International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

INFLUENCE OF ZINC OXIDE NANOPARTICLES ON GROWTH OF *SESAMUM INDICUM* L. IN ZINC DEFICIENT SOIL

S. NARENDHRAN*[1], P. RAJIV[1], RAJESHWARI SIVARAJ[2]

[1]Department of Biotechnology, School of Life Sciences, Karpagam Academy of Higher Education, Eachanari Post, Coimbatore 641021, Tamil Nadu, India, [2]Department of Chemistry, Government Arts College, Udumalpet, Tamil Nadu, India
Email: narendhransumathi@gmail.com

Received: 28 Dec 2015 Revised and Accepted: 03 Feb 2016

ABSTRACT

Objective: A comparative examine of *L. aculeate* mediated ZnO (BZnO) and synthesized chemical ZnO (CZnO) nanoparticles became done which will decide the impact of seed germination on *Sesamum indicum* (CO-1).

Methods: Zinc oxide nanoparticles had been synthesized by the biological and chemical methods. Synthesized nanoparticles have been confirmed with Ultra Violet–visible spectroscopy (UV-Vis), Fourier transform infrared spectrometer (FT-IR), Energy dispersive X-ray spectrometer (EDX), X-ray diffractometer (XRD), Field Emission Scanning Electron Microscopy (FESEM) and High-Resolution Transmission Electron Microscopy (HRTEM). Different concentration of synthesized zinc oxide nanoparticles (0.1, 0.25, 0.5, 1 and 2 g/l) solution were prepared and applied to *Sesamum indicum* by soaking approach. The impact of ZnO nanoparticles treated plants were studied by using the standard procedure on plant growth attributes (shoot and root length, dry and fresh weight of shoot and root), photosynthesis pigment (chlorophyll a, chlorophyll b and total chlorophyll) and biochemical analysis (protein, carbohydrate and reducing sugar).

Results: The characterization analysis revealed that BZnO & CZnO NPs were spherical in shape with a mean particle size of 12±3 nm and 18±2 nm. The maximum observation of growth attributes was recorded in 0.5 g/l concentration of biologically synthesized ZnO nanoparticles which was compared to chemically synthesized ZnO nanoparticles.

Conclusion: Results of this experiment revealed that *Lantana aculeate* mediated zinc oxide nanoparticles are an enhanced useful resource of *Sesamum indicum*. It is able to use as nano fertilizer in agriculture development.

Keywords: Plant growth attributes, *Sesamum indicum*, Seed germination, Zinc oxide nanoparticles.

INTRODUCTION

Plants are very essential to human and their environment but very few studies were performed with ecological terrestrial test species to assess the potential toxicity of nanoparticles [1]. Plants are predicted to be uncovered to nanoparticles due to uptake and accumulation of plant biomass [2]. Nanoparticles have been absorbed to plant surface and soak up via natural nano or micrometer scale plant opening and could significantly have an effect on their delivery inside the environment.

Zinc oxide naturally occurs as mineral zincite and is mostly used as a white powder. Its material science, ZnO is an extensive band gap semiconductor of the II-VI semiconductor group. Zinc oxide is an amphoteric oxide almost insoluble in water and alcohol, but it is soluble in most acid including hydrochloric acid [3]. Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zincblende. The wurtzite structure is most stable at ambient condition. Various studies have been carried out on the effect of zinc on the growth and metabolism of the plant [4]. Zinc is an essential micronutrient for the growth and improvement of plants and human beings. Zinc performs the most important position in various metabolic techniques. Zinc is essential to trigger several enzymes and activate enzymes such as superoxide dismutase, tryptophan synthetase and dehydrogenases [5, 6].

Sesamum indicum is a member of the Pedaliaceae family and considered as a drought tolerant crop [7]. Sesame oil is used as foods (cooking and salad), medicine, soap manufacturing and so on. Its seeds and young leaves are eaten as stews and soups in Asia [8]. Sesamum oil is used as active ingredients in antiseptics, bactericides, disinfectants and antitubercular agents because they incorporate natural herbal antioxidants such as sesamin and sesamolin [9].

In the previous study, A simple, rapid biological procedure has been evolved to synthesize ZnO nanoparticles from *Lantana aculeate* leaf broth extracted solution and chemical synthesize ZnO nanoparticles by a precipitated method using Zn (NO_3) as a precursor. The synthesized nanoparticles have been characterized by various techniques which include UV-Vis, FTIR, XRD, EDX, FESEM and HRTEM. The biological and chemical synthesis of ZnO nanoparticles was spherical in shape with an average size of 12±3 nm and 18±2 nm. These results clearly indicate the benefits of using biological method synthesized ZnO nanoparticles have antimicrobial activities and also it could be effectively in agricultural development [10, 11].

