Environmental Technology & Innovation 14 (2019) 100325

Contents lists available at ScienceDirect

Environmental Technology & Innovation

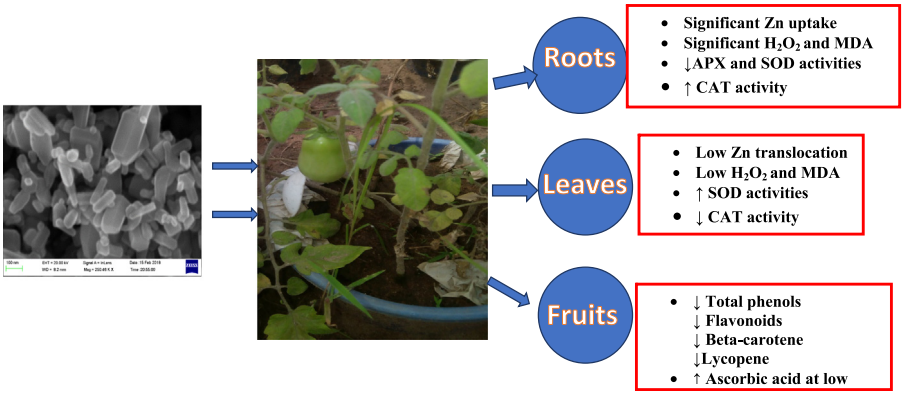
journal homepage: www.elsevier.com/locate/eti

Phytotoxicity of nano-zinc oxide to tomato plant (*Solanum lycopersicum* L.): Zn uptake, stress enzymes response and influence on non-enzymatic antioxidants in fruits

Mariam Abiola Akanbi-Gada [a,b], Clement O. Ogunkunle [a,*], Vinita Vishwakarma [c], Kanagasabai Viswanathan [c], Paul O. Fatoba [a]

[a] Environmental Biology unit, Department of Plant Biology, University of Ilorin, Ilorin 240003, Nigeria
[b] Department of Biological Sciences, Kwara State University, Malete, Nigeria
[c] Centre for Nanoscience and Nanotechnology, Sathyabama Institute of Science and Technology, Chennai 600119, India

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Zn uptake was significant in roots with reduced translocation to shoots.
- Increased APX and SOD activity levels, coupled with lipid peroxidation in roots.
- Contents of non-enzymatic antioxidants were significantly altered in fruits.

ARTICLE INFO

Article history:
Received 13 October 2018
Received in revised form 21 January 2019

ABSTRACT

The production of metallic nanoparticles is greatly increasing due to its wide range of applications in agricultural formulations. The present pot experiment investigated the uptake of Zn from nano-zinc oxide (n-ZnO)-amended soil at 300, 600 and 1000 mg n-ZnO/kg concentrations, and its effects on the enzymatic and non-enzymatic antioxidants

Abbreviations: APX, Ascorbate peroxidase; SOD, Superoxide dismutase; CAT, Catalase

* Corresponding author.
E-mail address: ogunkunle.co@unilorin.edu.ng (C.O. Ogunkunle).

https://doi.org/10.1016/j.eti.2019.100325
2352-1864/© 2019 Elsevier B.V. All rights reserved.

Accepted 31 January 2019
Available online 7 February 2019

Keywords:
Nano-zinc oxide
Oxidative stress
Stress enzymes
Zinc
Solanum lycopersicum
Antioxidants

in tomato tissues and fruits respectively. Results showed that root uptake of Zn increased with increasing n-ZnO concentrations. The enzyme activity showed that n-ZnO, through the generation of H_2O_2 and induction of oxidative stress, significantly reduced the activity of stress-controlling enzymes (APX and SOD) in the root. Conversely in leaves, despite alteration in chlorophylls in the early growing stage, APX activity was only significant at 1000 mg n-ZnO/kg) while SOD activity was enhanced at all treatments. CAT activity was significantly reduced, unlike in the roots where CAT activity was significantly enhanced. Contents of total phenols, flavonoids, β-carotene and lycopene in fruits were significantly reduced by at least 4.8% while ascorbic acid was promoted at low n-ZnO treatments. In conclusion, the toxic effect of n-ZnO on stress enzymes was prominent in tomato roots, and there was also inhibitory effect on induction of non-enzymatic antioxidants in the tomato fruits.

© 2019 Elsevier B.V. All rights reserved.

