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EFFECT OF NANO-ZnO ON GROWTH OF MUNG BEAN (*Vigna radiata*) AND CHICKPEA (*Cicer arietinum*) SEEDLINGS USING PLANT AGAR METHOD

Pramod Mahajan, S.K. Dhoke, A.S. Khanna and J.C. Tarafdar*

Department of Metallurgical Engineering and Material Science, Indian Institute of Technology, Mumbai, Maharastra, 400 076 India;
**Central Arid Zone Research Institute, Jodhpur, Rajasthan, 342 003, India*
*email: *jctarafdar@yahoo.in; tarafdar@cazri.res.in*

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ABSTRACT

The present study demonstrates the effect of nano-ZnO particles on growth of mung bean (*Vigna radiata*) and chickpea (*Cicer arietinum*) seedlings. The study was conducted in plant agar media to prevent precipitation of water-insoluble nanoparticles in test units. Various concentrations of nano-ZnO particles in suspension form were introduced to the agar media and its effect on root and shoot growth of seedlings examined. Analytical techniques like optical microscopy and scanning electron microscopy were used to study the architecture of root. Inductive coupled plasma (ICP) was used to determine the uptake of nano-ZnO particles by root. The best response of nano-ZnO on mung bean was observed at 20 ppm and on chickpea at 1 ppm, beyond these concentrations the seedling showed retardation in growth and development thereby suggesting the toxic effect of nano-ZnO particles.

Keywords: Biomass, chickpea, *Cicer arietinum,* mung bean, nano-ZnO, toxicity, *Vigna radiata*

INTRODUCTION

Nanotechnology is a versatile field and has found application in almost all the existing fields of science. The use of nano-particles in plant growth and for disease control is a recent practice (Zheng, 2000; Zheng *et al*., 2005; Park *et al*., 2006; Galbraith, 2007; Shah and Belozerova, 2009). However, whether the effects of nano-particles are beneficial or harmful to plant growth is yet an unresolved issue (Yang and Watts, 2005; Lee *et al*., 2008). The mixture of nano-SiO_2 and nano-TiO_2 reportedly increases nitrate reductase activity in soybean seeds thereby increases seed germination and growth (Lu *et al*., 2002). A proper concentration of nano-TiO_2 was found to improve spinach growth by promoting photosynthesis and nitrogen metabolism (Hong *et al*., 2005a,b; Yang *et al*., 2006). Nano-functionalized carbon nano-tubes enhanced root elongation in onion and cucumber (Cañas *et al*., 2008). Nano-ZnO has widely been used in industry for several decades. However, no study has been made on potential use of nano-Zn in agriculture. Zinc is one of the plant micro-nutrient and its phytotoxic effects are known (Lin and Xing, 2007). The present study deals with the effect of nano-ZnO particle suspension, as micro-nutrient, on the growth of mung bean and chickpea seedlings at various concentrations.

MATERIALS AND METHODS

Preparation of nano-ZnO particle suspension

Nano-ZnO particles (size 20 nm) were synthesized in the Department of Metallurgical Engineering and Material Science, IIT, Mumbai, India using Zn(II) acetate and potassium hydroxide as the starting material, and methanol as solvent. The material was confirmed after characterization by X-ray powder diffraction (XRD), UV-visible spectroscopy and dynamic light scattering analysis (DLS). Particle size was characterized using transmission electron microscopy (TEM, Philips CM200 electron microscope)**.** Nano-particles were directly suspended in deionized water and dispersed using mechanical stirrer and ultrasonicator (100W, 40 khz) for 30 min. Each nano-particle suspensions of different concentration were prepared separately by weighing particles and dispersing them in deionized water.

