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Nano-Sized Zinc Oxide's Effect on Plant Sprouting and Development

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Abstract - Zinc oxide (nano ZnO) and its counterpart, bulk ZnO, were tested for their effects on the germination and root growth of eight crops widely utilised in agriculture around the world as sources of human nourishment (Rice, Soybean, Wheat, Cucumber, Sunflower, Lettuce, Maize, and Tomato). Nano ZnO and bulk ZnO were tested at concentrations of 0, 2, 10, 20, 50, 200, and 1000 mg/L on the germination rate and root growth of seeds. Root length and root growth were shown to be inhibited, while seed germination was unaffected across the board. Different plant species showed widely varying levels of root development inhibition when exposed to metal oxides (nano ZnO and bulk ZnO). All of the plant species evaluated had their root development nearly halted when exposed to nano ZnO suspensions of 1000 mg/L. An IC50 of micro ZnO was determined to be close to 50 mg/L for lettuce, cucumber, sunflower, soybean, and tomato, and close to 100 mg/L for maize, wheat, and rice. This suppression lasted for the entire time the seeds were incubated, not just during the soaking phase. ZnO nanoparticles are more harmful to plants than their counterparts, as shown in the above study. Thus, it is important to exercise caution while releasing various items containing micro particles into the environment and ecosystem.

Keywords - Metal Oxide Nanoparticles, Phytotoxicity, Root length, Inhibition, Seed germination

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INTRODUCTION

Products based on nanotechnology have seen rising demand in recent years, especially in healthcare and related fields. Particles with a size of 100 nm or less are in the "nano" transition zone, where they produce both beneficial and deleterious effects on live cells (1,2).

Corn, cucumber, carrot, cabbage, and soybeans were all affected by exposure to even trace amounts of alumina micro particles. The researchers concluded that all of the plants evaluated were negatively affected by alumina nanoparticles. Similarly, (3) discovered that larger concentrations of aluminium, alumina, multi-walled carbon nanotube, zinc, and zinc oxide nano particles have a phytotoxic effect on the studied species, inhibiting root growth and preventing seed germination. In contrast, researchers looked into how both functionalized and non-functionalized single-walled carbon tubes affected the root growth and development of cabbage, carrot, cucumber, onion, lettuce, and tomato (4). More and more studies are being conducted today on the biological effects of nano particles on higher plants (5). It is a relatively new trend to employ nanoparticles in plant cultivation and disease prevention (6,7). Zinc (Zn) is one of many metals that plays a critical role in subthreshold biochemical, physiological, and anatomical responses. Many different kinds of products once contained nanoparticles of zinc oxide (ZnO). These included paints, coating materials, medicines, cosmetics, and more. Additionally, ZnO NPs were utilised as a UV absorber and protective. Interactions with biological and chemical materials raise the potential for negative effects on the environment and human health (8). The presence of ZnO NPs in the environment has an effect on the structure, physiology, and biochemistry of plants, although there have been few research reporting on ZnO's toxicity to plants. Internalization of NPs, accumulation in root tissue and root surface, dissolution of zinc ions from NPs, and other physiochemical parameters were taken into account to determine toxicity (9). Rape, corn, lettuce, radish, ryegrass, cucumber (3, 10), garden cress, broad bean (11), wheat (12), and rapeseed (13). There has been an increase in their environmental emission due to their widespread use in industrial products, such as cosmetics, sunscreen, and other personal care items (13). The rye grass studies demonstrated that ZnO nanoparticles accumulate and adsorb on root surfaces (14). Nanoparticles were observed in the apoplast, cytoplasm, and nuclei of endodermal cells, as well as the vascular system, in transverse electron microscopy pictures of rye grass roots. Subsequently, (15) saw 500 mg/L ZnO NPs being taken up and accumulated by soybean seedlings. It has been reported that increased agglomeration forms in response to high concentrations of nano particles may have impeded the uptake and accumulation of nano particles within plant tissues. ZnO NPs were discovered to have a deleterious effect on test plants' root development, as both root elongation and growth were severely impacted by the presence of ZnO. Soybean plants that were subjected to ZnO NPs saw a decline in both leaf area and root mass, and the soil bacterial communities where they were grown were disrupted.

