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Impact of Zinc oxide nanoparticles on eggplant (*S.melongena*): Studies on growth and the accumulation of nanoparticles.

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Abstract: The increasing use of nanoparticles and their occurrence in the environment has made it imperative to elucidate their impact on the environment. Although several studies have advanced our understanding of nanoparticle-plant interactions, our knowledge on the exposure of plants to nanoparticles and their effects on edible crop plants remains meager and is often paradoxical. The aim of this study was to increase our knowledge on the effect of zinc oxide nanoparticles on eggplant seed germination and seedling growth. Zinc oxide nanoparticles had a negative effect on the growth of eggplant in plant tissue culture conditions, as the growth of seedlings decreased with the increase in the concentration of zinc oxide nanoparticles. In contrast, zinc oxide nanoparticles enhanced eggplant growth under greenhouse conditions. The accumulation of zinc oxide nanoparticles in various parts of eggplant was observed through scanning electron microscopy of both plant tissue-culture and greenhouse-raised eggplant seedlings. To the best of our knowledge, this is the first paper to report on zinc oxide nanoparticle accumulation in eggplant and its effect on seed germination and seedling growth.

Keywords: eggplant, SEM, zinc oxide nanoparticles, zinc oxide nanoparticles accumulation, effect of nanoparticles, seed germination.

1. Introduction

Nanomaterials, with a peripheral dimension of 100 nanometers or less, have various applications in agricultural, medical, and environmental fields [1][2][3]. Its physicochemical properties can significantly differ from the corresponding bulk material, thus, indicating the unknown biological effects of nanomaterials in living cells [4]. Nanotechnology is rapidly gaining industrial importance due to its enormous revolutionizing potential in fields like electronics, medicine, and agriculture. On the other hand, there is a lack of clarity regarding of nanomaterial decay, deposition, and effects in the environment [5]. It should be noted that the increasing production and use of nanoproducts have resulted in their accumulation and spread in the environmental system [6].

Although the impacts of various nanomaterials on environment and ecosystem have been elucidated, studies on plants exposure to nanoparticles remain limited and often contradictory. It has been reported that plants produce certain mineralized nanomaterials required for their growth under certain conditions [7]. Understanding the interactions between nanoparticles and plant systems environment is important in understanding the impact of nanomaterials on the environment, especially in agriculture, with a focus on toxicity, plant disease, and nutrient management applications.

From the past two decades, several studies have reported the effect of nanoparticles on the plants. Both positive and negative or non-significant effects of various nanoparticles on plants have been reported [8][9]. This is because the effect of nanoparticles on plants varies from plant to plant and species to species [10]. Even the properties of nanoparticles vary with the method of synthesis and the solvents used in the synthesis of nanoparticles [11]. Easy penetration into organisms and living cells is the major factor for nanoparticles interaction, which, in turn, induces morphological variations [12] and produce pathological changes at the molecular level [13].

Zinc oxide (ZnO) nanoparticles have exceptional UV absorption and reflective properties. They are being used extensively in numerous products such as paints, coating materials, medical and personal care products [14] and are also applied in various fields of food and agriculture sectors as pesticides [15], fungicides [16], and fertilizers [17]. Nano-ZnO has been reported to improve the germination, root growth, shoot growth, dry weight of *Vigna radiata*, *Cicer arietinum*

[18], *Solanum lycopersicum* L. [19], and pod yield of the peanut seeds [20]. However, ZnO nanoparticles have shown toxicity in *Raphanus sativu*s, *Brassica napus*, *Lolium multiflorum*, *Lactuca sativa*, *Cucumis sativus*, *Brassica nigra, Zea mays*, *oryza sativa*[21], and *Ipomoea batatas* [22] by inhibiting seed germination and root growth [23]. The ZnO nanoparticles have been shown to enter ryegrass cells and pass all the way through the epidermis and cortex through an apoplastic pathway [24]. Capped ZnO and CuO nanoparticles enhanced the production of sweetner compounds by enhancing the shoot growth in *Stevia rebaudiana*. [25] The observed genotoxicity of ZnO nanoparticles in *Allium cepa*, [26] and *Lathyrus sativus* L., [27], and its cytotoxicity and genotoxicity in plants generally occur as a result of deregulation of components of the ROS-antioxidant machinery, and cell-cycle arrest, leading to DNA damage and cell death [28].

Although these studies have advanced our perception and understanding of nanoparticle-plant interactions, there is still a lack of knowledge on the mechanisms of nanoparticles transport from root areas to other plant parts. No relevant references are available on vegetables like eggplant (*Solanum melongena*) or aubergine. The current study will examine and assess the impact of ZnO nanoparticles on seed germination and seedling growth of the eggplant and their accumulation.

2. Materials and methods:

2.1. Nanomaterial:

ZnO nanoparticles with an average particle size of 35 nm were used in this study (Sigma-Aldrich, California, USA). Stock solution was first prepared by dispersing 2 g of ZnO nanoparticles in 500 ml Milli-Q water and homogenized by a sonicator. Five concentrations of ZnO nanoparticles were used for the seed germination experiment: 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, and 100 mg/L against control with equal volumes of sterile distilled water.

2.2. Plant material and media:

Seeds of Arka Anand variety of eggplant were obtained from the Indian Institute of Horticultural Research, Bangalore, India were used in the experiment. Seeds were surface sterilized in 0.05% Tween-20 and then washed with distilled water for 3-4 times and 0.05% mercuric chloride followed by several washes with sterile distilled water. Sterilized seeds were then placed on ZnO nanoparticles-enriched Murashige and Skoog medium (MS basal salts, 3% sucrose, 0.8% agar, pH 5.7). Plates were sealed and incubated in the dark till germination. Five types of media were used: standard MS medium (control), supplemented with 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L ZnO nanoparticles. Three replications were used for the treatments. The seeds were sown in germination trays filled with coco peat enriched with ZnO nanoparticles (0 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg and 100 mg/kg of coco peat), and the trays were covered with black sheet and incubated in the dark. After germination, the seedlings were transferred and allowed to grow in pots filled with 1:1 agricultural soil and coco peat under green house conditions of 22/16 °C (day/night) and 60% relative humidity.

Germination percentage was calculated using the following equation:
Germination % = (number of seeds germinated) / (no. of seeds inoculated) × 100 [29]

2.3. Scanning electron microscopy:

Environmental scanning electron microscopy (ESEM) Quanta 200 FEI was used to detect the transportation and accumulation of ZnO nanoparticles in various parts of eggplant plants. Root and stem samples were collected from 2 to 3-weeks-old seedlings grown in MS media. Stem, leaf, and flower samples were obtained from the potted plants at the flowering stage. 1-2 mm sized samples were obtained using a fine scalpel, and then placed on a specimen stub over an adhesive carbon tape. Then the samples were gold coated using sputter coating machine for better conductivity. Energy dispersive spectrometry is used to detect the chemical composition of observed nanoparticles in various plant parts.