Plant agar method

The experiment on the effect of nano-ZnO particle suspension on seedling growth was conducted in petri-dish test units (87 × 18 mm). Based on our preliminary observations for root and shoot growth in mung bean and chickpea, the test units showing highest and least seedling growth for corresponding nano-ZnO concentrations with respect to control were selected for the present study. Specific concentration of nano-ZnO particles in suspension (0, 10, 20, 50, 100, 500, 1000 and 2000 ppm for mung bean seeds and 0, 1, 2, 5, 10, 20, 50, 100, 500, 1000 and 2000 ppm for chickpea seeds) was added individually to the test units. The test units were prepared by pouring 20 ml of 2.5% agar solution into a pertidish and hardened immediately in a freezer to avoid any possible precipitation of nano-particles. Then 10 ml of 1% agar solution was distributed evenly over 2.5% agar medium. To each test unit 10 plant seedlings of 72 h old, raised from surface sterilized mung bean and chickpea seeds on wet cotton under controlled incubation conditions (25±1°C in dark for 24 h), were just placed above the surface of agar medium. The test units were placed in incubator (25±1°C) in dark. For each concentration three replicates were prepared and test units exposed for 60 h. The agar media separated from agar media and the growth response of test species, exposed to different range of nano-ZnO particle concentrations, measured with root image analyzer (Delta T Scan, GRLYB-0206). The concentration of nano-ZnO particles in root was determined by inductively coupled plasma mass spectrometer (model: Perkin Elmer ELAN DRC II).

Sample preparation for microscopy

The root sample sections were observed under light microscopy (Leica, DM500 B, Germany) for scanning electron microscopy. The nano-ZnO particle treated and control seedlings were separated into root and shoot. The roots were frozen in liquid nitrogen, and then fractured into smaller pieces with blunt knife. The pieces were freeze dried at overnight to -30°C under vacuum (15-25 torr) using freeze dry system (ALPHA1-2 LD plus, Germany). The morphology of roots was examined by scanning electron microscope and quantitative elemental analysis of nano-particles was carried out with electron diffraction spectrum (SEM/EDS, Model No. S3400, Hitachi).

Biomass assay and zinc concentration determination

The seedlings after 14 days growth were washed with deionized water, and roots and shoots separated for biomass determination. Initial fresh weight of roots and shoots was measured and samples dried in oven at 70°C for 24 h to determine their dry weights. Zn contents in roots were determined by ICPAES (Model Ultima, Jobin-Yvon Company, France) after HNO_3 digestion of dry roots. Student's t-test was used as statistical tool for comparison of experimental values with their corresponding controls.

RESULTS AND DISCUSSION

The average germination rate of mung bean and chickpea seeds was greater than 90% which reflects that the seeds were of good quality.

TEM study of nano-ZnO particles

The TEM micrograph image of ZnO particles revealed that the zinc oxide particles were monodispersed with a narrow size distribution and near spherical morphology (Fig. 1a). Analysis of particles in TEM

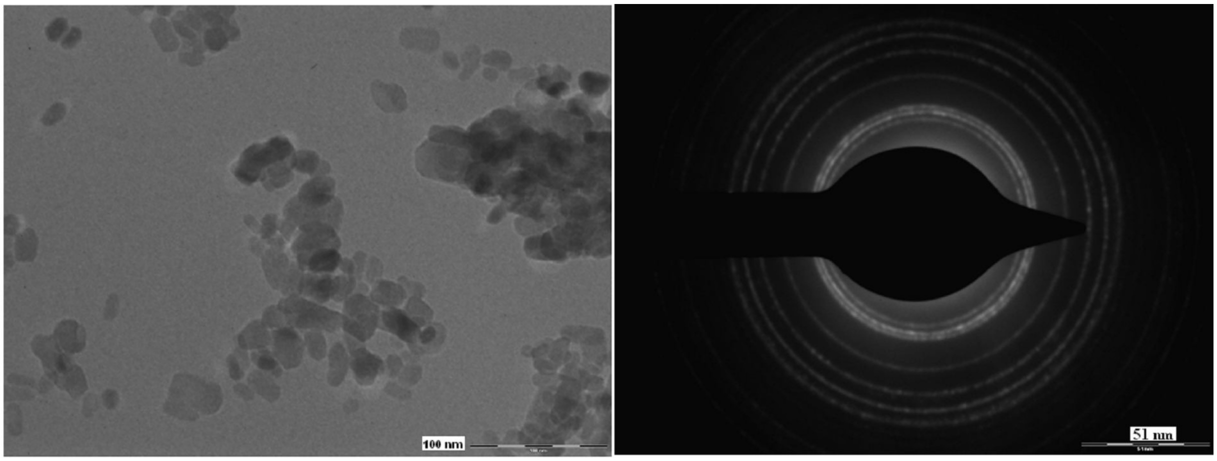


Fig. 1: TEM image (left) and selected area electron diffraction (SAED) pattern of nano-ZnO (right) in mung bean

monograph indicated hexagonal particles with the average size of 20 nm. The SAED pattern of ZnO particles showed rings composed of dots suggesting the crystalline nature of ZnO particles (Fig. 1b).