1. Introduction

Agriculture is the main nexus between humans and the environment, and portends major cause of soil and ecosystem modification. Agriculture is also an economic activity that is recently engaging the applications of nanotechnology and consequently leading to a new emission source of nanoparticles and nanomaterials into the environment and food chains (Scott and Chen, 2013). Nanoparticles have great potential to improve the agricultural productivity, especially in the area of nano-fertilizers (NF). Mineral nutrients encapsulated in NF forms promote plant nutrition (Fedorenko et al., 2015; Mohammad et al., 2017), and offer greater solubility with rapid absorption or less leaching, compared with conventional fertilizers. It is imperative to state that the problem associated with plants' mineral nutrition is not actually the amount of element (s) present in the soil but rather their phytoavailability (Plibeam, 2015). For this reason, the encapsulation of fertilizers and other compounds in inorganic nanomaterials such as nanotubes of carbon and metals has been explored (Liu et al., 2009).

Nanoparticles (NPs) are introduced into soil environment from multiple human activities, and the accompanying eco-risks have not been fully understood (Cai et al., 2006). Zinc nanoparticles (Zn NPs) are among the most commonly used nanomaterials, especially in agriculture, cosmetology, medicine and as antibacterial agent. Zinc oxide nanoparticles (ZnO NPs) are reported to possess efficient screen for a broader UV range than any of the molecular UV-absorbers, photocatalytic degradation of environment pollution, biocompatibility in drug delivery mechanisms and as nanofertilizers and nanopesticides (Kulkarni and Muddapur, 2014; Sabir et al., 2014).

However, aside the potential importance, ZnO NPs pose increased ecological and human health risks because of possible interaction with various components of the environment (Chithrani et al., 2006). The presence of elevated content of ZnO NPs in soil may affect plant growth and functioning due to its potential toxicity when internalized and accumulated in large amounts in plant tissues (Ma et al., 2009, 2011). Several studies have reported the toxicity potential of ZnO NPs to crop plants such as broad bean (Manzo et al., 2011), lettuce, radish, cucumber (Lin and Xing, 2007), wheat Du et al. (2011) and zucchini (Stampoulis et al., 2009).

Tomato is of great importance worldwide with more than 80% of grown tomatoes processed into edible products such as tomato juice, paste, sauce etc. (Kadar et al., 1977). Fruit of tomato is rich in nutrients such as organic acids, sugars, dietary fiber, pectic substances, proteins, minerals, vitamins, and carotenoids possessing antioxidant qualities (lycopene, β-carotene, etc.). Although, the toxicity of nanoparticles has been evaluated through a variety of routes such foliar and hydroponic applications, to the best of the authors knowledge, no study has evaluated the effect of nano-zinc oxide (particle size $<$ 100 nm) through particulate application via soil medium on tomato plant and its fruits. Therefore, the study aimed to (i) investigate the uptake of Zn and bioaccumulation, (ii) evaluate the response of stress enzymes in roots and leaves to n-ZnO and, (iii) assess the level of selected non-enzymatic antioxidants in the fruit of tomato grown in nano-ZnO-amended soil. It is the authors' belief that the results of the experiment would further enrich scientific information on the ZnO NPs-plant system interactions.

2. Materials and methods

2.1. Characterization and growth experimental

Nano-zinc oxide (n-ZnO) particles ($<$100 nm) was purchased from (Sigma-Aldrich, USA) in powder form with properties reported in **Table S1.** Further morphological characteristics of the particles was carried using the field emission scanning electron microscopy (model type-FESEM-Carl Zeiss, SUPRA® 55 equipped with GEMINI® Technology) coupled with an EDX spectroscopy (OXFORD-X Act). Test soil used in this study was collected from the topsoil layer (0–15 cm) at University of Ilorin Botanical garden, Ilorin (Nigeria), air-dried and sieved through a-2 mm mesh. Selected properties of the soil have earlier been reported in Ogunkunle et al. (2018) and presented in **Table S2**. The study was performed in a screen house under normal

environmental conditions at the Botanical garden to simulate real effects of nanoparticles in the natural environment. The sieved soils were potted as 1.7 kg dry soil/pot and a total of 16 pots were used for the experiment. The potted soils except the control were treated with varying concentrations of nano-zinc oxide (300, 600 and 1000 mg n-ZnO/kg) as dry powders and allowed to agglomerate for 48 hr before planting. The maximum concentration of 1000 mg/kg was adopted based on ISO guideline 11269–2 recommendation for ecotoxicological test of chemical substances (ISO, 2012). The application of n-ZnO to the dry soils followed the method described by Ogunkunle et al. (2018). Seeds of tomato (*Solanium lycopersicum* L.*)* used for the study were initially sterilized by soaking in sodium hypochlorite solution for 10 min (USEPA, 1996). Tomato seeds were initially broadcast on the potted soils and after 2 weeks of growth, seedlings were thinned to two plants per pot. Plants were allowed to grow under normal environmental conditions and irrigated with tap water as required by the plants. The experiment lasted for 90 days after which plant samples were collected for analysis.