The goal of this study is to evaluate the effects of ZnO nanoparticles on the germination rate and root growth of eight different crop seeds and plants, including rice (Oryza Sativa L), soybean (Glycine max), wheat (Triticumaestivum), cucumber (Cucumis sativa), sunflower (Helianthus annus), lettuce (Lettucia Edibelia), maize (Zeamays), and tomato (Solanumlycopersicum).

SUBSTANCES AND TECHNIQUES

Nanoparticles

Sigma-Aldrich USA supplied us with nano- ZnO that was pure to 99.9%, and SEM and TEM analysis established the nanoparticle size. Nano ZnO had an average size of 50–70 nm (nm). RFCL, Ltd. supplied us with 99% pure ZnO in bulk.

Seeds

At Sriram Seeds in Kancheepuram, India, we bought seeds for eight different plant species: rice (Oryzasativa L), soybeans (Glycine max), wheat (Triticumaestivum), cucumbers (Cucumis sativus), sunflowers (Helianthus annus), lettuce (Lettucia Edibelia), maize (Zeamays), and tomatoes (Solanumly copersicum). The seeds came from the United States. Five of the plant species (Rice, Soybean, Wheat, Sunflower, and Tomato) were widely grown for human use, and three of the species (lettuce, maize, and cucumber) are among the ten plants recommended by USEPA(17) for determining the ecological effects of pesticides and harmful compounds. An initial investigation confirmed that the average germination rate for all plant seeds was higher than 90%. The seeds were stored dry, in the dark, and at room temperature until they were ready to be used.

Cleaning Up the Test Solution

As a standard for Zn, we employed chelated bulk $ZnSO_4$. Chelated $ZnSO_4$ is commonly used by farmers in place of bulk ZnO due to the latter's inability to be dissolved in water and absorbed by plants. After being suspended in distilled water for about 45 minutes, the materials (nano ZnO and bulk ZnO) were dispersed using ultrasonic vibration (100 W and 40 KHz). Small magnetic bars were positioned to prevent the particles in suspension from clumping together.

Germination

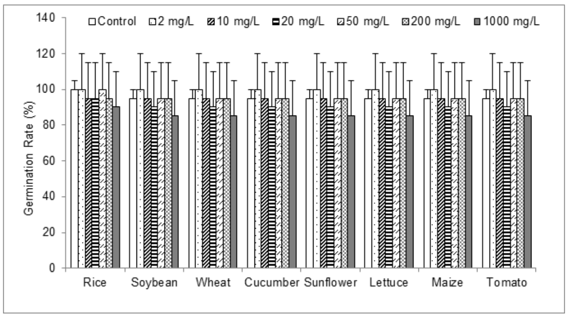
To assure surface sterility (17), seeds were submerged in a 10% sodium hypochlorite solution for 10 minutes, then soaked in DI-water for two hours, washed four times with distilled water, and then soaked in a series of produced nanoZnO and bulk ZnO Suspensions for around 2 hours. A small piece of lter paper was placed in a Petri dish, and 5 mL of test solution was added. The greater spacing between seeds and the 10 seeds per dish were moved to this lter paper (2). These dishes were sealed properly and incubated at room temperature. More than 90% of the control seed germinated after 10 days in the dark at ambient temperature, and their roots were as least 10 millimetres long. The rate of germination was then determined by pausing the process and measuring the root length of the emerging seedlings.

Statistical analysis

Three independent experiments were performed for each treatment (concentration), and the results were expressed as the mean standard deviation (standard deviation). The t-test, with a significance level of 0.05, was used to examine the results of the various therapies. The experimental values were contrasted with their matching baseline values.