Effect of nano-ZnO particles suspension on root and shoot growth

The doze response curves of nano-ZnO on root and shoot of mung bean and chickpea seedlings revealed that root and shoot growth increased with the increase in nano-ZnO concentration (Fig. 2). However, after certain concentration level the growth of root and shoot declined. In mung bean seedlings the best growth response for root (42.0%, p value 0.0498) and shoot (97.9%, p value 0.0444) was observed at 20 ppm. At highest concentration (2000 ppm) retardation in root (93.3%, p value 1.736×10^{-14}) and shoot growth (14.8%, p value 2.46×10^{-11}) in comparison to control was observed in mung bean seedlings. Similarly, for chickpea seedlings, 1 ppm concentration exhibited significant increase in root (53.1%, p value 1.125×10^{-7}) and shoot (6.4%, p value 0.026) as compared to control. However, beyond this level root and shoot growth was retarded. At 2000 ppm significant reduction in root (74.2%, p value 1.32×10^{-10}) and shoot growth (22.5%, p value 0.020) was observed. The reduction in

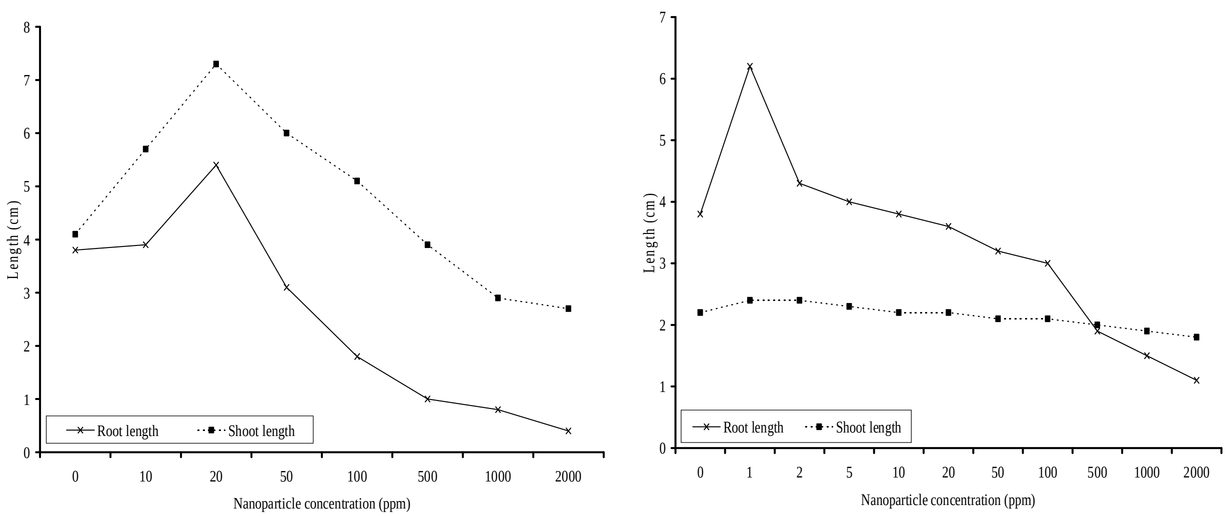


Fig. 2: Doze response curves of nano-ZnO on root and shoot of mung bean (left) and chickpea (right) seedlings after 14 days growth (The values given are mean ± SD of triplicate samples with 10 seeds each).

root and shoot growth at higher doses may be attributed to the toxic level of nano-particles. This demonstrates that mung bean and chickpea seedlings respond to added nano-particles in a limited range, beyond which decline in growth occurs owing to the toxic effect.

