

Biochemical Responses of *Cicer arietinum* Seedlings to Zinc And Bioengineered Zinc Nanoparticles Stress

*¹K.V. Pavani, ²V. Divya, ³I. Veena, ⁴M. Sravanthi, ⁵D. Chandana, ⁶B. Pooja Naidu, ⁷K. Sanjana
1, 2, 4, 5, 6, 7 Department of Biotechnology, Gokaraju Rangaraju Institute of Engineering and Technology, Hyderabad-500 090,
Andhra Pradesh, India

*Email: pavani_20042003@yahoo.co.in

Received: October 24th 2013, Accepted: November 15th 2013, Published: January 1st 2014

Abstract:

The effect of bioengineered zinc nanoparticles and zinc on biochemical analysis was investigated on the *Cicer arietinum* Seedlings. The extracellular synthesis of zinc nanoparticles with an average size of 47.6 nm was characterized by Transmission electron microscopy. Heavy metals are important and essential components in plant growth. However, when they increase in concentration, heavy metals show symptoms such as delay in growth and inhibition of biochemical reactions. Zinc treated seedlings showed decreased total sugars, reduced sugars and proteins compared to zinc nanoparticles treated seedlings. The positive effect of Bioengineered Nano Particles on seeds and seedlings could have significant economic impact on agriculture and horticulture.

Key words: *Cicer arietinum* Seedlings, Zinc, Zinc nanoparticles, Total sugars, Reducing sugars, Proteins.

Introduction:

Increasing numbers of commercial products, from cosmetics to medicine, incorporate manufactured nanomaterials (MNMs) that can be accidentally or incidentally released into the environment. ^[1, 2, 3] Though the use of nanoparticles is increasing, yet their environmental impact is poorly understood. There are many gaps in our knowledge on the effect of NPs. There are many unresolved problems and new challenges concerning the biological effects of these NPs. Concern over the potentially harmful effects of such nanoparticles have stimulated the advent of nanotoxicology as a unique and significant research discipline ^[4]. However, the majority of the published nanotoxicology articles have focused on mammalian cytotoxicity or impacts to animals and bacteria and only a few studies have considered the toxicity of MNMs to plants ^[5].

There is a critical knowledge gap in development of phytotoxicity. Nanoparticles entering wastewater

streams may predominantly be incorporated into sewage sludge and agricultural land ^[6]. Most of the studies are focused on the potential toxicity of NPs to plants where, both positive and negative effects have been reported ^[7]. Among the positive effects recorded, nano-TiO₂ was observed to promote the growth of Spinach ^[8]. Some of the research found that Carbon nanotubes (CNTs) could enhance root growth of onion (*Allium cepa*) and cucumber (*Cucumis sativa*) ^[9]. The fate and transport of nanoparticles in the environment and also the impacts of those materials on plants has not yet been fully characterized. The present investigation shows positive effect of bioengineered zinc nanoparticles on growth and biochemical characteristics of Bengal gram seedlings.

Materials and methods:

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 4°C in a refrigerator. A loopful of fungal culture was inoculated into a flask with 1000 ml of fungal media and Zinc sulphate (KH₂PO₄-7.0 g/L, KH₂PO₄-2.0 g/L, MgSO₄ · 7H₂O-0.1 g/L, (NH₄)₂SO₄- 1.0g/L, yeast extract-6.0 g/L, glucose-10 g/L Zinc sulphate (ZnSO₄) - 5.0mM/L). The flask was incubated at 28^o 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).

Seed germination:

Certified seeds of *Cicer arietinum* were obtained from the local market. Seeds with uniform size are chosen for experimental purpose. *Cicer arietinum* seeds were immersed in 0.1% mercuric chloride solution for 5 min for surface sterilization. After rinsing three times in sterile distilled water, they were

soaked in three different media containing zinc nanoparticles, deionised water (control), ZnSO₄ metal solution (5mM/L) respectively for 48hours. A piece of filter paper (Whatman No. 42) was put into each Petri dish (90 mm × 15 mm), 4 ml of deionised water, 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.

Estimation of Total Sugars:

0.5 g of plant sample (shoot) was homogenized in 10 ml of 80% Ethyl Alcohol (C₂H₅OH). The homogenate was centrifuged for 20 minutes at 200 g. 1 ml of the supernatant was taken in a test tube and 1 ml of 5% phenol and 5ml of 96% sulphuric acid (H₂SO₄) was added. The solution was mixed well and kept in water bath at 25^oc for 20 minutes. The sample was read at 490 nm in spectrophotometer.

Estimation of Reducing Sugars:

0.5 g of plant sample (shoot) was homogenized in 10 ml of 80% Ethyl Alcohol (C₂H₅OH). The homogenate was centrifuged for 20 minutes at 200 g. One ml of the filtrate was taken in a test tube and made up to 3ml with distilled water and 2ml of Dinitro salicylic acid (DNSA) was added. The solution was mixed well and kept in the water bath at 25^oc for 5 minutes. The sample was read at 540 nm in spectrophotometer.