2.4. Statistical analysis:

All the data obtained were represented as an average of 3 replicates with standard error. Data were analyzed by Web Based Agricultural Statistics Software Package (WASP 2.0) with ANOVA to find the differences between the treatments at 5% level of significance.

3. Results and Discussion:

3.1. Seed germination and seedling growth:

Zinc is an essential element for plant growth and development. It acts as a binding domain in many proteins, such as transcriptional regulatory proteins [30]. However, it becomes toxic when its amount is above certain concentrations, affecting many cellular processes. The effect of ZnO nanoparticles on seed germination and seedling growth of eggplant may be due to zinc ions resulting from ZnO dissolution [31].

The germination percentage was recorded after 5 days of germination. Seed germination of eggplant was negatively affected by ZnO nanoparticles in MS medium, but it showed positive results by enhancing seed germination in the soil medium. The percentage of seed germination of eggplant in MS media decreased as the concentration of ZnO nanoparticles increased. In soil media, the germination percentage was lowered at initial concentrations of ZnO nanoparticles (5 mg/kg and 10 mg/kg), but it increased with the increase in concentrations of ZnO nanoparticles from 15 mg/kg onwards. A higher toxicity of ZnO nanoparticles was observed in MS medium than in soil medium. The variations in the germination percentage are given in (see Fig.1). At lower concentration (5mg) of ZnO nanoparticles, the germination percentage was 88% in MS medium and 80% in soil medium, but at higher concentration (20mg) of ZnO nanoparticles, the germination percentage was 45% in MS medium and 100% in soil medium.

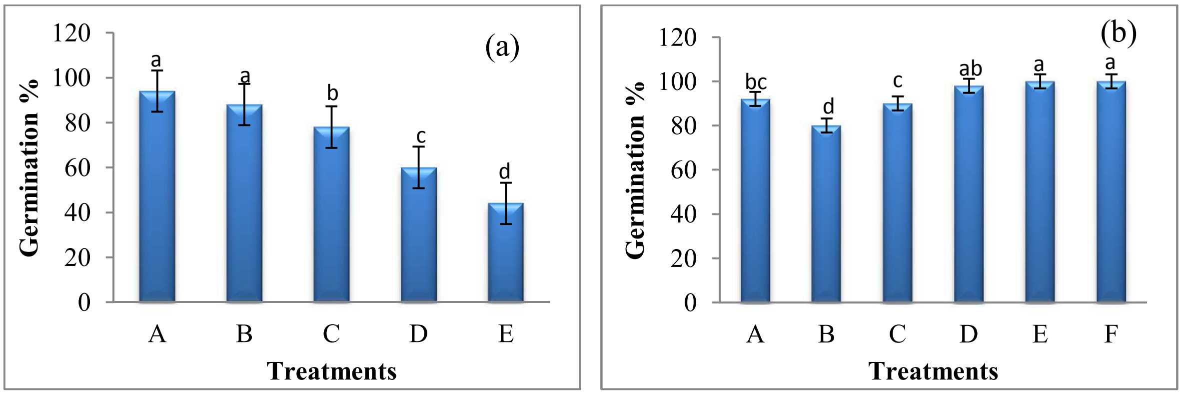

Fig.1. *Germination percentage variations of eggplant exposed to zinc oxide nanoparticles in (a) MS medium and (b) soil medium.Concentration of ZnO nanoparticles* ***(a)****: A-0 mg/l, B-5 mg/l, C-10 mg/l, D-15 mg/l, E-20 mg/l; Concentration of ZnO nanoparticles in* ***(b)****: A- 0 mg/kg, B-5 mg/kg, C-10 mg/kg, D-15 mg/kg, E-20 mg/kg, F-100 mg/kg; Error bars represent standard error. Different letters represent significant differences among treatments ($p < 0.05$).*

The observations for morphological traits were recorded after 2-3weeks of germination. The variations in the growth of seedlings have been observed with varying concentrations of ZnO nanoparticles in the MS media. The major effect was observed in the root growth (see Fig. 2 and Fig. 3). As the concentration of ZnO nanoparticles increased, a decrease in the number of lateral roots and thickening of the roots were observed. Thus, there is a decrease in leaf length, shoot and root mass with the increase in concentration of ZnO nanoparticles (see Fig. 4).

The response of ZnO nanoparticles on the test plants in soil differed from that observed in plant tissue cultures in MS media. ZnO nanoparticles have not shown toxicity towards the growth of plants in soil medium, rather it enhanced the plant growth. Shoot length, leaf length, and leaf width along with plant mass were found to be decreased at lower concentration of ZnO nanoparticles (5 mg/kg). However, these parameters increased with the increase in concentration of ZnO nanoparticles till 15 mg/kg and then decreased with further increase in ZnO nanoparticles concentration to 20 mg/kg and 100 mg/kg. ZnO nanoparticles showed a positive effect on root traits; this is because with the increase in the concentration of ZnO nanoparticles, the root length and root mass also increased. ZnO nanoparticles did not show significant effect on shoot length (see Fig. 5 & Fig. 6). Statistical analysis by ANOVA using WASP2.0 revealed that the treatments are significant at 5% level of significance.

The results obtained were consistent with the findings of a previous study [32] in *Phaseolus radiates* and *Sorghum biocolor*, where varied silver nanoparticles toxicity caused by media was observed. Negative effect on seed germination of *B. nigra* by ZnO nanoparticles in culture media has been demonstrated [33]. Enhanced plant growth and development by ZnO nanoparticles in wheat has been reported [34]. TiO_2 nanoparticles showed varied effects on tomato when exposed through soil and aerosolized foliar spray; the foliar spray had a negative effect and soil means exposure enhanced the growth of tomato [35].

