Original Article **Asian J Agri Biol, 2014, 2(4):216-223.**

INFLUENCE OF BIOENGINEERED ZINC NANOPARTICLES AND ZINC METAL ON *CICER ARIETINUM* SEEDLINGS GROWTH

K. V. Pavani*, V. Divya, I. Veena, M. Aditya, G. V. S Devakinandan

Department of Biotechnology, Gokaraju Rangaraju Institute of Engineering and Technology, Bachupally, Hyderabad 500090, Telangana, India

ABSTRACT

Metal nanoparticles are newly emerging alternate source of carriers to transmit and maintain the desired plant food sources available for plant growth and development. Their accumulation in agriculture lands is accompanied by both positive and negative effects on plants. Considering their impact on plants, it is necessary to evaluate them on seed germination and seedling growth. The main objective of this study was to investigate whether the treatment of *Cicer arietinum* seeds with relatively low concentrations of bioengineered ZnNPs and zinc metal would affect the seed germination and seedlings growth. Zinc nanoparticles were characterized by transmission electron microscopy (TEM). There were differences in germination (2%), root length (0.18cm), shoot length (0.17cm), fresh weight (0.52cm) and dry weight (0.03cm) between seeds grown in control and Zn NPs. There also existed significant differences in germination (2.67%), root length (4.94cm), shoot length (1.29cm), fresh weight (0.50cm) and dry weight (0.09cm) between seeds grown in control and $ZnSO_4$. *Cicer arietinum* seeds grown in zinc nanoparticles showed increase in germination, shoot length, root length, fresh weight and dry weight, whereas seeds grown in zinc metal decreased growth. Our findings suggest that bioengineered zinc nanoparticles showed positive effect on seedlings growth.

Keywords: Zinc nanoparticles, zinc *metal, Cicerarietinum,* positive effect, seedlings growth

INTRODUCTION

Nanotechnology has become a dynamically developing industry with increasing numbers of commercial products such as materials manufacturing, computer chips, medical diagnosis, energy and cosmetics (Ahsan et al., 2007). Due to rapid industrialization and intensive agriculture contamination of heavy metals in soil has been increased. Excessive level of heavy metals in the soil environment adversely affect the germination of seeds, plant growth and interfere with the activities of enzymes related to normal metabolic and developmental processes (Ali and Madhavan, 2012; Arora et al., 2012) . Engineered nanoparticles (ENPs) synthesized by Microorganisms in contaminated soils play a critical role in plant growth.

Manufactured nanomaterials can be accidentally or incidentally release into waste water streams and agriculture fields (Auffan et al., 2010). The impact of MNMs on different plant species can vary greatly, and there are reports of both positive and negative effects (Blaser et al 2013). Positive effects on germination of aged spinach seeds and on the growth of seedlings were obtained if the seeds were soaked in high-strength TiO2-nanoparticles-solution (Elena and Katarina, 2013). Increase of the electron transfer, oxygen evolution, and photophosphorylation was observed in the chloroplasts of spinach treated with anoanatase-TiO2 (John et al.., 2009). Carbon nanotubes (CNTs) enhanced root growth of onion (*Allium cepa*) and cucumber (*Cucumis sativa*) (Kuriakosa and Prasad, 2008). However, majority of the reports available in the literature indicate phytotoxicity of NPs (Laure et al., 2011). Application of MNPs such are silica, palladium, gold and copper nanoparticles significantly influenced the growth of lettuce plants (Lee et al. 2010). *Lecanicillium lecanii* based silver nanoparticles showed less effect on seedling emergence, shoot length, leaf surface area, chlorophyll content and phyllosphere microflora of economic important pulses such as **c**owpea (*Vigna unguiculata*), black gram (*Vigna mungo*), green gram (*Vigna radiate)* , sorghum (*Sorghum vulgare*) and horse gram *Macrotyloma uniflorum*) (Ling and watts, 2005). Scientific investigation on plant uptake and accumulation of ENPs in the environment

**Corresponding author:* e-mail: pavani_20042003@yahoo.co.in

is still in its rudimentary stage, few publications have been reported in the past few years in the area of ENP toxicology and uptake by plants. The use of engineered nanoparticles is one of the recent advances in the field of agriculture biotechnology.The objective of our study is to assess the effect of bioengineered Zinc nanoparticles synthesized by fungi and Zinc metal on the growth of *Cicer arietinum* seeds.