2.2. Determination of chlorophyll and Zn contents in plant

Determination of chlorophyll contents was carried out using acetone incubation method of Makeen et al. (2007), and described in Supplementary Information 1 (**SI 1**). Samples of root and shoot that were collected from n-ZnO treated and control plants were dried at 60 °C for 24 h and finely powdered using ceramic mortar and pestle. 1 g of sample was then digested with 9 ml concentrated HNO_3 and 3 ml HCl. The sample was heated on a hot plate until all brownish fumes were expelled out and solution filtered. The filtrate was diluted to 25 ml-mark with a deionized water and analyzed for Zn content by flame atomic absorption spectrophotometry (Bulk Scientific- ACCUSYS 211) using an air-acetylene flame. All samples were extracted and analyzed in triplicate using the above procedure. Detection limit for Zn was 0.005 mg/l and FAAS calibration for the element was done using a working standard solution of Zn and calibration graph with correlation coefficient (r^2) > 0.995. Replicate digestion and the use of reference material (IAEA 359-cabbage leaves) from International Atomic Energy Agency were carried out to ensure quality control and quality assurance. The percentage recovery from analysis of reference material ranged from 95%–99% for the analyzed elements

2.3. Determination of hydrogen peroxide (H_2O_2) content and malondialdehyde (MDA)

To determine the H_2O_2 content according to Islam et al. (2008), fresh sample leaf or root (1 g) was homogenized with 3 ml of 0.1% (w/v) trichloroacetic acid (TCA) using a vortex. The homogenized solution was later centrifuged at 12,000 x g for 30 min at 4 °C and supernatant was decanted to measure the H_2O_2 content. Supernatant (∼0.5 ml) was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The concentration of H_2O_2 was taken in a spectrophotometer at 390 nm wavelength. Lipid peroxidation, determined as malondialdehyde (MDA) content in sample was determined by homogenizing 0.5 g of fresh plant sample (root/leaf) with 3 ml of 0.5% thiobarbituric acid in 20% thiochloroacetic acid (w/v) using a ceramic pestle and mortar according to Li (2000). The reacting mixture was incubated for 30 min at 100 °C and the resulting solution was centrifuged for 10 min at 10 000 x g before the absorbance was measured spectrophotometrically at wavelength of A_{450} A_{532} and A_{600}.

2.4. Antioxidant enzyme activities and bioactive compounds

For ascorbate peroxidase-EC 1.11.1.11 (APX) activity, 1 g of fresh sample (root/leaf) was homogenized in 4 μl 0.5 Mm ascorbate, 886 μl 50 mM potassium phosphate buffer of pH 7.4 and 10 μl 0.2 mM H_2O_2, according to Nakano and Asada (1981). The absorbance was read for 3 min at 290 nm with an extinction coefficient of 2.8 mM cm^{-1}. For the determination of catalase- EC 1.11.1.6 (CAT) and superoxide dismutase- EC 1.15.11 (SOD), the buffers were prepared according to Lee and Lee (2000). 1 g of sample (root/leaf) was homogenized in 100 mM potassium phosphate buffer of pH 7.8 that contained 0.1 mM ethylenediamine-tetraacetic acid (EDTA), 0.5% Triton X-100 and 1% polyvinyl-pyrrolidone (PVP) at 4 °C. The resulting homogenate was filtered and centrifuged at 18,000 x g for 20 min at 4 °C. The supernatant was collected and used appropriately for CAT and SOD determination. CAT activity in plant tissues (root and leaf) was determined according to Aebi (1974). A reaction mixture of 0.98 ml 10 mM H_2O_2 in 25 mM phosphate buffer with 20 μl of crude extract was assayed and absorbance was taken at wavelength of 240 nm with extinction coefficient of 39.4 mM cm^{-1}. Activity of SOD plant sample was determined (Beyer and Fridovich, 1987) using a reaction assay of 3 ml containing 450 μl of 500 μM nitroblue tetrazolium (NBT), 0.5 ml of 78 mM methionine, 300 μl of 0.02 mM riboflavin, 0.2 ml of 1.5 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer (pH 7.8) and 50 μl crude extract. The absorbance was taken spectrophotometrically at wavelength of 560 nm for 5 min. The amount of enzyme extract that produced 50% inhibition of NBT is considered a unit of SOD.