Table 1: Nutritional value of deficient soil

Parameter	Deficiency soil
Soil texture	Sandy loam
Soil type	Reddish brown
Lime status	Calcareous
pH	8.12
EC	0.06 dS/m
Nitrogen	1.37%
Phosphorous	1.40%
Potassium	5.41%
Copper	0.60 ppm
Zinc	0.21 ppm
Iron	6.96 ppm
Manganese	3.65 ppm

MATERIALS AND METHODS

Materials

L. aculeate mediated (BZnO) nanoparticles and chemical mediated (CZnO) nanoparticles were synthesized with an average particle size (12±3 nm and 18±2 nm) and which determined through high-resolution transmission electron microscope (HRTEM) (JEOL JEM-3100F) (fig. 1). Experimental chemical was purchased from Sigma-Aldrich chemicals, India. *Sesamum indicum* (CO-1) seeds were obtained from Department of Oil Seed, Tamil Nadu Agriculture University, Coimbatore, India. The zinc-deficient soil was collected from Karur (10.9580 ° N, 78.0800 ° E) (D. T), Tamil Nadu, India (table 1).

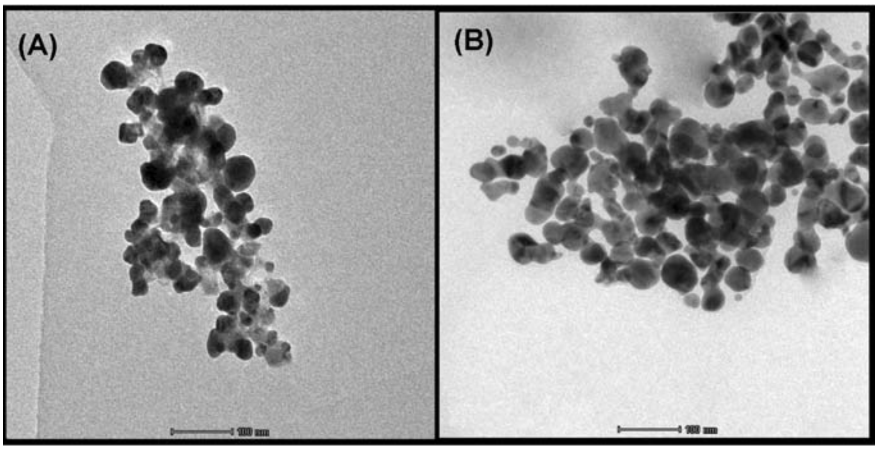

Fig. 1: (A) HRTEM image of zinc oxide nanoparticles synthesized from *L. aculeate* leaf extract (B) Chemical synthesis of zinc oxide nanoparticles

Preparation of nanosuspension and seed treatment

Zinc nanoparticles have been suspended using Milli-Q water and dispersed by ultrasonic vibration. For the present study five concentrations viz. 0.1, 0.25, 0.5, 1 and 2 g/l of both BZnO and CZnO NPs were used. *Sesamum indicum* (CO-1) seeds were immersed in a 2.5% sodium hypochlorite solution for 15 min for sterilization and experimental consistency following Rossi *et al.* [17]. After rinsing three instances with Milli-Q water, sesame has been soaked in BZnO and CZnO suspensions at the soaking duration of 1 d. Milli-Q water was used in the soaking method for a better control of the media.

Pot experiments and treatment details

A pot experiment was conducted at Karpagam Academy of Higher Education Campus, Eachanari, Coimbatore, Tamil Nadu, India, during July 2015. Ten seeds had been sown in each pot (30 cm diameter and 25 cm deep) on zinc-deficient soil. There were three replication pots for each treatment with factorial completely randomized design (FCRD) (table 2).

Table 2: Treatment details of green and chemical ZnO nanoparticles

Treatment	Concentration
T1	Control
T2	0.1g/l ZnO-treated seed
T3	0.25g/l ZnO treated seed
T4	0.5 g/l ZnO-treated seed
T5	1 g/l ZnO-treated seed
T6	2 g/l ZnO-treated seed
T7	0.25 g/l $ZnSO_4$ treaded seed

Effect of zinc oxide nanoparticles on the morphological and biochemical parameters of *Sesamum indicum*

The plant morphological parameters like shoot length, root length, dry weight and fresh weight of test crops treated with zinc oxide nanoparticles were carried out by using the standard procedures [12]. Chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll) [13], total carbohydrates [14], reducing sugars and protein [15] were determined by standard procedure. Statistical analysis was used to analyze the significant differences among different treatments for studied parameters.

RESULTS AND DISCUSSION

Confirmation of zinc level in NPs treated leaf

Zinc level was significantly high in zinc oxide nanoparticles soaked sesame plant leaves (i.e. 2 g/l>1 g/l>0.5 g/l>0.25 g/l>0.1 g/l) (fig. 2) when compared to control leaves, results are very similar to Prasad *et al.* (2012). Brassica is known to be a metal hyperaccumulator with enormous metal accumulation being reported for Zn [16].