MATERIALS AND METHODS

1. Bioengineered nanoparticles

Aspergillus species (BankIt ID: 1526725) isolated from the soil samples collected from Hyderabad Metal Plating Industry, I.D.A, Balanagar, Hyderabad, A.P. were preserved at 4ºC in a refrigerator. A loop full of fungal culture was inoculated in a 250 ml of Erenlymer flask containing 100 ml of fungal media. The flask was incubated at 28^0 C, for 2 days on a rotary shaker at 160 rpm, and the cells were filtered using filter paper. The filtered solution was autoclaved and the size of the nanoparticles was determined by Transmission Electron Microscopy (TEM) (JEOL JEM 2010, Japan, operated at 120 kV).

2. Seed germination

Cicer arietinum seeds were immersed in a 0.1% mercuric chloride solution for 5 min for surface sterilization. After rinsing three times in sterile distilled water, they were soaked in the media containing zinc nanoparticles, deionised water (control), $ZnSO_4$ metal solution (5mM/L) for 48hours. A piece of filter paper (Whatman No. 42) was put into each Petri dish (90 mm × 15 mm), 4 ml of deionized water or nanoparticle media and ZnSo4 solution were added, and 25 seeds were then transferred onto each dish. Petri dishes were sealed with parafilm and placed in an incubator. The seeds were kept under a photoperiod of 12 hr, and 25/18 °C day/night temperature. The seedlings were harvested after 14 days and the germination percentage, root and shoot length, fresh weight and dry weight was measured for all replicates. The number of seeds germinated was counted and the germination percentage was calculated by using the following formula

$$\text{germination percentage} = \frac{\text{number of seeds germinated}}{(\text{total number of seeds sown})} \text{X } 100$$

Fresh weight (g/plant) was taken with the help of an electrical single pan balance. The collected plant materials were kept in hot air oven at 50°C for 48 hours and their dry weight (g/plant) was recorded.

STATISTICAL ANALYSIS

Each treatment was conducted in at least triplicate, and the results were presented as mean values with respective standard errors mean (SEM). Statistical significance of differences between treatments was determined by using paired T test at 95% confidence level.

Table.1: The impact of bioengineered zinc nanoparticles and zinc metal on growth of *Cicer arietinum* seedlings growth. Each value represents the mean of 3 replicates ± standard error in mean.

	% of germination	Root length	Fresh weight	Dry Weight	Shoot length
Control	94.67 ± 3.528	6.308 ± 0.312	1.378 ± 0.238	0.216 ± 0.021	5.38 ± 0.580
Zn NPS	96.00 ± 2.309	6.48 ± 0.652	1.896 ± 0.0145	0.24 ± 0.016	5.550 ± 0.399
$ZnSO_4$	92.00 ± 4.619	1.36 ± 0.356	0.772 ± 0.055	0.125 ± 0.0098	4.09 ± 0.420

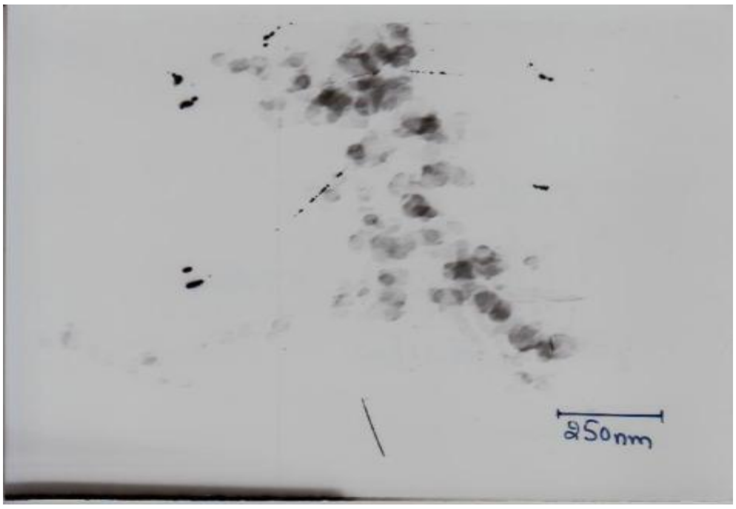

Fig.1: Transmission Electron Microscope showing the size of Zinc nanoparticles. Magnification 77200 X