Effect of nano-ZnO particles suspension on biomass

The root and shoot biomass production in mung bean and chickpea seedlings were found in accordance with the root and shoot length for corresponding nano-ZnO treatment (Fig. 3a,b). For nano-ZnO at 20 ppm treatment mung bean seedlings showed 40.9% increase in root mass and 76.0% increase in shoot mass over control. The chickpea seedlings at 1 ppm treatment exhibited 37.1% increase in root mass and 26.6% increase in shoot mass in comparison to control. At highest concentration of 2000 ppm, 44.7% decrease in root mass and 31.9% in shoot mass in mung bean seedling was observed over control. While in chickpea seedlings 32.2% decrease in root mass and 52.8% decrease in shoot mass was observed. The increase in biomass at certain concentration suggests the optimum doze limit for the growth of mung bean and chickpea seedlings. However decrease in biomass beyond this concentration suggests the toxic effect of nano-ZnO particles.

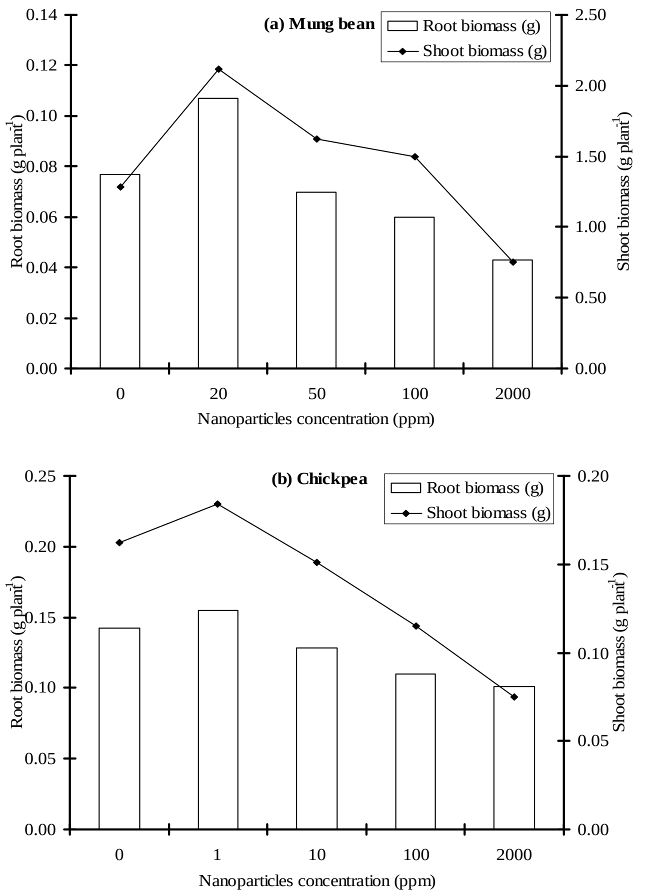


Fig. 3: Dry biomass of root and shoot of mung bean seedlings (left) and chickpea seedlings (right) after 14 days growth

Elemental determination

Table 1 shows Zn content in the roots of mung bean and gram seedlings at optimum and highest concentration. It was observed that the Zn content of roots in mung bean and chickpea increased significantly with the increase of nano-ZnO application. It indicated that the ZnO nano-particles were absorbed and translocated in seedlings resulting in increase in Zn content at corresponding nano-ZnO treatment.

Microscopic analysis

The examination of transverse sections of roots under light microscopy revealed that at 20 ppm concentration of nano-ZnO in mung bean (Fig. 4) and at 1 ppm in chickpea (Fig. 5) the roots developed very well with usual three tissue system (epidermis, cortex and vascular cylinder) as compared to their controls. This indicated that nano-ZnO particles had no adverse effect on root architecture. However, disruption of epidermis, cortex and vascular cylinder in both the seedling roots were noticed at higher concentrations which was more detrimental above 50 ppm in case of mung bean and above 20 ppm in case of chickpea. The cortical cells were highly vacuolated and collapsed; and vascular cylinder also shrank. The damage to the basic architecture of roots at higher concentrations may be a direct reason for growth inhibition in mung bean and chickpea seedlings.