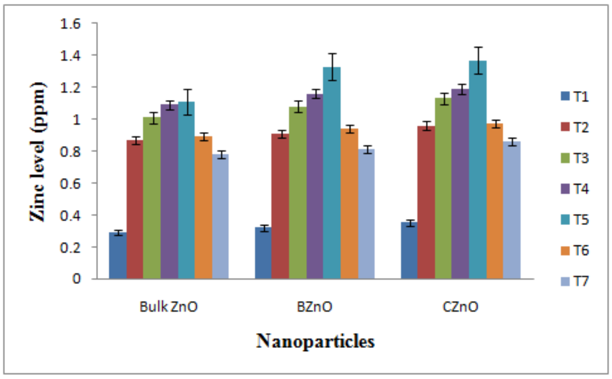

Fig. 2: Zinc level in different concentration of zinc oxide nanoparticles treated *Sesamum indicum* leaves. Data represent as mean±SE

Impact of zinc oxide nanoparticles treatment on growth profile of *Sesamum indicum*

Shoot and root elongation, the fresh and dry weight of sesame seedlings (fig. 3 and 4) were influenced by the application of various doses of zinc oxide nanoparticles. On 15 DAS, The root length (6.93 cm), shoot length (4.82 cm), fresh weight (33.30 g) and dry weight (11.93 g) was excessive in BZnO treated plants (T4 treatments) and found to be statistically significant. The plant sample treated with CZnO; maximum growth profile was observed at the concentration of 0.5 g/l at the end of 15 DAS. From the growth profile of sesame plant, CZnO confirmed less growth effect followed through BZnO nanoparticles treated plant sample while as compared to sesame grown in zinc-deficient soil by without soaked zinc oxide nanoparticles (T1 treatments). Lee *et al.* [20] on the early growth of buckwheat (*Fagopyrum esculentum*) substantially decreased on treatment with ZnO NPs at 1g/l and 2 g/l. Boonyanitipong *et al.* [21] observed the determined impact of nano-ZnO on rice root at the early seedling stage with stunt root length along with a reduction in a number of roots.

Manivasagaperumal *et al.* [22] confirmed a gradual decline in the dry matter production of the plant sample at higher concentration. This result indicated that application of ZnO slightly increased dry and fresh weight at a lower concentration while an excess of ZnO nanoparticles reduced the biomass [23].

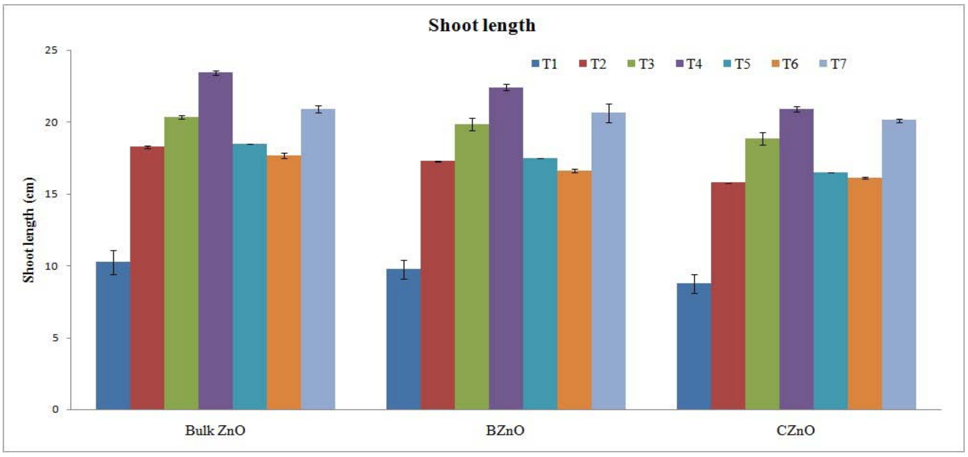

Fig. 3(A)

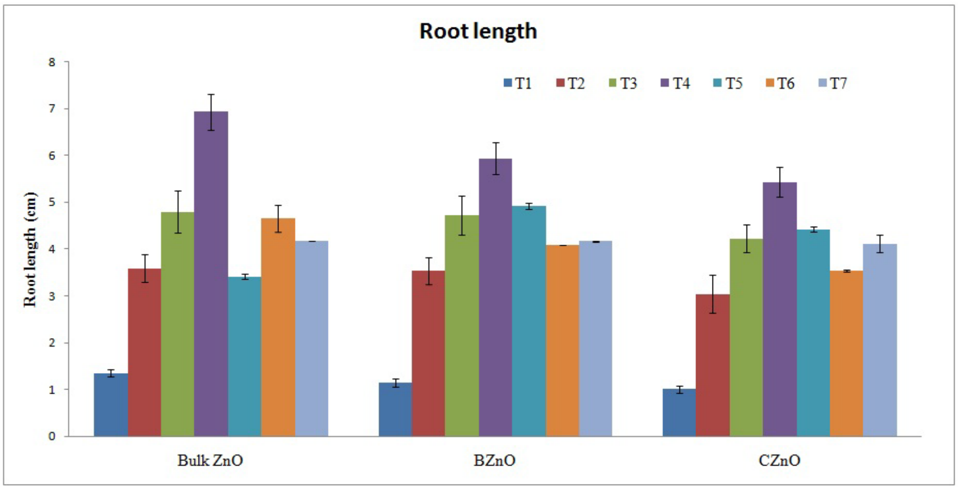

Fig. 3(B)