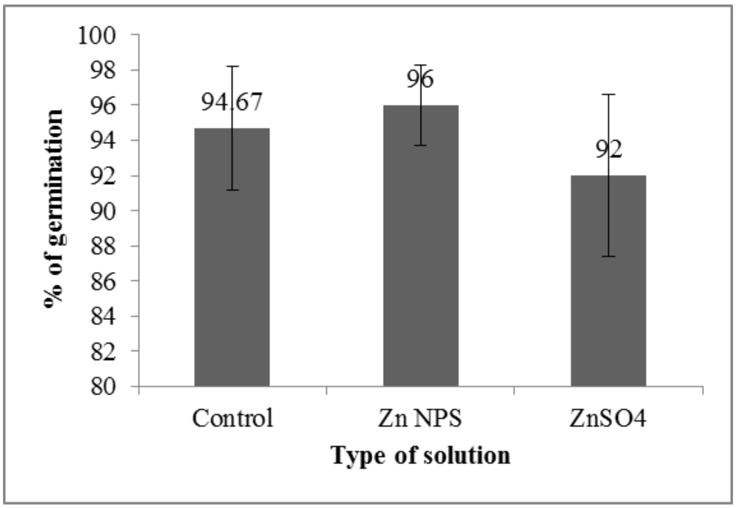

Fig.2: Changes in the germination % of *Cicer arietinum* grown in Zn and Zn NPS

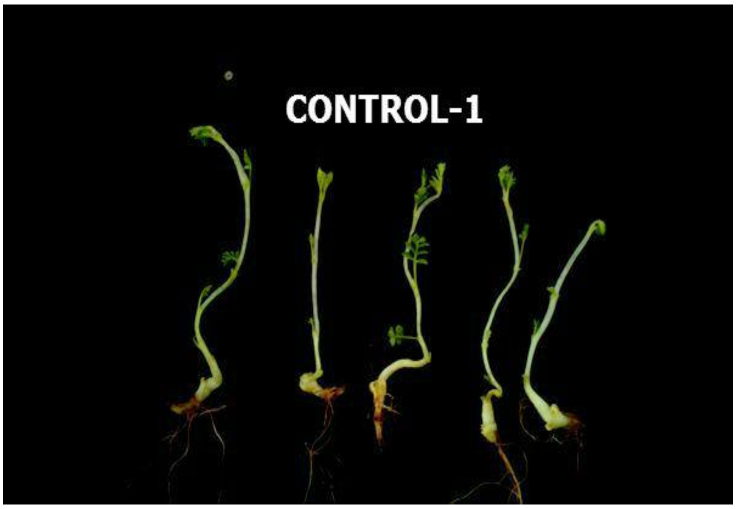

Fig.3: Comparative morphology of *Cicer arietinum* grown in water

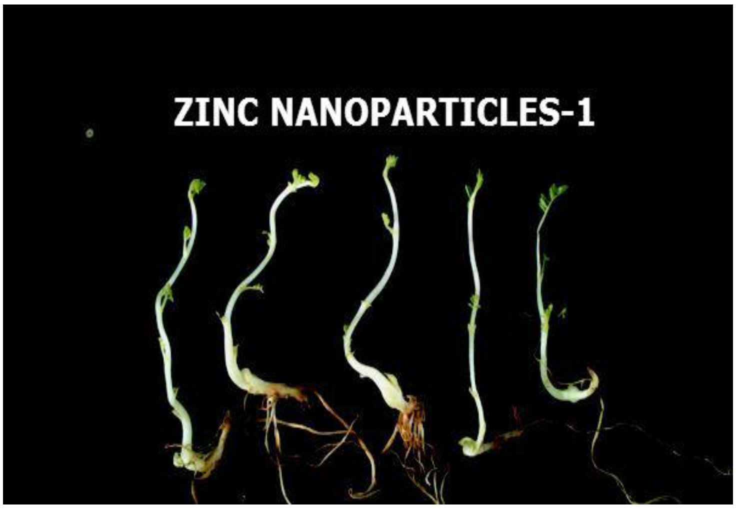

Fig.4: Comparative morphology of *Cicer arietinum* grown in zinc nanoparticles

Fig.5: Comparative morphology of *Cicer arietinum* grown in zinc sulphate

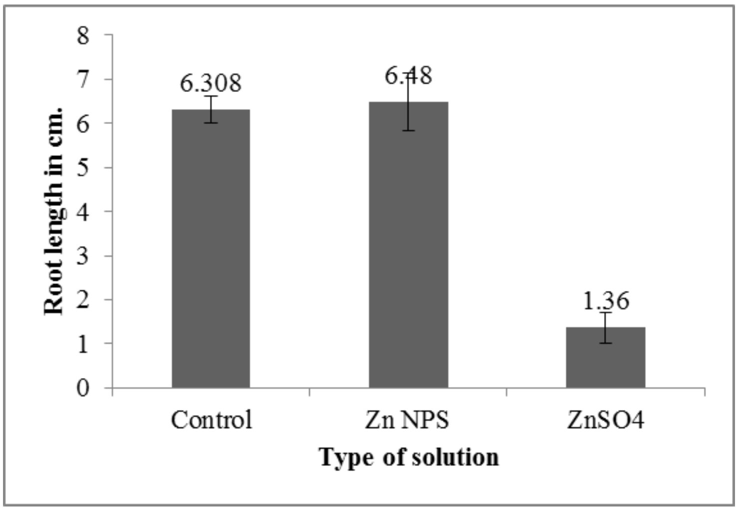

Fig.6: Changes in the root length of *Cicer arietinum* grown in Zn and Zn NPS

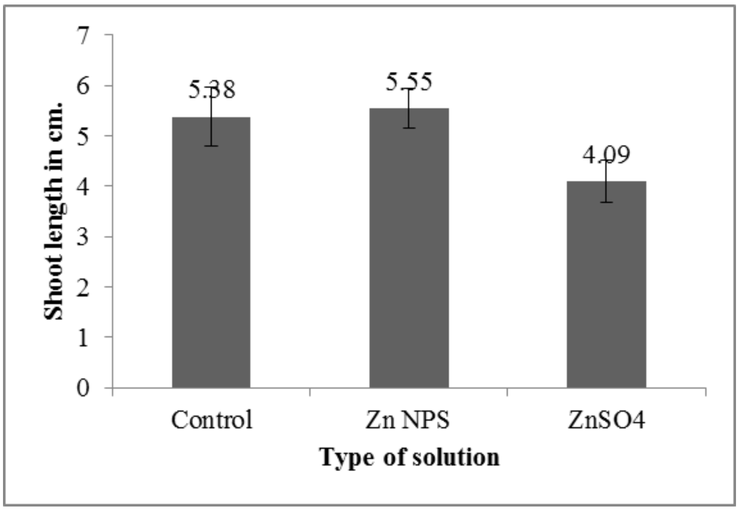

Fig.7: Changes in the shoot length of *Cicer arietinum* grown in Zn and Zn NPS

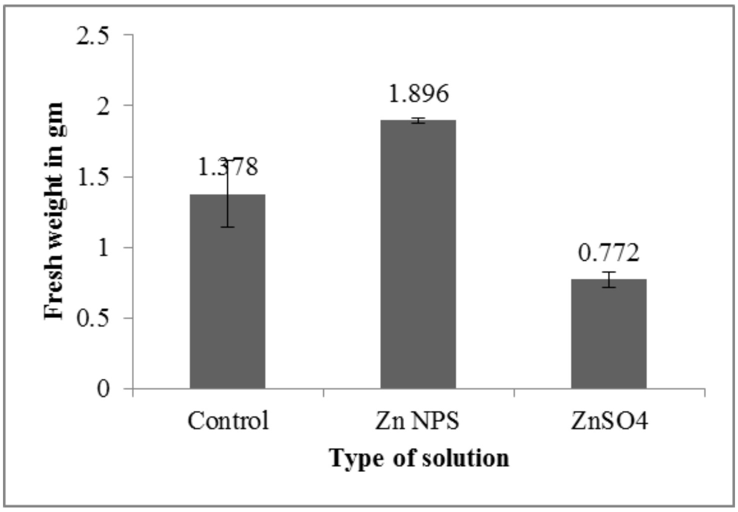

Fig.8. Changes in the fresh weight of *Cicer arietinum* grown in Zn and Zn NPS

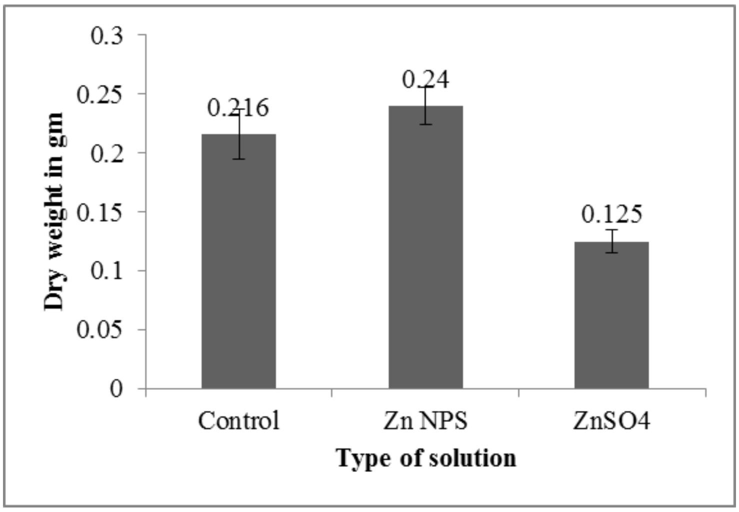

Fig.9: Changes in the dry weight of *Cicer arietinum* grown in Zn and Zn NPS

RESULTS AND DISCUSSION