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Fig.2. *Eggplant seedlings with variations in root traits. Concentration of ZnO nanoparticles: Control – 0 mg/l, A-5 mg/l, B-10 mg/l, C-15 mg/l, D-20 mg/l*

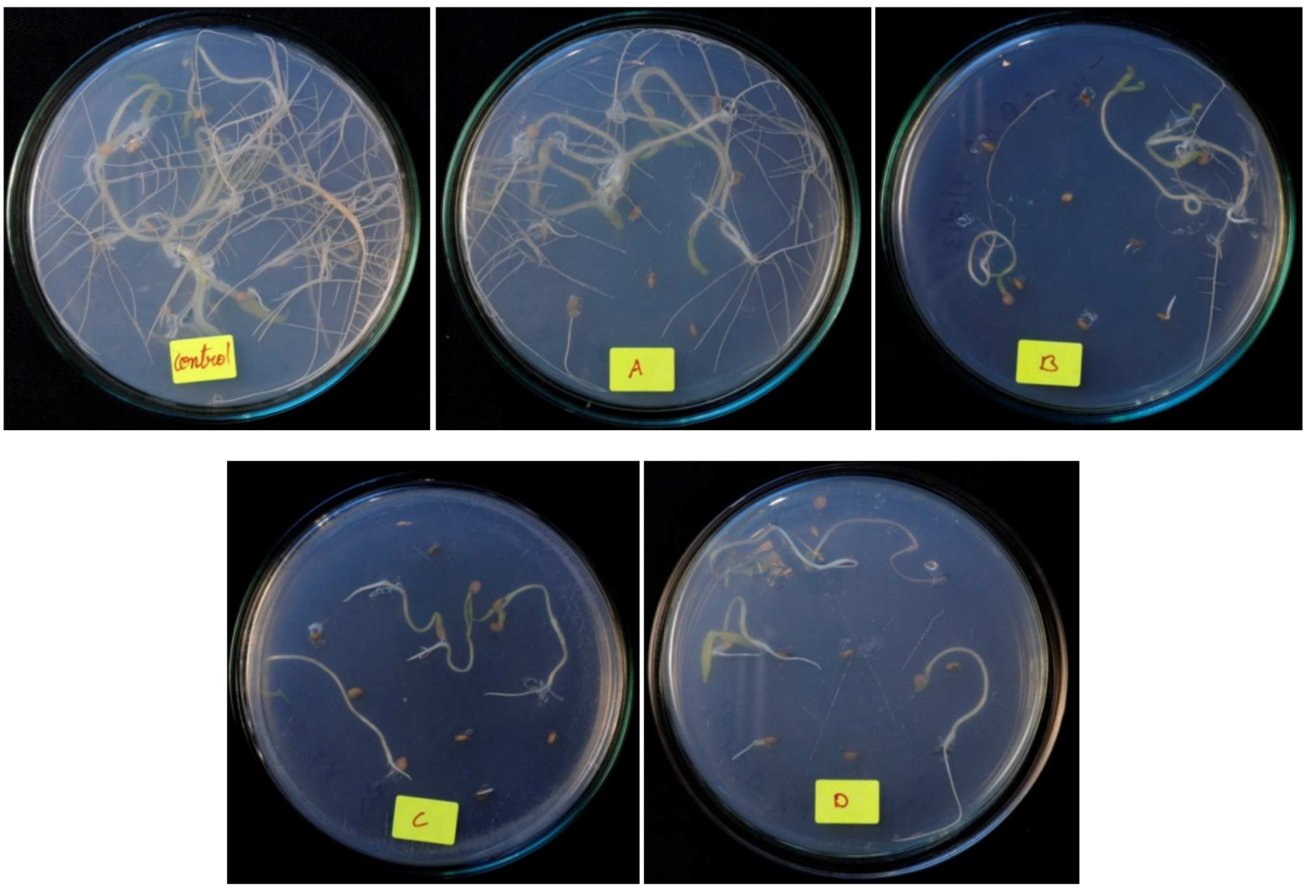

Fig.3. *View of seedlings of eggplant in different concentrations of ZnO nanoparticles. Concentration of ZnO nanoparticles: Control – 0 mg/l, A-5 mg/l, B-10 mg/l, C-15 mg/l, D-20 mg/l*

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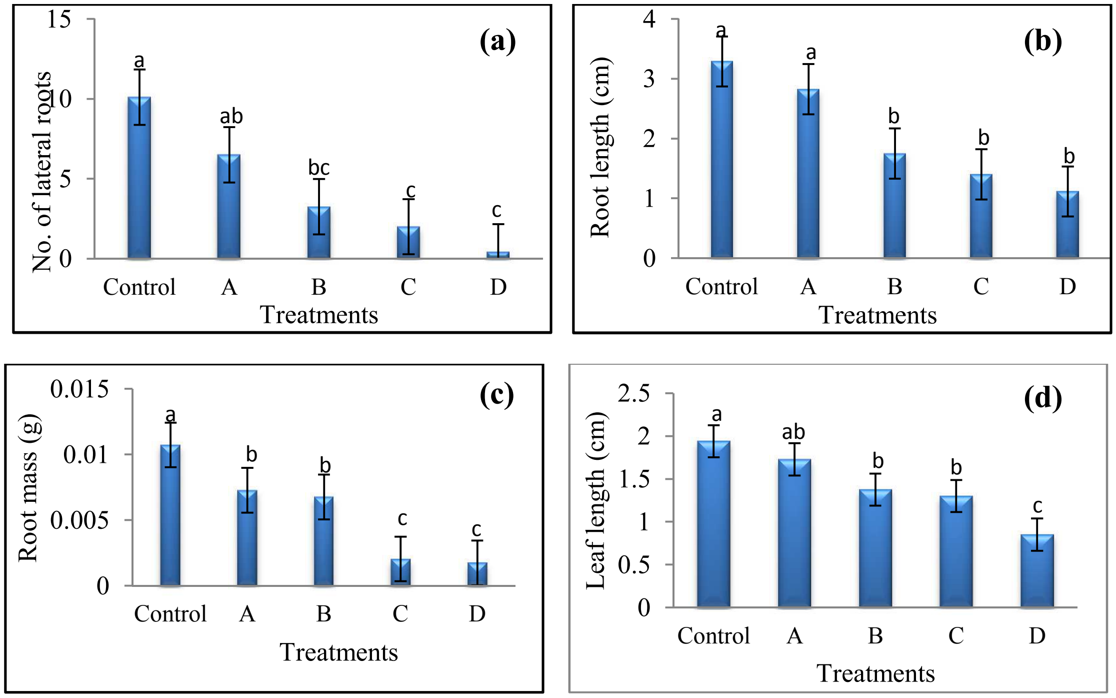

***Fig.4.** Effect of ZnO nanoparticles on (a) number of lateral roots, (b) root length, (c) root mass and (d) leaf length; Concentration of ZnO nanoparticles: Control – 0 mg/l, A-5 mg/l, B-10 mg/l, C-15 mg/l, D-20 mg/l; Error bars represent standard error. Different letters represent significant differences among treatments (p < 0.05).*