Estimation of Protein:

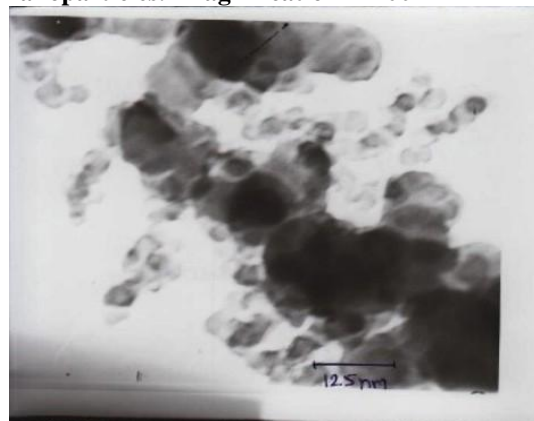
0.4 g of plant sample (shoot) was homogenized in 8 ml of 20% Trichloro Acetic Acid (TCA). The homogenate was centrifuged for 10 minutes at 300 g. Protein content present in the filtrate was determined by the method of Lowry et al [10].

Results and Discussion:

Due to the widespread production and use of NPs, it is expected that they find their way into the environment, be taken up by plants and consequently enter into the food chain. [11, 12] Objectives of the present study were to investigate the effects of nano-scale zinc and zinc metal on the biochemical responses of Bengal grams, as it is important to analyse the connections among nanoparticles and plants. TEM (Transmission Electron Micrograph) revealed the presence of mono and polydispersive

nanoparticles. The zinc nanoparticles synthesized are spherical with an average particle size of 47.6 nm. It was also observed that bioengineered nanoparticles are aggregated (Fig.1).

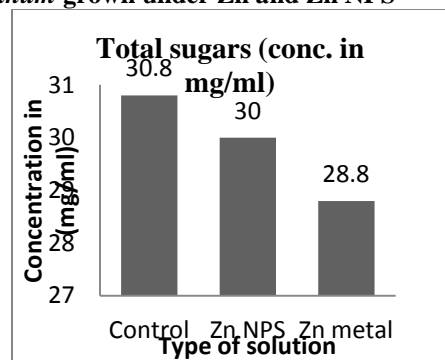
Fig 1: Below shows the Transmission Electron Microscope showing the size of Zinc nanoparticles. Magnification 77200 X



Total sugars:

Total sugar content of seedlings of Bengal gram grown under Zinc and Zinc NPs stress is represented in Fig.2. It is worth to mention that total sugars content in Zn NPs treated seedlings was almost similar to that of control. Sugar content was found to be highest in the seedlings grown in ZnNPs (30mg/ml) than zinc (28.8 mg/ml). Aldoobie [13] also reported that sugar content was hindered in lead, chromium and zinc stressed plants. Such inhibition may be due to decrease in photosynthesis or diversion of metabolites to other synthetic process. [14]

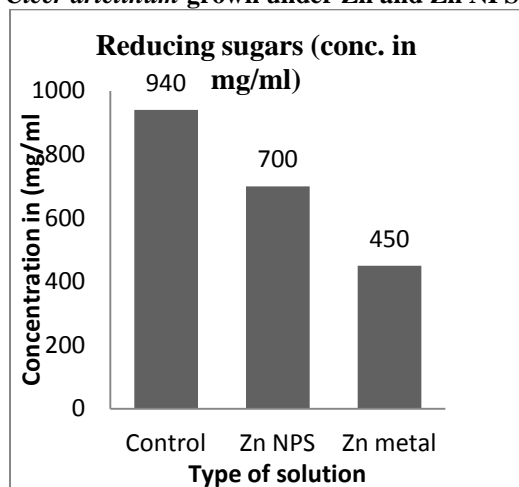
Fig 2: Changes in the total sugar contents of *Cicer arietinum* grown under Zn and Zn NPS



Reducing sugars:

Chemical analysis of carbohydrates showed significant decrease in reducing sugars in response to zinc stress (Fig.3). Aldoobie also mentioned that the contents of non-reducing sugars decreased in lead and cadmium-treated plants.

Fig 3: Changes in the reducing sugar contents of *Cicer arietinum* grown under Zn and Zn NPS



Protein estimation:

Protein concentration was decreased effectively in zinc treated seedlings than zinc nanoparticles treated seedlings (Fig.4). The results showed that the effect of zinc nanoparticles on *Cicer arietinum* seedlings was significant. The decrease in protein content in seedlings exposed to zinc suggests the toxic effect of the metal. Metals are essential for many proteins in plants, although they are toxic in excess. Proteins are important constituents of the cell that are easily damaged by the environmental stress conditions [15]. It is well-known that the active or passive uptake of nanoparticles can cause cellular effects and dynamic interaction between nanoparticles and protein cascades which are essential for the most cellular reactions.