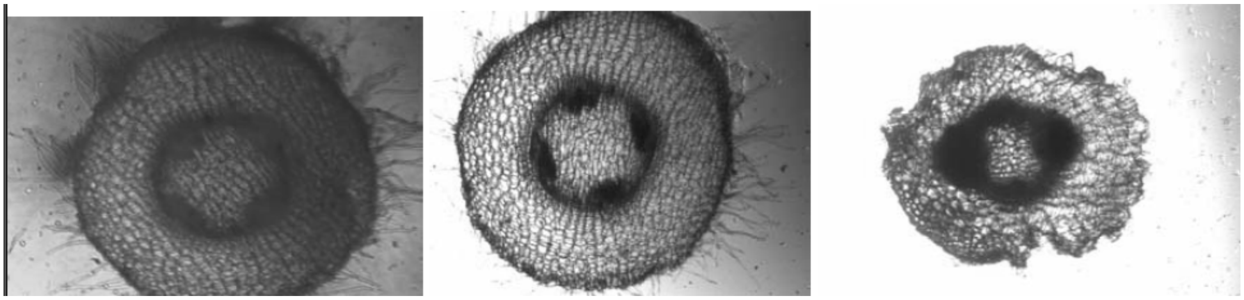


Fig. 4: Transverse section of mung bean roots; control (left), 20 ppm nano-ZnO treated (middle) and 2000 ppm nano-ZnO treated (right)

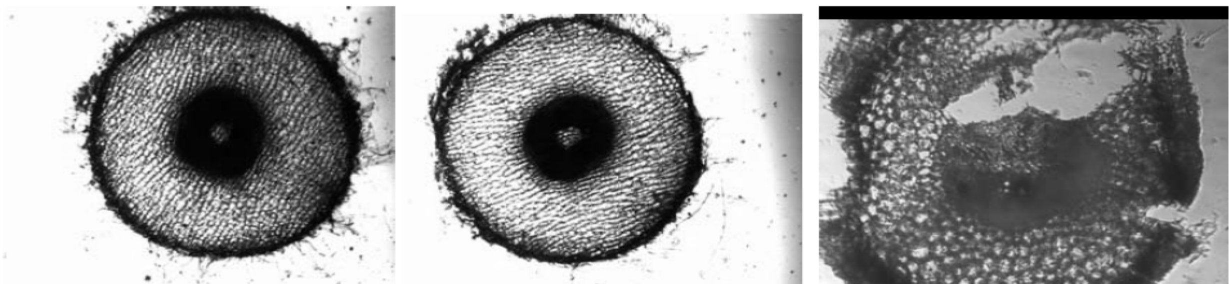
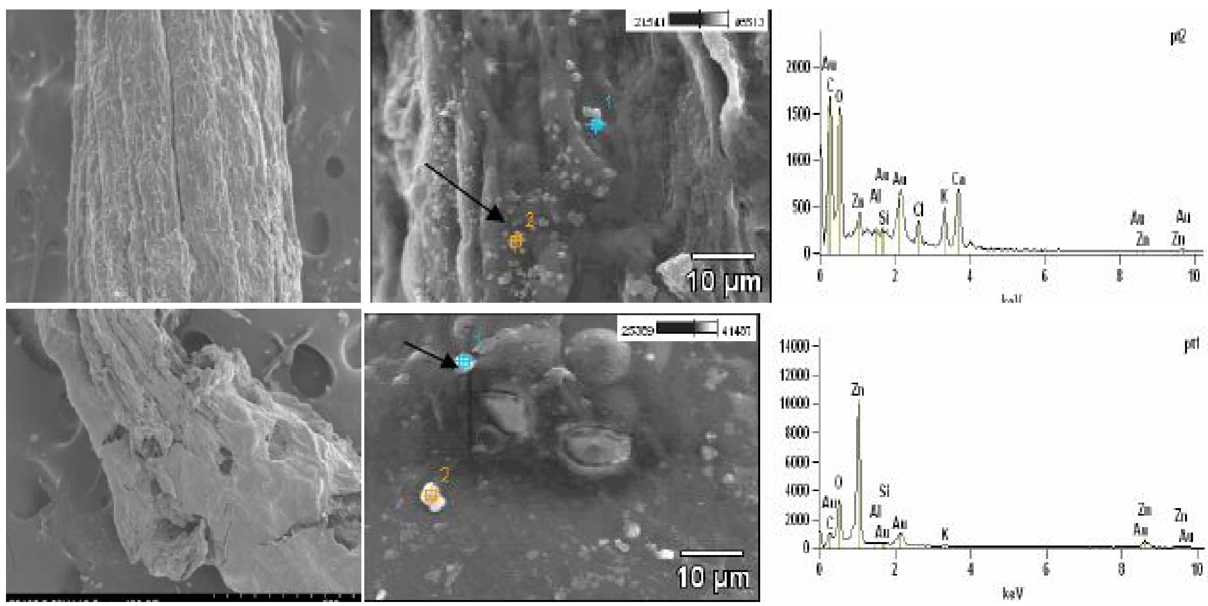


