nano ZnO suspension

To examine which process (seed soaking or incubation after the soaking) primarily retarded the root growth, three treatments were used on five plant species (Wheat, Soybean, Cucumber, Lettuce and Tomato) : (1) both seed soaking and incubation were performed in nano particle suspensions; (2) seeds were soaked in nano particle suspensions for 2 h, and were then transferred into Petri dishes with 5 ml DI-water for incubation after being rinsed three times with DI-water; and (3) seeds were incubated in Petri dishes with 5 ml nano particle suspensions after being soaked in DI-water for 2 h. As described above, Fig.3; the root growth was almost halted by seed soaking and incubation in the suspensions of nano ZnO (the first treatment) also, root growth of all the plants were nearly terminated under the third treatment (soaking in water, then incubation in suspension), while roots could grow relatively well under the second treatment (soaking in suspension, then incubation in water).Though the root development of the eight plants was significantly inhibited by nano ZnO. To further clarify the phytotoxicity of nano ZnO, the following experiments were carried out using the nano particle suspensions.

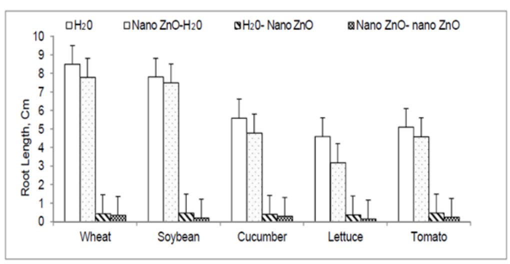

Fig.3. Effect of seed soaking under different treatments on root growths. Treatments of H_2O, nano ZnO- nano ZnO are those with seed soaking and incubation in DI- water, 1000 mg/L suspensions of nano ZnO respectively; nano ZnO-H_2O are the treatments where seed incubation was done in DI- water after being soaked in 1000 mg/L suspensions of nano ZnO respectively. The values were given as mean ± SD (Standard Deviation) of triplicate samples with 10 seeds each

Dose-response relationship of nanoZnO

Dose-response curves of nano ZnO suspensions on root growth of all eight plants are displayed in Fig. 4. No significant root growth inhibition was observed under low concentrations (less than 20 mg/L for lettuce, Cucumber, Sunflower, Soybean, Tomato) and 50 mg/L for Maize, Wheat, and Rice). Root growth was restricted with increasing concentrations and was almost terminated at 1000 mg/L. Fifty percent root growth inhibitory concentrations (IC_{50}) of nanoZnO was estimated to be near 50 mg/L for lettuce, Cucumber, Sunflower, Soybean, Tomato and near 100 mg/L for Maize, Wheat, Rice.

(a)

(b)

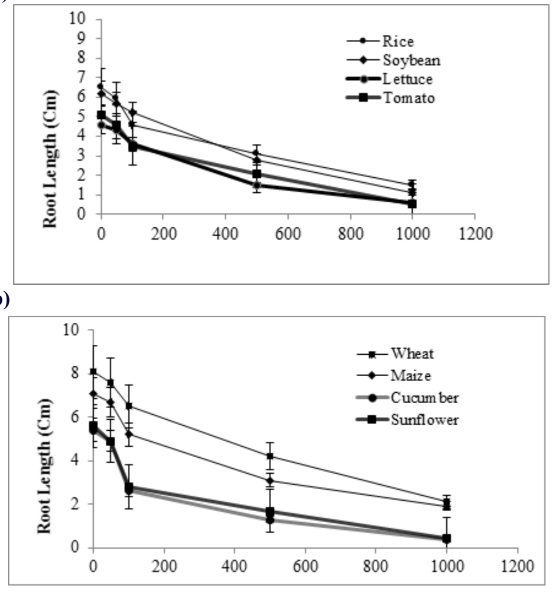

Fig. 4; Dose response curves of nano ZnO on root growth of eight different plant species. The values were given as mean ± SD (Standard Deviation) of replicate samples with 10 seeds each.

Discussion

Nano particles generally showed the negative effect on the tested target organisms or cells despite different test methods used. Phytotoxicity in higher plants should be investigated to develop a comprehensive toxicity profile for nanoparticles [18]. Seed root elongation and germination is a rapid and widely used acute phytotoxicity test with several advantages: sensitivity, simplicity, and low cost and suitability for unstable chemicals or samples [19,20]. In this study, seeds showing the emergence of radicle or cotyledon coming out of the seed coat were recorded as being germinated. Seed coat plays a very vital role in protecting the embryo from harmful external factors. Seed coats can have selective permeability [21]. Pollutants, though having an inhibitory effect on root growth, may not affect germination if they cannot pass through seed coats. This experiment was explaining that seed germination was not greatly altered by metal oxides (Fig. 1 and Fig.2). The selective permeability by seed coat is supported by the observation that root growth of all seeds was incubated in DI-water after being soaked in the nano ZnO suspension was significantly inhibited. (Fig.3). Significant retardation of root was also observed when the seed soaking process was done in nano ZnO suspensions. In our study, shoots could grow to a certain degree even though root elongation was halted in the presence of nano ZnO in water. The dose-response curves of nano ZnO are 'T" shaped [22]. There was a threshold below which no obvious symptom appeared. However, root length decreased with increasing dose after the threshold (Fig. 4). The IC_{50} of nano ZnO on the eight test plant species (less than 50 mg/L) was lower than that of bulk ZnO on some forb species (65-156 mg/L) [23], indicating their remarkable phytotoxicity. Only nano ZnO particles were observed to have significant inhibition on seed germination and root growth of the eight plant species. The inhibition occurred for the duration of the seed incubation process rather than soaking stage was estimated to be with IC_{50} of about 50 mg/L for lettuce, Cucumber, Sunflower, Soybean and Tomato, and near 100 mg/L for Maize, Wheat, and Rice. However, compared with the bulk ZnO, the ZnO NPs showed greater toxicity to root elongation of all the plants at the higher concentrations. The high surface/volume ratios of Nano Particles with their smaller size make them highly reactive and with better catalytic properties, which increases their toxic potential compared with their bulk counterparts.

Conclusion

The seed germination of Cucumber, Rice, Soybean, Sunflower, Wheat, Lettuce, Maize and Tomato was not affected by all the two metal oxides (nano ZnO and bulk ZnO), but the root elongation was significantly inhibited by nano ZnO at 1000 mg/ L. No negative effects were observed in the bulk ZnO solutions on root growth, suggesting the phytotoxicity was mainly due to the nano particles. Also, ZnO NPs showed greater toxicity to root elongation of all crop plants than bulk ZnO. Thus, it could be concluded that the phytotoxicity of nano ZnO particles was not directly from their dissolution in bulk aqueous solutions though dissolution right on the root surface cannot be ruled out at this point, which needs further investigation. Further studies are supposed to conduct to phytotoxicity mechanism, for example, the size distribution of nano particles in solution and its results on phytotoxicity possible uptake and translocation of nano particles by plants and physical and chemical properties of nano particles in the rhizo sphere and on root surfaces. These results will help to further understand the phytotoxicityof various nano materials and are significant regarding the use and disposal of engineered nano particles.

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