RESULTS

Inhibition of seed germination and root development caused by a nano- and bulk-zinc-oxide suspension Nano ZnO and bulk ZnO were both used, although at varying quantities. To examine the phytotoxicity of ZnO nano particles, the following concentrations were used: 0, 2, 10, 20, 50, 200, and 1000 mg/L. Images 1 and 2 depict the detrimental effects of nanoparticles on seed germination and root development at the concentrations used in the experiments. All seed types showed similar germination rates when treated with nano ZnO or bulk ZnO. Metal oxide suspensions at 1000 mg/L had varying effects on root growth among metal oxide types and plant species. There was no statistically significant difference between the ZnO suspension and the distilled water (Control). It was clear that micro ZnO had a harmful effect on plants. All eight plant species were significantly stunted in their root growth and development by these suspensions. As nano ZnO was having the most noticeable effect on all eight crop varieties, it was chosen for additional testing.



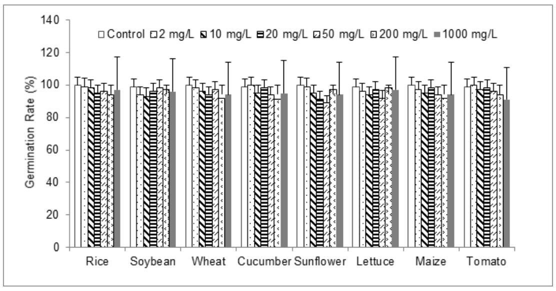
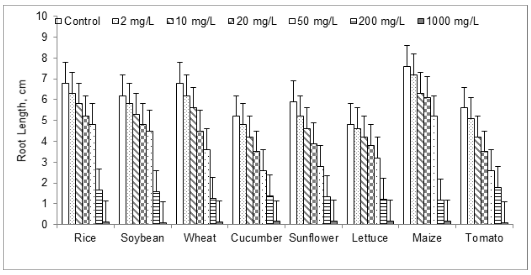


Figure 1: (a) The germination rates of seeds soaked and incubated with different concentrations of Nano ZnO, (b) Bulk ZnO particle suspensions. The values were given as mean ± SD (standard Deviation) of triplicate samples with 10 seeds each.



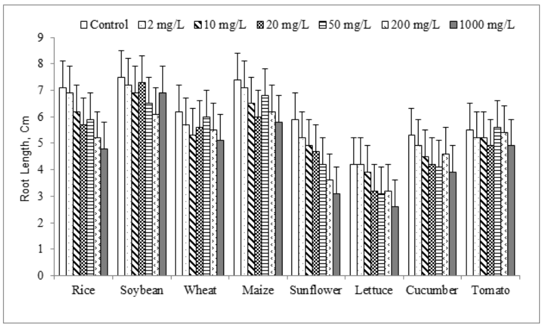


Figure 2: (a) Root length of the seeds soaked and incubated with different concentrations of nano ZnO, (b) Bulk ZnO particle suspensions. The values were given as mean ± SD (Standard Deviation) of triplicate samples with 10 seeds each. The ZnO nano particles at 1000 mg /L almost terminated the root growth

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 onve 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 □ rst 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.

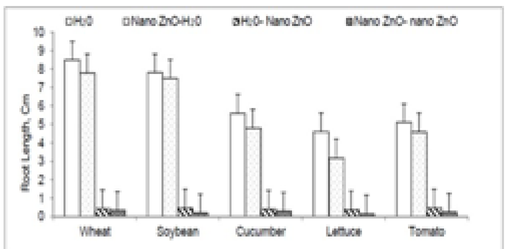
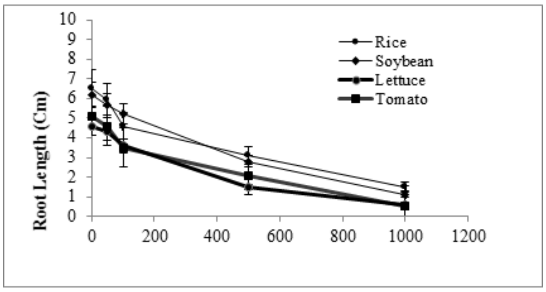