Fig. 3: (A) shoot length and (B) root length in *Sesamum indicum* treated with different concentrations of zinc oxide nanoparticles. Data represent as mean±SE

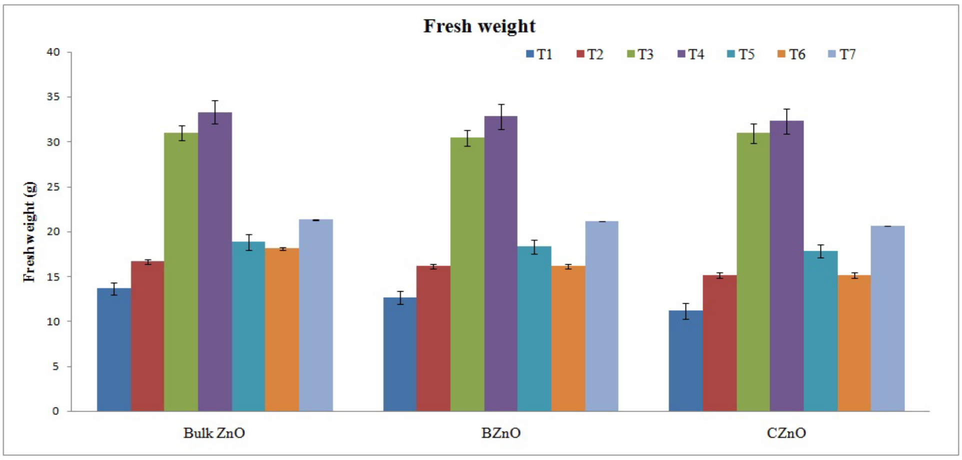

Fig. 4 (A)

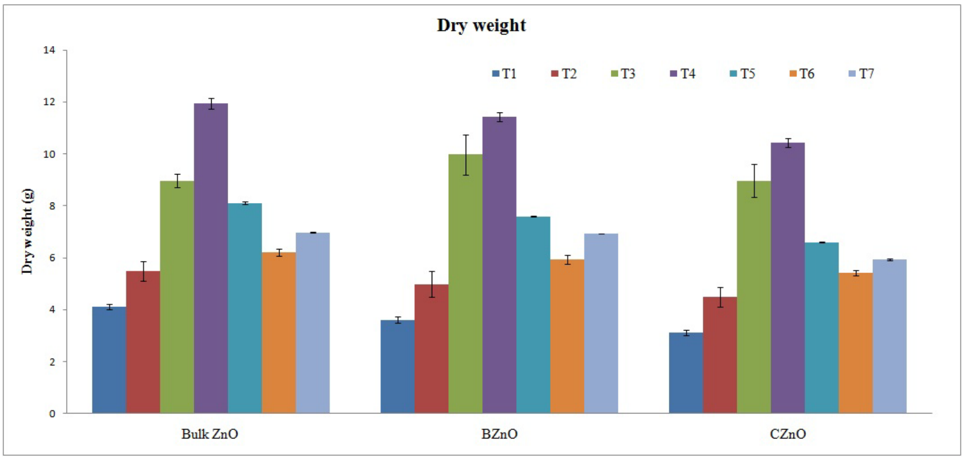

Fig. 4 (B)

Fig. 4: (a) fresh weight (b) dry weight in *Sesamum indicum* treated with different concentrations of zinc oxide nanoparticles. Data represent as mean±SE

Quality parameters for *Sesamum indicum*

The effect of various doses of zinc oxide nanoparticles on diverse treatments on photosynthetic pigment (chlorophyll a, chlorophyll b and total chlorophyll) contents of sesame (fig. 5). On 15 DAS, the plant sample treated with BZnO nanoparticles indicates chlorophyll 'a' (T1: 0.06 and T4: 0.27 mg/g), chlorophyll 'b' (T1: 0.07 and T4: 0.20 mg/g) and total chlorophyll content (T1: 0.13 and T4: 0.43 mg/g) level had been high in T4 treatment, whereas the plant samples treated with higher concentration of T5 and T6 showed decreased level of chlorophyll. CZnO treated plant samples showed maximum chlorophyll 'a' (T1: 0.06 and T4: 0.24 mg/g), chlorophyll 'b' (T1: 0.07 and T4: 0.16 mg/g), total chlorophyll content (T1: 0.13 and T4: 0.43 mg/g) level were high in T4 treatment. These results are confirmed with the results obtained from other studies carried out by Karthick and Chitrakala [18].