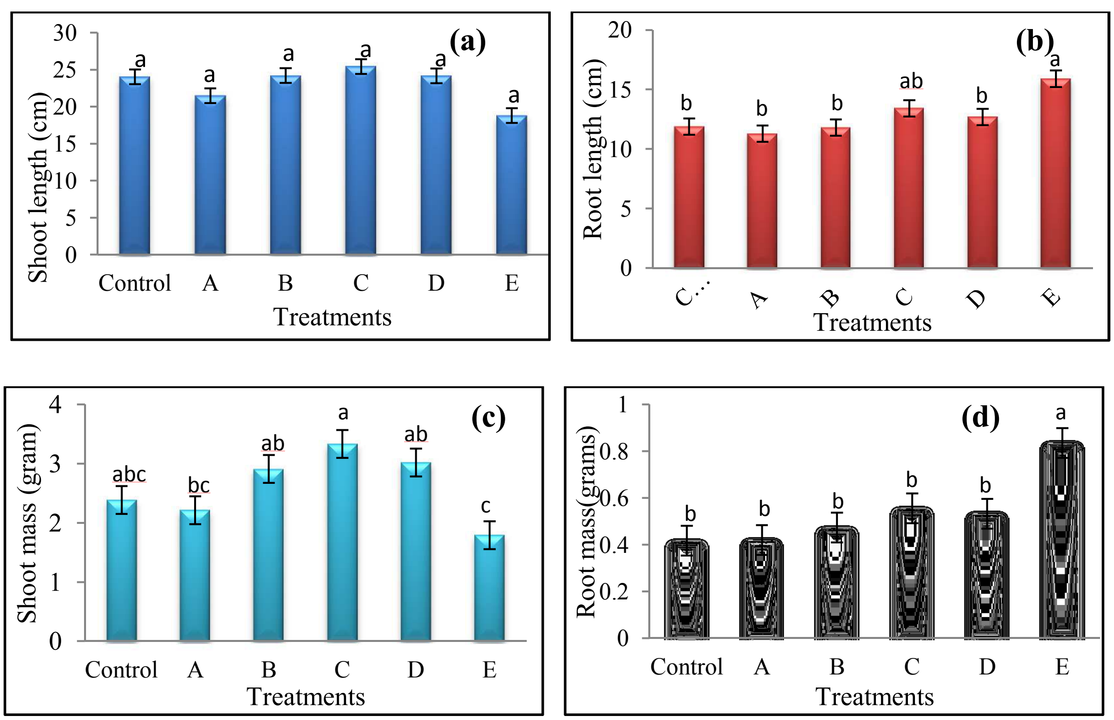

***Fig.5.** Effect of ZnO nanoparticles on (a) shoot length, (b)root length, (c)plant mass, (d)root mass; Concentration of ZnO nanoparticles: Control- 0 mg/kg, A- 5 mg/kg, B- 10 mg/kg, C- 15 mg/kg, D- 20 mg/kg, and E- 100 mg/kg; Error bars represent standard error. Different letters represent significant differences among treatments (p < 0.05).*

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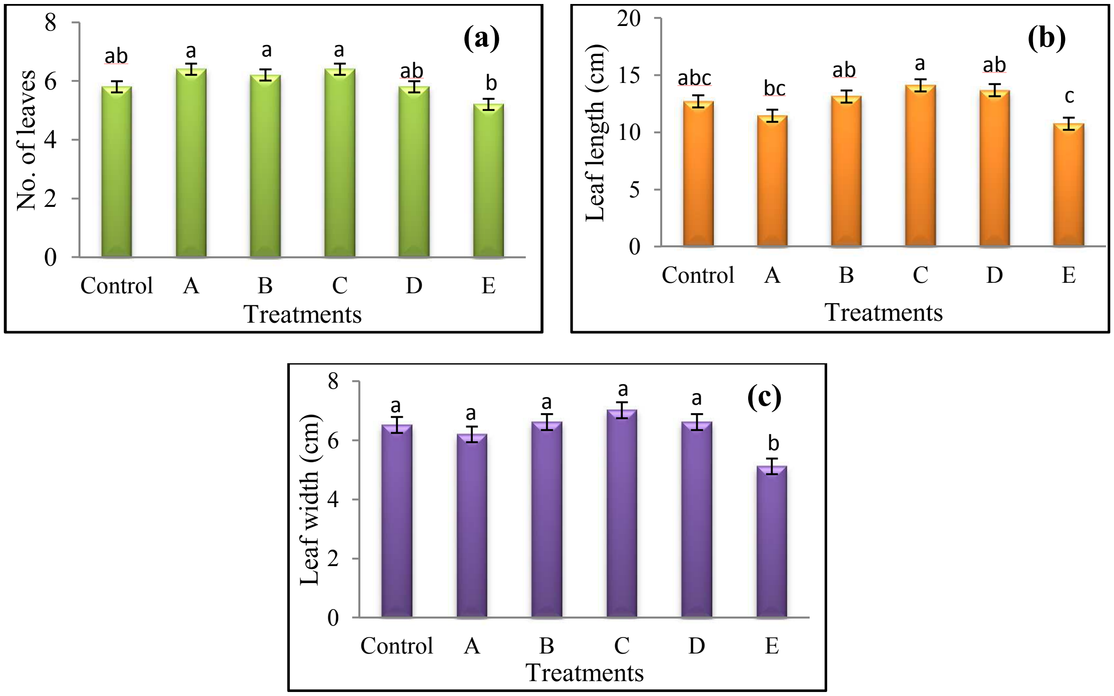


Fig.6. *Effect of ZnO nanoparticles on (a) number of leaves, (b) leaf length, (c)leaf width; Concentration of ZnO nanoparticles: Control- 0 mg/kg, A- 5 mg/kg, B- 10 mg/kg, C- 15 mg/kg, D- 20 mg/kg, and E- 100 mg/kg Error bars represent standard error. Different letters represent significant differences among treatments (p < 0.05).*