Fig. 5: Transverse section of chickpea seedling roots; control (left), 1 ppm nano-ZnO treated (middle), and 2000 ppm of nano-ZnO treated (right).



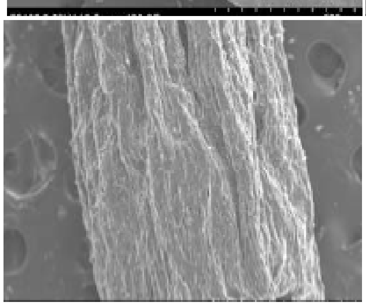
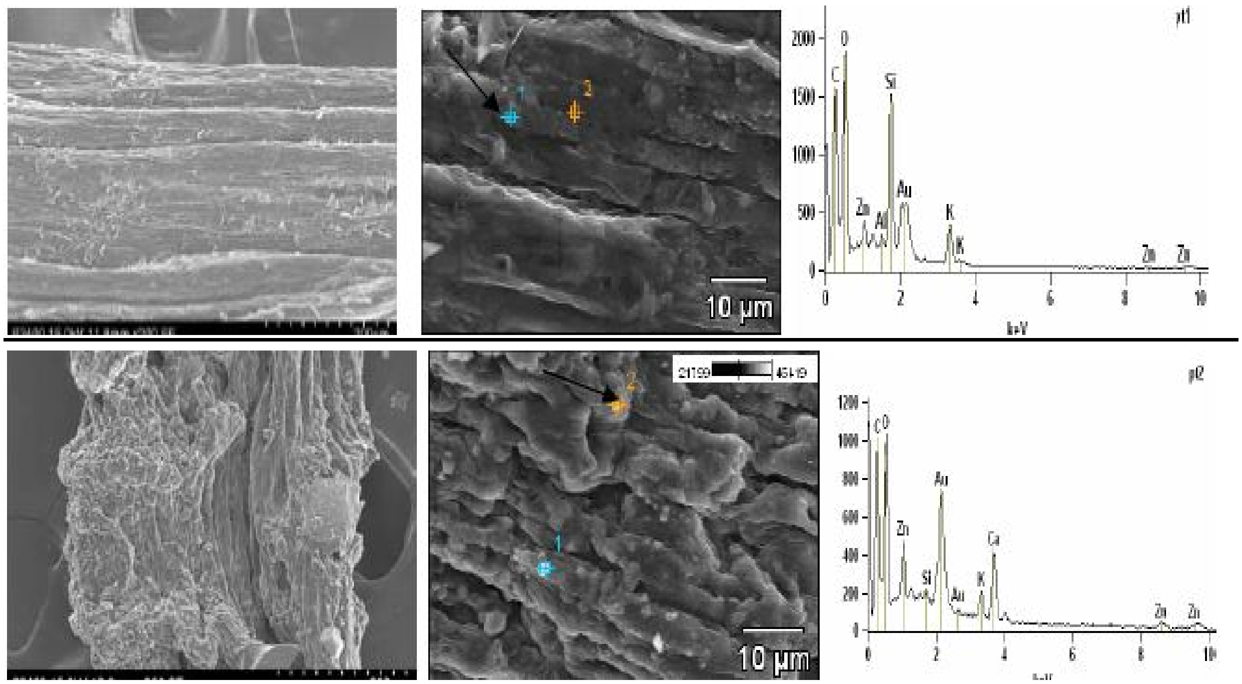


Fig. 6: Scanning electron micrographs of roots of mung bean; at 20 ppm nano-Zn (top row) and at 2000 ppm of nano-Zn treatment (middle row), and control (bottom, left side); The energy dispersive spectroscopy shows the Zn peaks obtained from points indicated by the arrows.