Nanoparticles are introduced into the soil as a result of human activities, and by conversion of metals into nanoparticles by microorganisms present in the soil. So in the present study we tried to analyse the effect of zinc nanoparticles synthesized by *Aspergillus species* on bengal gram, as it is important to analyse the connections among nanoparticles, microbes and plants.

TEM studies

TEM (Transmission Electron Micrograph) revealed the presence of mono and polydispersive nanoparticles. The zinc nanoparticles synthesized are spherical and the size ranges from 13.2nm – 19.2nm. The average particle size of 15.332 nm was observed. It was also observed that bioengineered nanoparticles are aggregated (Fig.1). The surface properties of engineered NPs are of essential importance for their aggregation behavior, and thus for their mobility in aquatic and terrestrial systems and interactions with microorganisms and plants (Mihoub et al. 2005; Muhammad et al. 2008).

Germination percentage

Germination percentage was calculated after 14 days of seed germination. Number of seeds germinated was counted from each triplicate and the percentage was calculated by the formula mentioned in the method. Exposure to zinc sulphate significantly affected seed germination rates of Bengal gram, compared to the seeds exposed to zinc nanoparticles and control. (Fig. 2-5). Germination response was more in seeds soaked in zinc nanoparticles than the control. The decrease in seed germination of bengal gram under metal treatment can be attributed to the metal stress (Arora et al. 2012), osmotic effect (Navarro, 2008) or alterations in permeability properties of cell membrane (Punz and seighardt, 1993) hydrolytic enzymes activity, which led to impaired hydrolysis of complex food material (starch) or obstruction in mobilization of sugars (Radha et al. 2011; Rahoui, 2008; Remedios et al. 2012).