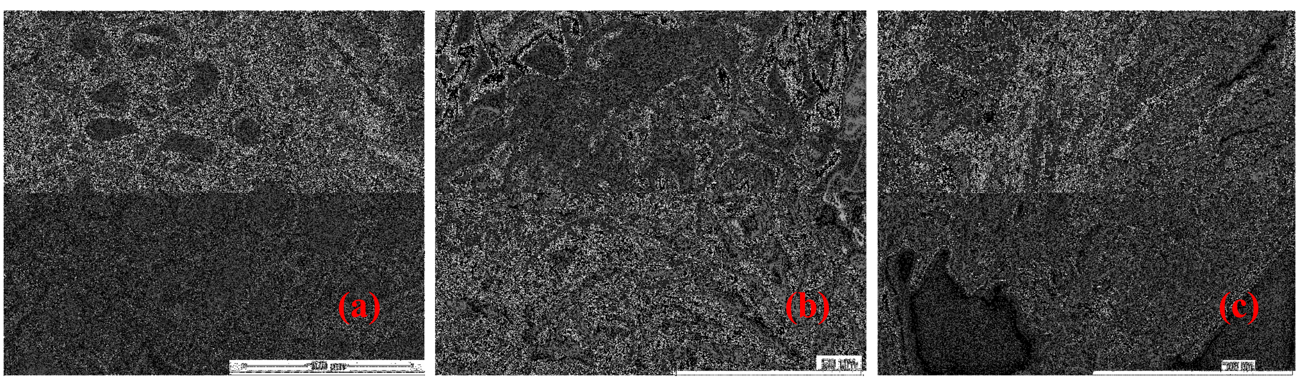
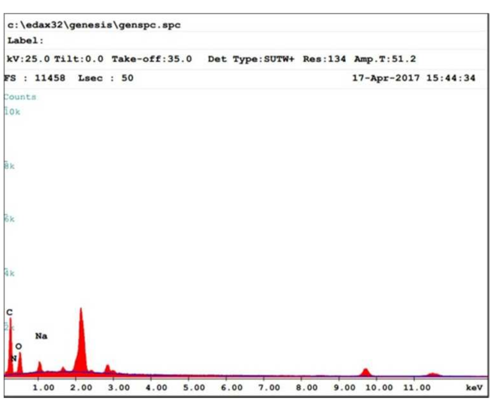


Fig.7. *SEM micrographs of control eggplant without ZnO nanoparticles (a) in vitro stem, (b) in vitro root, (c) in vitro leaf.*



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : User c:\edax32\eds\genuser.sec

Element	Wt %	At %	K-Ratio	Z	A	F
C K	58.16	65.13	0.3156	1.0085	0.5378	1.0003
N K	7.26	6.97	0.0080	1.0005	0.1104	1.0006
O K	30.03	25.24	0.0471	0.9933	0.1578	1.0001
NaK	4.54	2.66	0.0147	0.9318	0.3480	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	249.80	12.78	0.94	19.55
N K	8.68	14.56	10.02	0.60
O K	112.70	19.18	1.54	5.88
NaK	57.56	34.06	2.75	1.69

Fig.8. *Energy dispersive spectroscopy of control eggplant.*

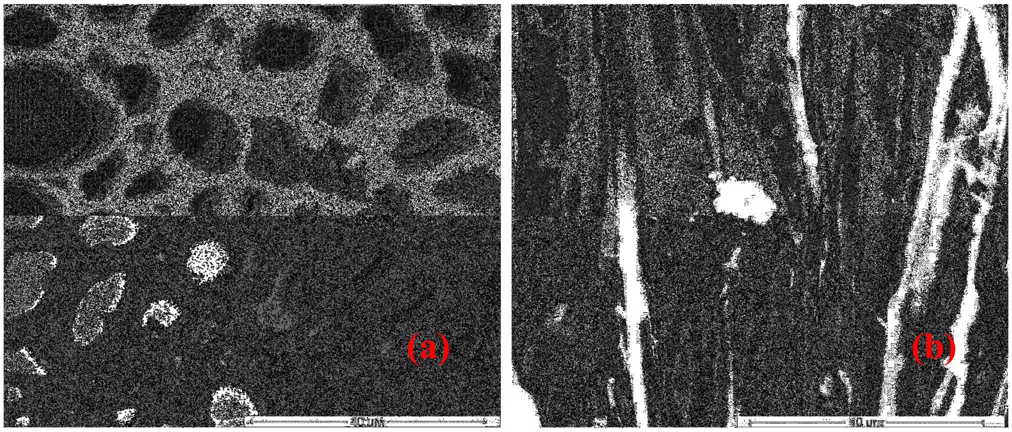
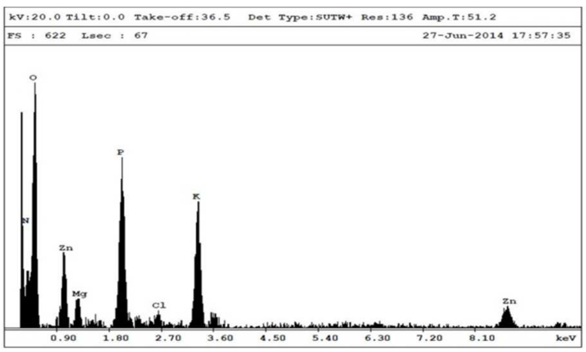


Fig.9. *SEM of in vitro cultured eggplant (a) stem and (b) root with ZnO nanoparticles accumulation*



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
N K	21.13	28.93	0.0498	1.0394	0.2267	1.0012
O K	46.12	55.29	0.1005	1.0311	0.2113	1.0003
MgK	2.20	1.73	0.0089	0.9893	0.4088	1.0017
P K	9.53	5.90	0.0707	0.9522	0.7763	1.0031
ClK	0.93	0.50	0.0075	0.9307	0.8559	1.0076
K K	8.89	4.36	0.0783	0.9373	0.9393	1.0000
ZnK	11.20	3.28	0.0949	0.8418	1.0073	1.0000
Total	100.00	100.00				

Fig.10. *Energy dispersive spectroscopy of observed ZnO nanoparticles of in vitro cultured eggplant*

3.2. Microscopic Studies:

Environmental scanning electron microscopy was used to detect the transportation and accumulation of ZnO nanoparticles in eggplant. Different plant samples like root, stem, and leaf of seedlings grown in MS media were freshly obtained, coated with gold for better conductivity and visualized for the detection of ZnO nanoparticles. Control samples of in vitro cultures without ZnO nanoparticles (see Fig.7) and the energy dispersive spectroscopy readings are given (see Fig.8). Triangularly shaped ZnO nanoparticles were detected in various parts of eggplant. The accumulation of ZnO nanoparticles was detected in stem and root (see Fig.9). Energy dispersive spectroscopy attached to scanning electron microscope was used to confirm the accumulated particles as ZnO nanoparticles (see Fig.10).