Photosynthesis pigment content was significantly ($p<0.05$) increased by BZnO in sesamum at a concentration of 0.5 g/l (T4 treatment). A similar trend of a drop in the chlorophyll level was noticed in the plant samples treated with increased concentration (1 and 2 g/l) of green and chemical NPs. The decline within the overall chlorophyll contents, as well as the growth inhibited, can be regarded as general responses associated with metal toxicity. Mukherjee *et al.* [24] observed lower in chlorophyll level in leaves, compared to control plant treated with ZnO NP_S or bulk ZnO in organic matter enriched the soil. Salama [25] proved the effect of AgNPs on the carbohydrate content of common bean (*Phaseolus vulgaris*) and corn (*Zea mays*) plant at higher doses which may be attributed to a toxic level of nanoparticles causing a subsequent decline in growth.

The carbohydrate content of the plant sample treated with various concentrations of ZnO NPs is predicted in the fig. 6. The control samples display carbohydrate level of 2.40 mg/g in zinc-deficient soil. Higher carbohydrate level of 4.82, 5.13 and 5.59 mg/g (BZnO) and 4.31, 4.63 and 5.08 mg/g (CZnO) was noted at a concentration of 0.1, 0.25 and 0.5 g/l at the end of 15th DAS. Likewise, 1 and 2 g/l of BZnO exhibited 4.39 and 4.58 mg/g of carbohydrate, whereas 4.33 and 4.08 mg/g carbohydrate was noted for the plant sample treated with CZnO NPs. The plant carbohydrate levels displayed are in the significant range ($p<0.05$)

On 15 DAS, the control samples showed protein level of 0.78 mg/g in zinc-deficient soil. The plant sample was treated with BZnO nanoparticles which show highest leaf protein content (0.71, 1.22 and 1.73 mg/g) was found to be in 0.1, 0.25 and 0.5 g/l. CZnO treated plant samples showed maximum protein content (0.69, 1.00 and 1.23 mg/g). But at the concentration of 1 g/l and 2 g/l treated plant showed a reduction in protein level (fig. 6).

The amount of reducing sugar present in the plant sample upon various treatments is shown in the fig. 6. The control samples display reducing sugar level of 4.07 mg/g in zinc-deficient soil. The plant sample was treated with 0.1, 0.25 and 0.5 g/l of BZnO recorded 4.23, 4.43 and 4.83 mg/g of reducing sugar, whereas 4.2 and 4.06 mg/g (1 and 2 g/l) of reducing sugar was noticed at the end of 15th DAS. The CZnO treated plant sample displayed maximum sugar level of 3.73, 3.93 and 4.33 mg/g at the concentration of 0.1, 0.25 and 0.5 g/l whereas 4.00 and 3.58 mg/g (1 and 2 g/l) of reducing sugar exhibited at the end of 15th DAS. Thus plant samples showed significant reducing sugar ($p<0.05$).

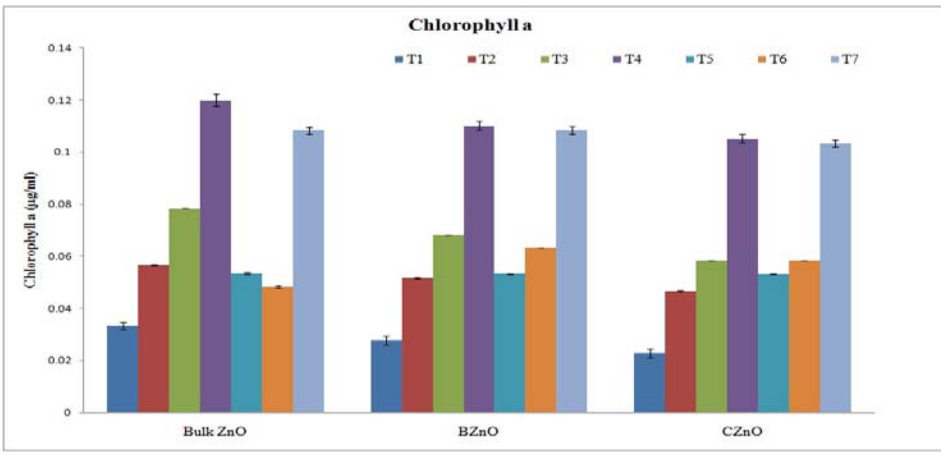

Fig. 5 (A)