Figure 3: Root development after seed soaking with various treatments

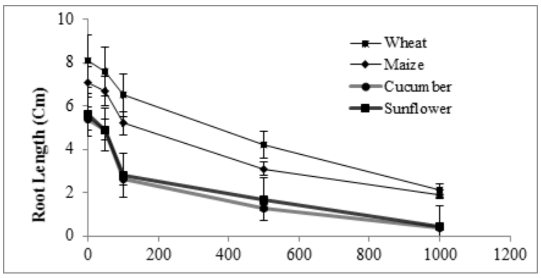
Seeds were soaked and incubated in DI-water or 1000 mg/L nano ZnO suspensions for the H2O and nano ZnO-nano ZnO treatments, whereas for the nano ZnO-H2O treatments, the seeds were soaked in nano ZnO suspensions and then incubated in DI-water. The values were presented as the mean SD (Standard Deviation) of replicated 10-seed samples.

The dose-response relationship of micro ZnO

Fig. 4 shows the dose-response curves for nano ZnO suspensions on the root development of all eight plants. In low doses (less than 20 mg/L for lettuce, cucumber, sunflower, soybean, tomato, and 50 mg/L for maize, wheat, and rice), no significant reduction of root growth was detected. The concentration that had the most significant effect on root development was 1000 mg/L, when growth slowed nearly to a halt. It was calculated that the IC50 of nanoZnO for inhibiting root growth was close to 50 mg/L for lettuce, cucumber, sunflower, soybean, and tomato, and close to 100 mg/L for maize, wheat, and rice.



(a)



(b)

Figure 4: The effects of nano ZnO on root development in eight plant species as a function of dose. Root length was significantly different between nanoscale and bulk ZnO, with values reported as means standard deviations (SDs).

DISCUSSION

In spite of variations in testing procedures, nanoparticles were consistently found to have a detrimental effect on the organisms or cells under investigation. To create a thorough toxicity profile for nanoparticles, research into their effects on higher plant life is required (18). Acute phytotoxicity testing using seed root elongation and germination is popular due to its speed, ease of use, low cost, and adaptability to volatile substances or samples (19, 20). Germinated seeds were identified in this study as having either a radicle or cotyledon emerging from the seed coat. The seed coat's primary function is to shield the developing plant from environmental hazards. Selective permeability in seed coats is a real thing (21). Root growth can be stifled by pollutants, but if they can't get through the seed coat, they might not hinder germination. The purpose of this study was to demonstrate experimentally that metal oxides do not significantly affect the germination of seeds (Fig. 1 and Fig.2). Supporting the selective permeability by seed coat is the finding that all seeds immersed in the nano ZnO suspension and then incubated in DI-water showed significantly reduced root development. (Fig.3). When seeds were soaked in nano ZnO suspensions, significant root retardation was also observed. Our research showed that even while micro ZnO in water prevented root elongation, shoot growth was still possible to some extent. Nano ZnO has 'T' shaped dose-response curves (22). Below a certain point, no noticeable symptoms would show up. However, doses beyond the threshold resulted in shorter root systems (Fig. 4).

CONCLUSION

Neither nano ZnO nor bulk ZnO influenced the germination of seeds for cucumber, rice, soybean, sunflower, wheat, lettuce, maize, or tomato, however nano ZnO at 1000 mg/ L significantly decreased root elongation. It appears that the nano particles are responsible for the phytotoxicity, as no deleterious effects on root growth were seen in the bulk ZnO solutions. Root elongation in all crop plants was more harmful to ZnO NPs than it was to bulk ZnO. It follows that nano ZnO particle phytotoxicity was not caused by their solubility in bulk water solutions; however, dissolving directly on the root surface cannot be ruled out at this time and requires additional study. Uptake and transport of nanoparticles by plants, as well as physical and chemical characteristics of nanoparticles in the rhizosphere and on root surfaces, are all potential mechanisms of phytotoxicity that need more research. These findings have important implications for the safe use and disposal of designed nanoparticles, and will aid in our understanding of the phytotoxicity of diverse nano materials.

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