The samples from eggplant grown in soil media were obtained at the flowering stage. Various samples from leaf, stem, root and flower were collected for the detection of transportation and accumulation of ZnO nanoparticles in eggplant (see Fig.11 & Fig.12). It has been found that ZnO nanoparticles were accumulated in various aerial parts of eggplant like leaf, stem and flower as confirmed by energy dispersive spectroscopy (see Fig.13). This provided evidence for the possibility of ZnO nanoparticles accumulation in fruits. So there is a need for further research on ZnO nanoparticles accumulation in subcellular organelles and their effect on physiological and biochemical parameters. The accumulation of metal and metal oxide nanoparticles in edible crops is of particular concern because most of the nanoparticles are toxic to living organisms. Numerous reports have documented nanoparticles uptake either through the soil or foliar contact, where nanoparticles adhere to the root surface and enter epidermis and cortex by means of an apoplastic pathway [36][37][38][39][40][41]. However, the uptake and translocation of nanoparticles in plants may vary with plant species, cultivars, and growth conditions [42][43][44][45]. Multi-walled carbon nanotubes directly penetrated periwinkle (*Catharanthus roseus*) protoplasts [46], while single-walled carbon nanotubes labeled with fluorescein isothiocyanate entered Bright Yellow (BY-2) cells through endocytosis [47]. Carbon-coated magnetic nanoparticles could be taken up by the roots of crops (pea, sunflower, tomato, and wheat) and distributed in the whole plant [48].

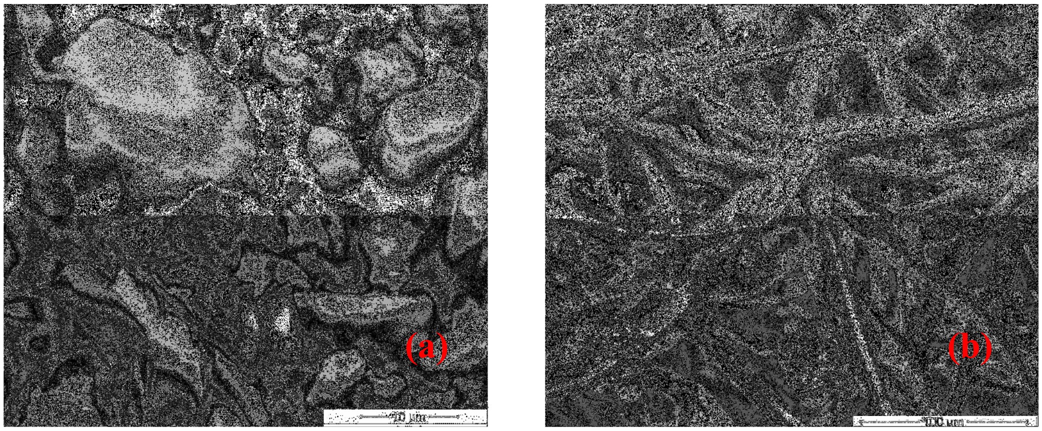


Fig.11. *SEM micrographs of eggplant with ZnO nanoparticles accumulation:* ***(a)*** *stem and* ***(b) leaf*** *of potted plan*

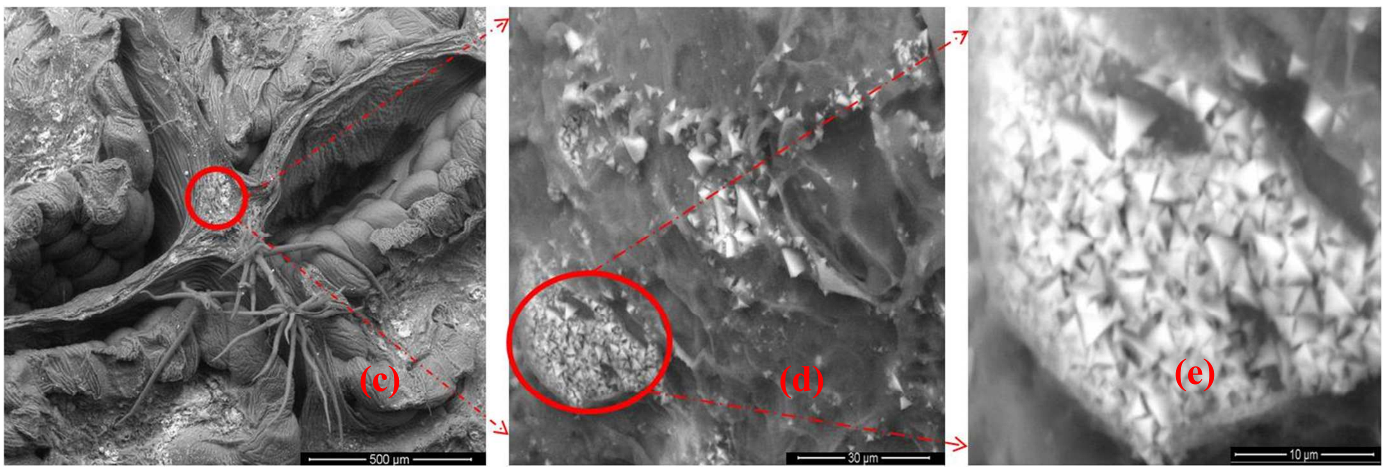
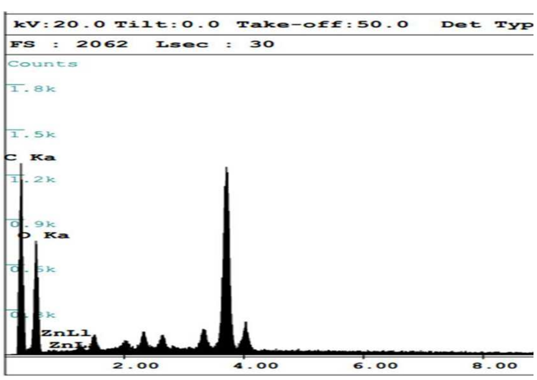


Fig.12. *SEM micrographs of eggplant with ZnO nanoparticles accumulation in* ***(c)(d)(e)*** *flower of potted plant with different magnifications.*



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	58.77	65.59	0.3807	1.0072	0.6429	1.0004
O K	41.01	34.36	0.0996	0.9904	0.2452	1.0000
ZnL	0.23	0.05	0.0011	0.8311	0.6066	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	243.96	0.92	1.17	264.57
O K	155.21	1.75	1.47	88.92
ZnL	1.12	6.36	60.28	0.18

Fig.13. *Energy dispersive spectroscopy of observed ZnO nanoparticles in eggplant of potted plant flower*

4. Conclusion:

The seed germination in this study was negatively affected by ZnO nanoparticles in MS-cultures and enhanced in soil medium. Plant growth was varied, showing a negative effect in culture media but positive effect in soil media. The uptake, transportations and accumulation of ZnO nanoparticles in both the media were observed in various parts of eggplant. Thus, it is clear that ZnO nanoparticles enter and accumulate in various parts of eggplant despite the use of growth media. The further effects of the accumulated ZnO nanoparticles on physiological and biochemical parameters on eggplant need to be investigated. To the best of our knowledge, this is the first paper to report the effect of seed germination, seedling growth, and accumulation of ZnO nanoparticles in eggplant.