The reason for reduced seedling growth under metal exposure may be due to low water potential and nutrient uptake (Karthick et al. 2011). Hydrolytic enzymes which break down the food reserves during seedling growth might be affected by metal stress which may lead to the reduction in seedling growth. Similar observations have been made by several authors under various metal toxicity (Ali and Madhva, 2012; Arora et al. 2012; Shah and Belozerova, 2009).

Root length

Length of the root was measured for all triplicates in centimetres (cm) using a measurement scale. Highest root length is taken among many roots. Root length was increased in seeds exposed to Zn NPS. The root length is significantly affected in the seeds exposed to Zn metal (Fig.6). This may be due to the roots are the first target tissue to the toxic pollutants (Shaukat et al. 1999). The inhibition of root growth can be attributed to the inhibition of mitosis, reduced cell wall synthesis and changes in metabolism (Smiri et al. 2013).

Al_2O_3 NPs at concentrations up to 4000 mg/L had no significant toxic effects on seed germination, root elongation, and number of leaves of *Arabidopsis thaliana* (Thul et al. 2013). Larue and collaborators (Yang et al. 2006) studied the effects of TiO_2-NPs on *Triticum aestivum*, *Brassica napus*, and *Arabidopsis thaliana*. They showed that the TiO_2-NPs did not affect the germination and root elongation.

Shoot length

Length of the shoot was measured for all triplicates in centimetres (cm) using a measurement scale. As shown in (Fig. 7) shoot growth was affected by NPs. Shoot lengths in the presence of Zn NPs were significantly larger than in control. The same results were obtained when seeds of *Triticum aestivum* were grown in the presence of 50 mg/L alumina nanoparticles (Zheng et al. 2007). Field experiments with foliar sprays of gold-nanoparticles on Brassica *juncea* plant shows positively affect the growth profile; including plant height, stem diameter, number of branches, number of pods and seed yield (Arora et al. 2012).

Fresh weight& Dry weight

Fresh weight (g/plant) was taken with the help of an electrical single pan balance (Fig.8). The collected plant materials was kept in hot air oven at 50°C for 48 hours and their dry weight (g/plant) was taken by using an electrical single pan balance(Fig.9). Fresh and dry weights were found to be in accordance With shoot and root lengths for corresponding treatments. The fresh and dry weights were both significantly higher than those of the untreated plants (controls) and Zn treated plants.

CONCLUSION

The present study clearly reveals nontoxic effect of bioengineered zinc nanoparticles on seedlings emergence, and toxic effect of zinc metal on seedlings emergence. Nanoparticles size plays important roles in the behaviour, in the reactivity and in the toxicity of NPs on plants. Considering these aspects it is not strange to find both positive and negative effects of nanoparticles on higher plants. Our findings suggest important new avenues of research for understanding the fate and transport of NPs in natural media, the interactions between NPs and plant tissues, and indirect and direct effects of NPs in mixed communities. Further studies will be helpful to assess toxicity of released nanoparticles on plant growth and other components of ecosystem.

REFERENCES

Ahsan N, Lee DG, Lee SH, Kang KY, Lee JJ. and Kim PJ, 2007. Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere. 67: 1182-1193.

Ali Riahi-Madvar, FatemehRezaee and VahidrezaJalali, 2012. Effects of alumina nanoparticles on morphological properties and antioxidant system of *Triticumaestivum*. Appl. Biochem Biotechnol.167:2225-2233.

Arora S, Sharma P, Kumar S, Nayan R, Khanna PK, Zaidi MGH, 2012. Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. Plant Growth Regul. 66: 303-310.

Auffan M, Bottero JY, Chaneac C and Rose J, 2010. Inorganic manufactured nanoparticles: how their physicochemical properties influence their biological effects in aqueous environments. Nanomedicine. 5: 999–1007.

Blaser SA, Scheringer M, Macleod M, Hungerbuhler K, 2008. Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. Sci. Total. Environ. 390:396–409.