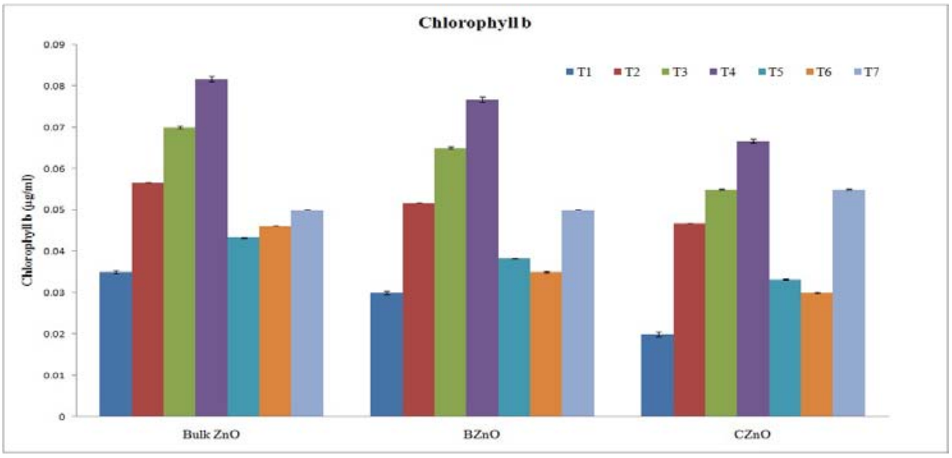

Fig. 5(B)

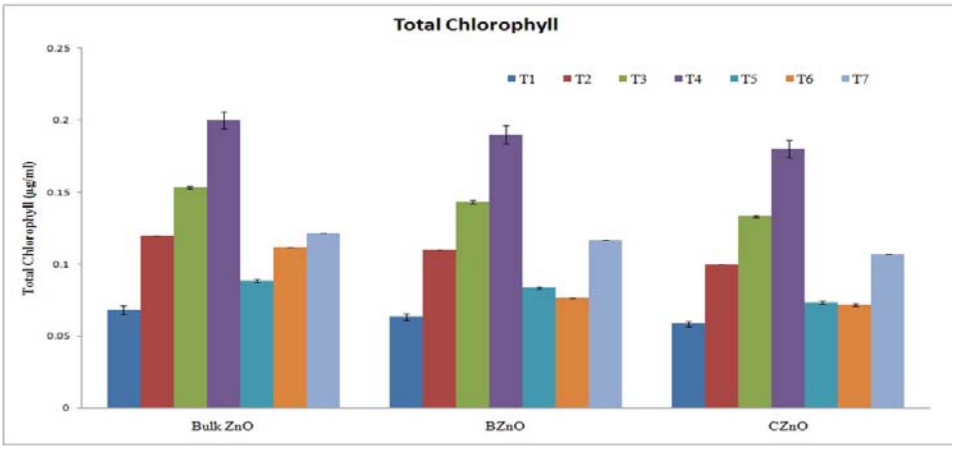

Fig. 5 (C)

Fig. 5: (A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll content in *Sesamum indicum* treated with different concentrations of zinc oxide nanoparticles. Data represent as mean±SE

Farshian *et al.* [19] showed a reduction in the protein total content of lettuce plant, which might be caused by the toxic effect of chemically synthesized ZnO nanoparticles on the protein synthesis. Protein concentration decreased in *Brassica juncea* seedlings due to metal treatment, particularly at the highest Zn concentration [26]. Protein and sugar were significantly reduced in this cereal wheat (*T. aestivum*) as reported by Tandon and Gupta [27] at increased doses of heavy metal. Qiang *et al.* [28] reported decreased in the sugar soluble content of wheat on the application of slow release fertilizer coated and felted by nanomaterials. The same result was obtained by Liu *et al.* [29] who demonstrated that low concentration of nano calcium carbonate caused increasing soluble sugar and peanut protein. From the phytotoxicity above studies prove that chemically synthesized nanoparticles are more toxic to the plant environment when compared green synthesis.

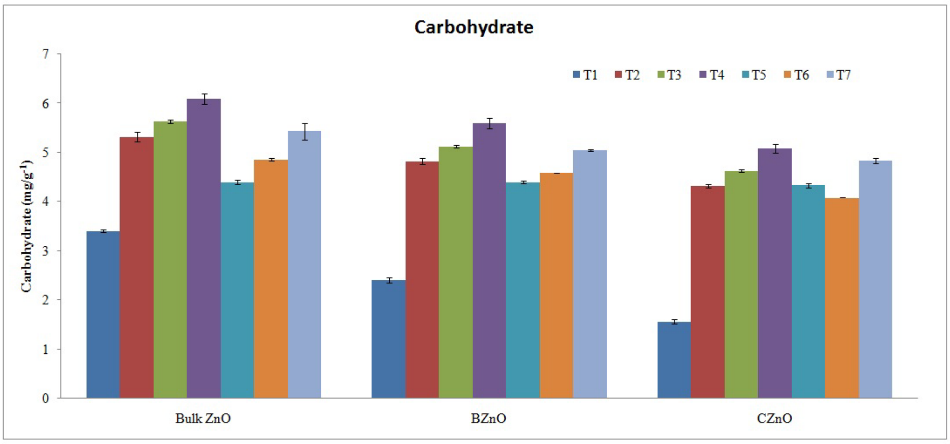

Fig. 6 (A)