5. Acknowledgements

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6. Reference:

[1]. Rogers, L., Safety fears over 'nano' anti-aging cosmetics. The Sunday Times. Available from: <http://www.timesonline.co.uk/tol/news/uk/article544891.ece> 17 July, 2005.

[2]. Lee, W.M., An, Y.J., Yoon, H. and Kweon, H.S.: 'Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles', Environmental toxicology and chemistry, 2008, 27(9), pp.1915-1921.

[3]. Lee, C.W., Mahendra, S., Zodrow, K., Li, D., Tsai, Y.C., Braam, J. and Alvarez, P.J.: 'Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*', Environmental Toxicology and Chemistry, 2010, 29(3), pp.669-675.

[4]. Dubchak, S., Ogar, A., Mietelski, J.W. and Turnau, K.: 'Influence of silver and titanium nanoparticles on arbuscular mycorrhiza colonization and accumulation of radiocaesium in *Helianthus annuus*', Spanish Journal of Agricultural Research, 2010, 8(S1), pp.103-108.

[5]. Khodakovskaya, M.V., de Silva, K., Nedosekin, D.A., Dervishi, E., Biris, A.S., Shashkov, E.V., Galanzha, E.I. and Zharov, V.P.: 'Complex genetic, photothermal, and photoacoustic analysis of nanoparticle-plant interactions', Proceedings of the National Academy of Sciences, 2011, 108(3), pp.1028-1033.

[6]. Gottschalk, F. and Nowack, B.: 'The release of engineered nanomaterials to the environment', Journal of Environmental Monitoring, 2011, 13(5), pp.1145-1155.

[7]. Wang, L.J., Guo, Z.M., Li, T.J. and Li, M.: 'The nano structure SiO2 in the plants', Chin. Sci. Bull, 2011, 46(8), pp.625-631.

[8]. Menard, A., Drobne, D. and Jemec, A.: 'Ecotoxicity of nanosized TiO 2. Review of in vivo data', Environmental Pollution, 2011, 159(3), pp.677-684.

[9]. Rizwan, M., Ali, S., Qayyum, M.F., Ok, Y.S., Adrees, M., Ibrahim, M., Zia-ur-Rehman, M., Farid, M. and Abbas, F.: 'Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review', Journal of hazardous materials, 2017, 322, pp.2-16.

[10]. Zhang, W., Ebbs, S.D., Musante, C., White, J.C., Gao, C. and Ma, X.: 'Uptake and accumulation of bulk and nanosized cerium oxide particles and ionic cerium by radish (*Raphanus sativus L.*)', Journal of agricultural and food chemistry, 2015, 63(2), pp.382-390.

[11]. Ali A, Ambreen S, Javed R, Tabassum S, ul Haq I, Zia M.: 'ZnO nanostructure fabrication in different solvents transforms physio-chemical, biological and photodegradable properties', Materials Science and Engineering: C, 2017, 74, PP.137-145.

[12]. Siddiqui, M.H. and Al-Whaibi, M.H.: 'Role of nano-SiO 2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.)', Saudi journal of biological sciences, 2014, 21(1), pp.13-17.

[13]. Auffan, M., Santaella, C., Thiéry, A., Paillès, C., Rose, J., Achouak, W., Thill, A., Masion, A., Wiesner, M. and Bottero, J.Y.: 'Ecotoxicity of inorganic nanoparticles: From unicellular organisms to invertebrates', in Encyclopedia of Nanotechnology (Springer Netherlands, 2012), pp. 623-636

[14]. Serpone, N., Dondi, D. and Albini, A.: 'Inorganic and organic UV filters: Their role and efficacy in sunscreens and suncare products', Inorganica Chimica Acta, 2007, 360(3), pp.794-802.

[15]. Goswami, A., Roy, I., Sengupta, S. and Debnath, N.: 'Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens', Thin solid films, 2010, 519(3), pp.1252-1257.

[16]. Rai, M. and Ingle, A.: 'Role of nanotechnology in agriculture with special reference to management of insect pests', Applied microbiology and biotechnology, 2012, 94(2), pp.287-293.

[17]. Kah, M. and Hofmann, T.: 'Nanopesticide research: current trends and future priorities', Environment international, 2014, 63, pp.224-235.

[18]. Mahajan, P., Dhoke, S.K. and Khanna, A.S.: 'Effect of nano-ZnO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method', Journal of Nanotechnology, 2011.

[19]. Raliya, R., Nair, R., Chavalmane, S., Wang, W.N. and Biswas, P.: 'Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum L.*) plant', Metallomics, 2015, 7(12), pp.1584-1594.

[20]. Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K.R., Sreeprasad, T.S., Sajanlal, P.R. and Pradeep, T.: 'Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut', Journal of plant nutrition, 2012, 35(6), pp.905-927.

[21]. Yang Z, Chen J, Dou R, Gao X, Mao C, Wang L.: 'Assessment of the phytotoxicity of metal oxide nanoparticles on two crop plants, maize (Zea mays L.) and rice (Oryza sativa L.)', International journal of environmental research and public health, 2015, 12(12), pp.15100-15109.

[22]. Bradfield, S.J., Kumar, P., White, J.C. and Ebbs, S.D.: 'Zinc, copper, or cerium accumulation from metal oxide nanoparticles or ions in sweet potato: yield effects and projected dietary intake from consumption', Plant Physiology and Biochemistry, 2017, 110, pp.128-137.

[23]. Lin, D. and Xing, B.: 'Phytotoxicity of nanoparticles: inhibition of seed germination and root growth', Environmental Pollution, 2007, 150(2), pp.243-250.

[24]. Lin, D. and Xing, B.: 'Root uptake and phytotoxicity of ZnO nanoparticles', Environmental science & technology, 2008, 42(15), pp.5580-5585.

[25]. Javed R, Zia M, Yücesan B, Gürel E.: 'Abiotic stress of ZnO-PEG, ZnO-PVP, CuO-PEG and CuO-PVP nanoparticles enhance growth, sweetener compounds and antioxidant activities in shoots of Stevia rebaudiana Bertoni', IET Nanobiotechnology, 2017, 11(7), PP.898-902.