Elena Masarovicova and KatarínaKralova, 2013. Metal nanoparticles and plants. Ecol. Chem. Engs. 20:9-22.

John RP, Ahmad K, Gadgil and Sharma S, 2009. Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. Int. J. Plant. Prod. 3: 65-76.

Karthick Raja Namasivayam S and Chitrakala K, 2011. Ecotoxicological effect of Lecanicilliumlecanii (Ascomycota: Hypocreales) based silver nanoparticles on growth parameters of economically important plants. J. Biopest. 4: 97 - 101.

Kuriakosa SV, Prasad MNV, 2008. Cadmium stress affects seed germinationand seedling growth in *Sorghum bicolor* (L.) Moench by changingthe activities of hydrolyzing enzymes. Plant Growth. Regul. 54:143-156.

Larue C, Khodja H, Herlin-Boime, N, Brisset F, Flank AM, Fayard B, Chaillou S, Carriere M, 2011. Investigation of titanium dioxide nanoparticles toxicity and uptake by plants. J. Phys. 304: 1-4.

Lee CW, Mahendra S, Zodrow K, Dong Li, Yu-chang T, Janet B, Pedro JJ, 2010. Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana. Environ. Toxic. Chem. 29: 669–675**.**

Ling Yang, Watts DJ, 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles, Toxic. Lett. 158:122–132.

Mihoub A, Chaoui A, and Ferjani E, 2005. Biochemical change associated with cadmium and copper stress in germinating pea seeds (*Pisum sativum* L.). C.R. Biol. 328: 33-41.

Muhammad SI, Zafar M and Mohammad A, 2008. Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. J. Appl. Sci. Environ. Manag. 12: 61-66.

Navarro E Baun, A. Behra R, Hartmann NB, Filser J, Miao AJ, Quiqq A, Santschi PH, 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology. 17: 372–386.

Punz WF and Sieghardt H, 1993. The response of roots of herbaceous plant species to heavy metals. Environ. Exp. Bot. 33: 85-98.

Radha Solanki, AnjuPoonam, Dhankhar R. 2011. Zinc and copper induced changes in physiological characteristics of *Vigna mungo* (L.). J. Environ. Biol. 32: 747-751.

Rahoui S, Chaoui A, Ferjani E, 2008. Differential sensitivity to cadmium in germinating seeds of three cultivars of faba bean (*Vicia faba* L.). Acta. Physiol. Plant. 30(4): 451-456.

Remedios C, Rosario F and Bastos V, 2012. Environmental Nanoparticles Interactions with Plants: Morphological, Physiological, and Genotoxic Aspects. J. Botany. Article ID 751686, 8 pages.

Shah V and Belozerova I, 2009. Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. Water Air Soil Pollut. 197:143-148.

Shaukat SS, Mushtaq M and Siddiqui ZS, 1999. Effect of cadmium, chromium and lead on seed germination, early seedling growth and phenolic contents of *Parkinsonia aculeate* L. and *Pennisetum americanum* (L.) Schumann. Pak. J. Biol. Sci. 2: 1307-1313.

Smiri M, Chaoui A and Ferjani E, 2009. Respiratory metabolism in the embryonic axis of germinating pea seed exposed to cadmium. J. Plant Physiol. 166: 259-269.

Sresty TVS and Rao KVM, 1999. Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeonpea. Environ. Exp. Bot. 41: 3–13.

Thul ST, Sarangi BK. and Pandey RA, 2013. Nanotechnology in Agroecosystem: Implications on plant productivity and its soil environment. Expert. Opin. Environ. Biol. 2: 1-7.

Yang F, Hong F, You W, Liu C, Gao F, Wu C and Yang P, 2006. Influences of nano-anatase TiO2 on the nitrogen metabolism of growing spinach. Biol. Trace Elem. Res.110:179-190.

Zheng L, Su MG, Liu C, Chen L, Huang H, Wu X, Liu XQ, Yang F, Gao FQ and Hong FH, 2007. Effects of nanoanatase TiO2 on the photosynthesis of spinach chloroplasts under different light illumination. Biol. Trace. Elem. Res. 119:68-76.