Fig 4: Changes in the total protein contents of *Cicer arietinum* grown under Zn and Zn NPS

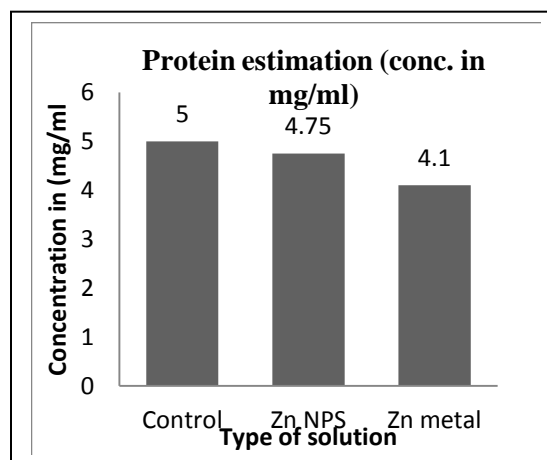


TABLE-1: Biochemical activities of Bengal gram (*Cicer arietinum*) treated with different solution

Type of Solution (Triplicates)	Biochemical Activities		
	Total Sugars Conc. (mg/ml)	Reducing Sugars Conc. (mg/ml)	Protein Estimation Conc. (mg/ml)
Control-1	29.6	860	8.25
Control-2	32.8	730	6.4
Control-3	29.6	1210	1.45
Average	30.8	940	5.0
Zn NPS-1	28.8	330	2.25
Zn NPS-2	28.8	450	4
Zn NPS-3	30.4	1300	7.1
Average	30	700	4.75
ZnSO ₄ -1	28.4	440	3.25
ZnSO ₄ -2	28.8	290	3.75
ZnSO ₄ -3	30	620	5
Average	28.8	450	4.1

Conclusion:

Based on the results obtained from the study, it can be speculated that the fate of the heavy metals that are transported into the soil can be reduced by the microorganisms in the soil. These bioengineered nanoparticles at low concentrations are non toxic to seed germination. High concentrations of metal nanoparticles may be toxic to the plants, but bioavailability of these nanoparticles to the plants is also important. It is therefore recommended to undertake further research on the potential impacts of manufactured nanoparticles on agricultural and environmental systems.

References:

[1]. Karthick Raja, Namasivayam, R. S., Arvind Bharani and Moinuddin Raqib Ansari (2013). Natural Occurrence of Potential Fungal Bio pesticide *Nomuraea Rileyi* (Farlow) Samson Associated with Agriculture Fields of Tamil Nadu, India and its Compatibility with Metallic Nanoparticles. *J Biofertil Biopestici*, 4, 1-7.

[2]. Behra, R. and H. Krug (2008). Nanoecotoxicology-Nanoparticles at large. *Nature Nanotechnology*, 3, 253-254.

[3]. Owen, R. and Handy, R (2007). Formulation the Problems for Environmental Risk assessment of Nanomaterials. *Environmental Science & Technology*, 41, 5582-5588.

[4]. Dreher, K.L (2004). Health and Environmental Impact of Nanotechnology: Toxicological Assessment of Manufactured Nanoparticles. *Toxicological Sciences*, 77, 3-5.

[5]. Wiesner, M.R., Lowry, G.V., Alvarez, P.J.J., Dionysiou, D., Biswas, P (2006). Assessing the risks of manufactured nanomaterials. *Environ.Sci.Technol*, 40, 4336-4345.

[6]. Karsten Schlich, Thorsten Klawonn, Konstantin Terytze, Kerstin Hund-Rinke (2013). Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. *Environmental Sciences Europe*, 25, 17.

[7]. Menard, A., Drobne, D., Jemec, A (2011). Ecotoxicity of nanosized TiO₂. Review of

In vivo data. *Environ. Pollut*, 159, 677-684.

[8]. Hong,F.S., Yang,F., Liu,C.,Gao,Q., Wan.Z., Gu,F., Wu, C., Ma, Z., Zhou,J.,Yang, P (2005). Influence of nano-TiO₂ on the chloroplast aging of spinach under light. *Biol Trace Elem Res*, 104,249-260.

[9]. Yang, L and Watts, D.J (2005). Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol Lett*, 158,122-132.

[10]. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275.

[11]. Leacoanet, H. F., J. Y. Bottero, J.Y., Wiesner, M. R (2004). Laboratory assessment of the mobility of nanomaterials in porous media. *Environ. Sci. Technol*, 38, 5164-5169.

[12]. Nowack, B and Bucheli, T.D (2007). Occurrence, behaviour and effects of nanoparticles in the environment. *Environ. Pollut*, 150, 5-22.

[13]. Aldoobie, N. F and Beltagi, M. S (2013). Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris L.*) plants to heavy metals stress. *African Journal of Biotechnology*, 12, 4614-4622.

[14]. Buzzes, F.A., Carlson, R.W., Rife, G.L (1975). Inhibition of corn and sunflower photosynthesis lead, *Physiology Plant arum*, 34, 326-329.

[15]. Wu, G., King H., Zhang, X., Shoo, H., Chu, L., Roan, C (2010). A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. *J. Hazard. Mater.* 174, 1-8.