[26]. Kumari, M., Khan, S.S., Pakrashi, S., Mukherjee, A. and Chandrasekaran, N.: 'Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*', Journal of hazardous materials, 2011, 190(1), pp.613-621.

[27]. Panda, K.K., Golari, D., Venugopal, A., Achary, V.M.M., Phaomei, G., Parinandi, N.L., Sahu, H.K. and Panda, B.B.: 'Green Synthesized Zinc Oxide (ZnO) Nanoparticles Induce Oxidative Stress and DNA Damage in *Lathyrus sativus L*. Root Bioassay System', Antioxidants, 2017, 6(2), p.35.

[28]. Ghosh, M., Jana, A., Sinha, S., Jothiramajayam, M., Nag, A., Chakraborty, A., Mukherjee, A. and Mukherjee, A.: 'Effects of ZnO nanoparticles in plants: Cytotoxicity, genotoxicity, deregulation of antioxidant defenses, and cell-cycle arrest', Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2016, 807, pp.25-32.

[29]. Ellis, R.H. and Roberts, E.H.: 'The quantification of ageing and survival in orthodox seeds. Seed Science and Technology' (Netherlands, 1981).

[30]. Kochian, L.V.: 'Molecular physiology of mineral nutrient acquisition, transport, and utilization', Biochemistry and molecular biology of plants, 2000, pp.1204-1249.

[31]. López-Moreno, M.L., de la Rosa, G., Hernández-Viezcas, J.Á., Castillo-Michel, H., Botez, C.E., Peralta-Videa, J.R. and Gardea-Torresdey, J.L.: 'Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants', Environmental science & technology, 2010, 44(19), pp.7315-7320.

[32]. Lee, W.M., Kwak, J.I. and An, Y.J.: 'Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: media effect on phytotoxicity', Chemosphere, 2012, 86(5), pp.491-499.

[33]. Zafar, H., Ali, A., Ali, J.S., Haq, I.U. and Zia, M.: 'Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: growth dynamics and antioxidative response', Frontiers in plant science, 2016, 7.

[34]. Ramesh, M., Palanisamy, K., Babu, K. and Sharma, N.K.: 'Effects of bulk & nano-titanium dioxide and zinc oxide on physio-morphological changes in *Triticum aestivum Linn*', J Glob Biosci, 2014, 3, pp.415-422.

[35]. Raliya, R., Nair, R., Chavalmane, S., Wang, W.N. and Biswas, P.: 'Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum L.*) plant', Metallomics, 2015, 7(12), pp.1584-1594.

[36]. Rui, Y., Gui, X., Li, X., Liu, S. and Han, Y.: 'Uptake, transport, distribution and bio-effects of SiO 2 nanoparticles in Bt-transgenic cotton', Journal of nanobiotechnology, 2014, 12(1), p.50.

[37]. Servin, A.D., Castillo-Michel, H., Hernandez-Viezcas, J.A., Diaz, B.C., Peralta-Videa, J.R. and Gardea-Torresdey, J.L.: 'Synchrotron micro-XRF and micro-XANES confirmation of the uptake and translocation of TiO2 nanoparticles in cucumber (*Cucumis sativus*) plants', Environmental science & technology, 2012, 46(14), pp.7637-7643.

[38]. Shi, J., Peng, C., Yang, Y., Yang, J., Zhang, H., Yuan, X., Chen, Y. and Hu, T.: 'Phytotoxicity and accumulation of copper oxide nanoparticles to the Cu-tolerant plant *Elsholtzia splendens*', Nanotoxicology, 2014, 8(2), pp.179-188.

[39]. Zhu, H., Han, J., Xiao, J.Q. and Jin, Y.: 'Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants', Journal of Environmental Monitoring, 2008, 10(6), pp.713-717.

[40]. Zhou, D., Jin, S., Li, L., Wang, Y. and Weng, N.: 'Quantifying the adsorption and uptake of CuO nanoparticles by wheat root based on chemical extractions', Journal of Environmental Sciences, 2011, 23(11), pp.1852-1857.

[41]. Zhao, L., Peng, B., Hernandez-Viezcas, J.A., Rico, C., Sun, Y., Peralta-Videa, J.R., Tang, X., Niu, G., Jin, L., Varela-Ramirez, A. and Zhang, J.Y.: 'Stress response and tolerance of *Zea mays* to CeO2 nanoparticles: cross talk among H2O2, heat shock protein, and lipid peroxidation', ACS nano, 2012, 6(11), pp.9615-9622.

[42]. Zhao, L., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Bandyopadhyay, S., Peng, B., Munoz, B., Keller, A.A. and Gardea-Torresdey, J.L.: 'ZnO nanoparticle fate in soil and zinc bioaccumulation in corn plants (*Zea mays*) influenced by alginate', Environmental Science: Processes & Impacts, 2013, 15(1), pp.260-266.
[43]. Du, W., Sun, Y., Ji, R., Zhu, J., Wu, J. and Guo, H.: 'TiO 2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil', Journal of Environmental Monitoring, 2011, 13(4), pp.822-828.
[44]. Slomberg, D.L. and Schoenfisch, M.H.: 'Silica nanoparticle phytotoxicity to *Arabidopsis thaliana*', Environmental science & technology, 2012, 46(18), pp.10247-10254.
[45]. Li, X., Gui, X., Rui, Y., Ji, W., Yu, Z. and Peng, S.: 'Bt-transgenic cotton is more sensitive to CeO 2 nanoparticles than its parental non-transgenic cotton', Journal of hazardous materials, 2014, 274, pp.173-180.
[46]. Serag, M.F., Kaji, N., Venturelli, E., Okamoto, Y., Terasaka, K., Tokeshi, M., Mizukami, H., Braeckmans, K., Bianco, A. and Baba, Y.: 'Functional platform for controlled subcellular distribution of carbon nanotubes', ACS nano, 2011, 5(11), pp.9264-9270.
[47]. Liu, Q., Chen, B., Wang, Q., Shi, X., Xiao, Z., Lin, J. and Fang, X.: 'Carbon nanotubes as molecular transporters for walled plant cells', Nano letters, 2009, 9(3), pp.1007-1010.
[48]. Cifuentes, Z., Custardoy, L., de la Fuente, J.M., Marquina, C., Ibarra, M.R., Rubiales, D. and Pérez-de-Luque, A.: 'Absorption and translocation to the aerial part of magnetic carbon-coated nanoparticles through the root of different crop plants', Journal of nanobiotechnology, 2010, 8(1), p.26.