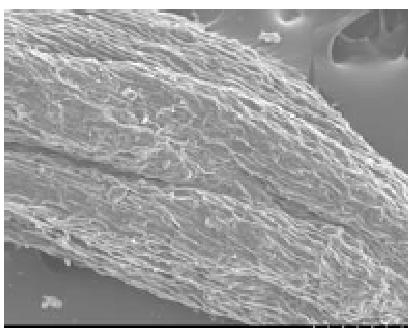


Fig. 7: Scanning electron micrographs of roots of chickpea: 1 ppm nano-Zn treated (top row) and 2000 ppm nano-Zn treatment (middle row) and untreated control (bottom, left side). The energy dispersive spectroscopy shows the Zn peaks obtained from points indicated by the arrows.

Table 1: Concentration of zinc element in mung bean and chickpea seedling by ICP-AES

Mung bean		Chickpea	
Concentration added (ppm)	Concentration by ICP-AES (mg L^{-1})	Concentration added (ppm)	Concentration by ICP-AES (mg L^{-1})
0	2.80	0	2.68
10	7.15	1	2.80
20	12.17	2	3.71
50	15.35	5	4.23
100	18.72	10	6.14
500	23.38	20	9.85
1000	28.28	50	13.13
2000	31.18	100	16.23
		500	18.96
		1000	20.17
		2000	22.11

Lin and Xing (2007) analyzed phytotoxicity of five types of multi-walled nano-particles at the level of seed germination and root growth in six higher plant species (*Rapbanus sativus, Brassica napus, Lolium multiflorum, Lactuca sativa, Zea mays* and *Cucumis sativus*). Seed germination remained unaffected, except for the inhibition of nanoscale zinc on *Lolium multiflorum* and nanoscale zinc oxide on *Zea mays*. Inhibition of root growth varied greatly among the nano-particles and plants and was partially correlated to nanoparticles concentration. Further, the inhibition occurred during seed incubation process rather than seed soaking stage. Lin and Xing (2008) analyzed the cell internalization and the upward translocation of ZnO nano-particles by *Lolium preenne*. In presence of ZnO nano-particles ryegrass biomass significantly reduced, root tip shrank and root epidermal and cortical highly vacuolated or collapsed cells. ZnO nano-particles greatly adhered onto the root surface, and individual nanoparticles were observed to be present in apoplast and protoplast of root endodermis and stele. Translocation factor of Zn from root to shoot remained very low under ZnO nano-particles treatments. However, the phytotoxicity of ZnO nano-particles was not directly correlated with their limited dissolution in bulk nutrient solution or rhizosphere.

The surface micrographs of seedling roots revealed that in mung bean at 20 ppm ZnO nano-particle concentration (Fig. 6) and in chickpea at 1 ppm concentration (Fig. 7) no structural alteration on root surfaces were observed in comparison to control root samples. However, structural alteration in root surface was seen in the seedlings treated with higher concentrations i.e. more than 50 ppm concentration for mung bean and 5 ppm for chickpea. The adsorption and aggregation of nano-ZnO particles were observed on root surface of treated seedling, which was located and identify on the root surface by SEM-EDEX analysis.

Conclusion: The effect of different concentrations of ZnO nanoparticles on the growth of mung bean and chickpea seedlings was maximum at 20 ppm in mung bean and at 1 ppm in chickpea seedlings beyond which the growth was retarded. The effective growth at optimum concentrations and inhibited growth beyond these may be attributed to the accumulation and uptake of nano-ZnO particle by the roots.

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