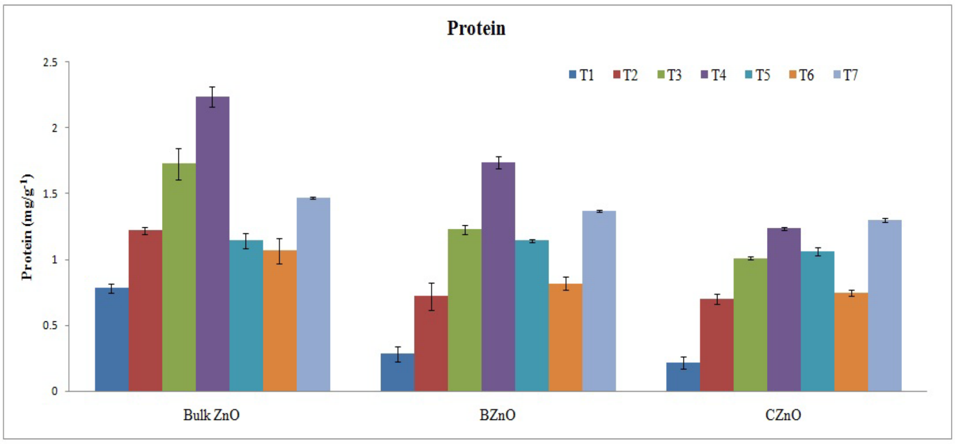

Fig. 6 (B)

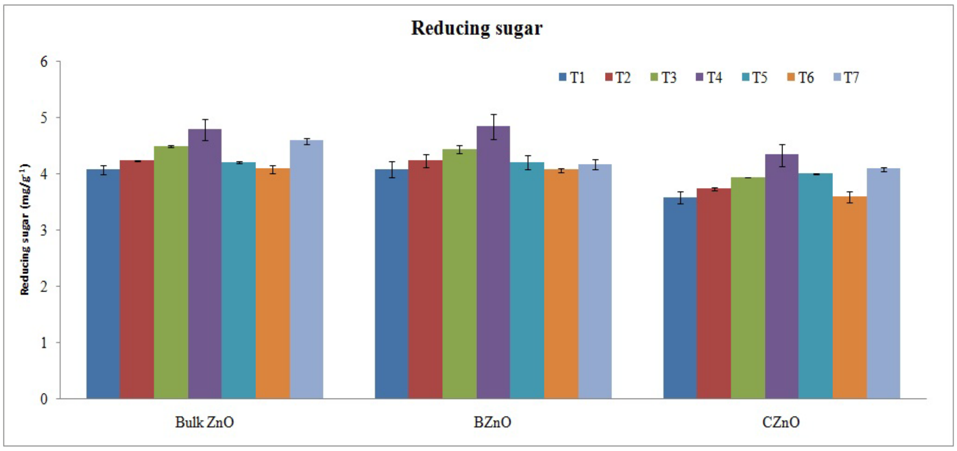

Fig. 6 (C)

Fig. 6: (a) carbohydrate, (b) Protein level and (c) Reducing sugar in *Sesamum indicum* treated with different concentrations of zinc oxide nanoparticles. Data represent as mean±SE

CONCLUSION

From the morphological and biometric analysis of sesame plants treated by biologically synthesized ZnO nanoparticles showed less toxic effects when compared with chemically synthesized ZnO nanoparticles. Total zinc accumulation in plant samples were found to be high in the order of T5>T6>T4>T3>T2>T1 in BZnO and CZnO nanoparticles treatment. The results of our studies indicate that the CZnO nanoparticles were toxic to sesame plant, resulting either from the more presence of the nanoparticles (at the concentration of 1 g/l and 2 g/l). The nanoparticles are induced tremendous changes in the plant environment. Our findings hence shows, chemically synthesized nanoparticles are toxic to the ecosystem. However, to confirm the size depended on effects of the toxicity induced by ZnO nanoparticles, further experiment with various sizes of nanoparticles, different species of plants and different exposure periods are needed.

ACKNOWLEDGEMENT

We thank to the management of Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India for providing necessary facilities to carry out this work.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Canas JE, Long MQ, Nations S, Vadan R, Dai L, Luo MX, *et al.* Effect of functionalized and non-functionalized single-walled carbon nanotube on root elongation of selected crop species. Environ Toxicol Chem 2008;27:1922-31.
2. Ma XM, Geiser Lee J, Deng Y, Kolmakov A. Interactions between engineering nanoparticles (ENP) and plants: phytotoxicity, uptake, and accumulation. Sci Total Environ 2010;408:3053-61.
3. Spero JM, Devito B, Theodore L. Regulatory chemical handbook. CRC press; 2000.
4. Auld DS. Zinc coordination sphere in biochemical zinc sites. Biometals 2001;14:271-313.
5. Brodley MR, White PJ, Hammond JP, Zelko J, Lux A. Zinc in plants. New Phytologist 2007;173:677-702.
6. Singh MV. Micronutrient nutritional problems in soils of India and improvement for human and animal health. Indian J Fert 2009;5:11-6.
7. Toan DP, Thuy-Duong TNA, Carlsson S, Bui TM. Effect of weeding frequencies and plant density on vegetative growth characteristic of groundnut (Arachis hypogaea L.) in North Kordofan of Sudan. Aust J Crop Sci 2010;4:498-504.
8. Narendhran S, Rajeshwari Sivaraj. Fourier transform infrared spectroscopy ananlysis of phytochemical constituent of various solvent extracts from lantana aculeate L. and its antibacterial activity. Int J Curr Res 2015;7:18177-80.
9. Fukuda Y, Nagata M, Osawa T, Namiki M. Contribution of lignin analogues to antioxidative activity of refined unroasted seame seed. J Am Oil Chem Soc 1986;63:1027-31.
10. Narendhran S, Rajeshwari Sivaraj. Biogenic ZnO nanoparticles synthesized using *L. aculeate* leaf extract and their antifungal

activity against fungal plant pathogens. Bull Mater Sci 2015. Doi:10.1007/s12034-015-1136-0. [Article In Press].
11. Vanathi P, Rajiv Narendhran S, Rajeshwari Sivaraj, Pattanathu KSMR, Venckatesh R. Biosynthesis and characterization of phyto mediated zinc oxide nanoparticles: a green chemistry approach. Mater Lett 2014;134:13-5.
12. Arnon DI. Copper enzymes in isolated chloroplasts: polyphernol oxidase in *Beta vulgaris*. Plant Physiol 1949;24:1-15.
13. Hedge JE, Hofreiter BT. Carbohydrate chemistry. In: Whistler RL, Be Miller JN. Editors. New York: Academic Press; 1962. p. 17.
14. Miller GL. Use of DNS reagent for determination of reducing sugar. Anal Chem 1972;31:426-8.
15. Lowry OH, Rosehmugh NJ, Farr AL, Randall KJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
16. Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Raja Reddy K, *et al*. Effect of nanoscale zinc oxide particles on the germination, growth, and yield of peanut. J Plant Nutr 2012;35:6905-27.
17. Rossi G, Figliola A, Socciarelli S, Pennelli B. Capability of *Brassica napus* to accumulate cadmium, zinc and copper from soil. Acta Biotechnol 2002;2:133-40.
18. Karthick Raja Namasivayam S, Chitrakala K. Ecotoxicological effect of *Lecanicillium lecanii* (Ascomycota: Hypocreales) based silver nanoparticles on growth parameters of economically important plants. J Biopesticides 2001;4:97-101.
19. Farshian S, Khara J, Malekzadeh P. Influence of *arbuscular mycorrhizal* fungus with lettuce plant under zinc toxicity in nutrient solution. Pak J Biol Sci 2007;10:2363-7.
20. Lee H, Chung S, Lee I. The genotoxic effect of ZnO and CuO nanoparticles on early growth of buckwheat, *Fagopyrum esculentum.* Water Air Soil Pollut 2013;224:1-11.
21. Boonyanitipong P, Kositsup B, Kumar P, Baruah S, Dutta J. Toxicity of ZnO and TiO2 nanoparticles on germinating rice seed *Oryza sativa* L. Int J Biosci Biochem Bioinform 2011;1:282-5.
22. Manivasagaperumal R, Vijayarengan P, Balamurugan S, Thiyagarajan G. Effect of zinc on growth, dry matter yield and nutrient content of *Vigna radiate* (L.) Wilczet. Int J Recent Sci Res 2012;3:687-92.
23. Xiong Z, Liu C, Geng B. Phytotoxic effect of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotoxicol Environ Saf 2006;64:273-80.
24. Mukherjee A, Peralta-Videa JR, Bandyopadhyay S, Rico CM, Zhao L, Gardea-Torresdey JL. Physiological effect of nanoparticles ZnO in green peas (*Pisum sativum* L.) cultivated in soil. Metallomics 2014;6:132-8.
25. Salama HMH. Effect of silver nanoparticles in some crop plant, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Int Res J Biotechnol 2012;3:190-7.
26. Sharma RK, Agarwal M, Marshall F. Heavy metal contamination of soil and vegetables in suburban area of Varanasi, India. Ecotoxicol Environ Saf 2007;66:258-66.
27. Tandon PK, Gupta S. Effect of cobalt and lead on the growth and metabolism of gram (*Cicer arietinum*) seeds. Indian J Agric Biochem 2002;15:55-8.
28. Qiang X, Fu-Dao Z, Yu-Jun W, Jian-Feng Z, Shuqing Z. Effect of slow/controlled releases fertilizers felted and coated by nanomaterial on crop yield and quality. Plant Nutr Fertilizer Sci 2008;14:951-5.
29. Liu XM, Zhang FD, Zhang SQ, He XS, Fang R, Feng Z, *et al*. Effect of nano-ferric oxide on the growth and nutrient absorption of peanut. Plant Nutr Fertilizer Sci 2005;11:14-8.