Non-antioxidant enzymes such as polyphenols (total phenols and flavonoids), provitamin A (Beta-carotene and lycopene) and ascorbic acid in mature green fruits were analyzed according to conventional methods and described in Supplementary Information 2 (**S2**).

All absorbance for determination of enzyme activities and bioactive compounds were taken using a UV/VIS spectrophotometer (2300 model, Segar Scientific, Japan).

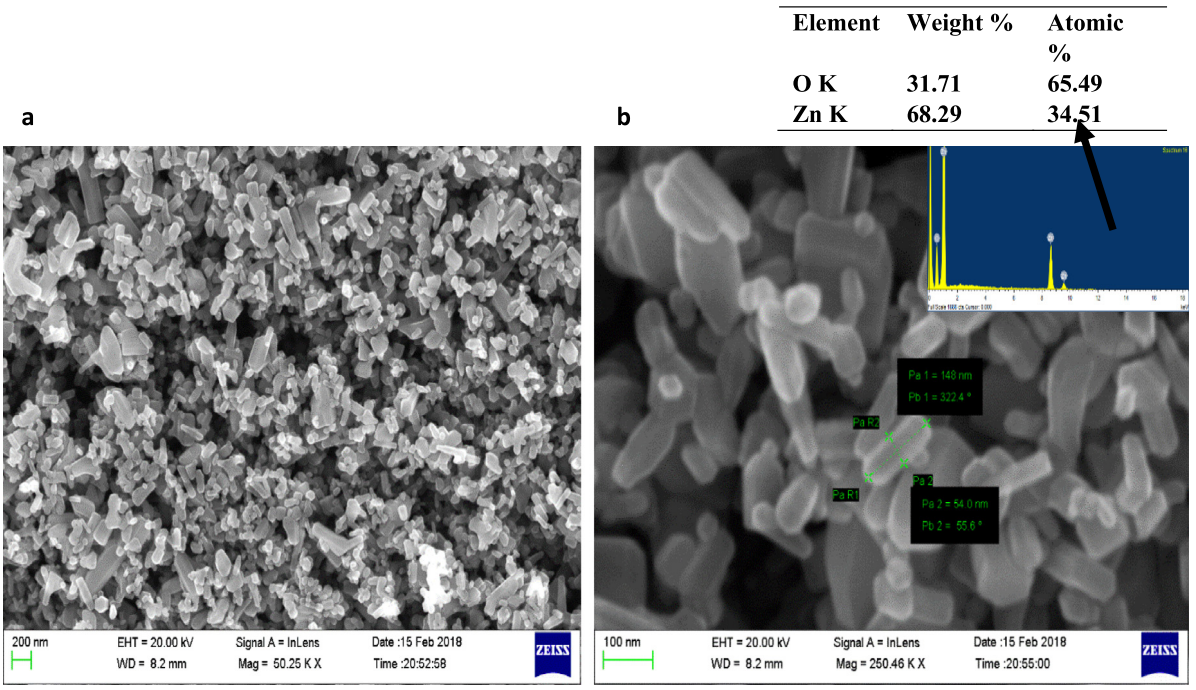


Fig. 1. FESEM micrograph of n-ZnO particles used for the experiment at (a) low and (b) high magnifications. (Inset: Energy-dispersive X-ray spectroscopy (EDS) of n-ZnO particles.)

2.5. Statistical analysis

Minitab® 17.1.0 software was used to carry out a one-way ANOVA test to evaluate the experimental variance. Significant means among treatments were separated by Turkey test at a $p < 0.05$. Data presented are means of triplicates and graphs were presented using OriginPro 8 SR0 (OriginLab Corporation, Northampton, USA).

3. Results discussion

3.1. Characterization of n-ZnO particles

FESEM of nano-zinc oxide particle at 50× and 250× magnifications are presented in Fig. 1a & b. The micrograms revealed uniform nano-rod morphology for nano-ZnO. The observed average length and length of n-ZnO was (148 nm) and (54.0 nm), respectively. Jahan et al. (2018) also revealed from FESEM examination that ZnO nanomaterials have uniform nano-rod morphology, though the dimension varied from the result of our study due to different methods of production (nanomaterial was synthesized).

3.2. Effect of n-ZnO on Zn accumulation in roots and shoots

Zinc concentrations in dry tissue biomass of tomato are shown in Fig. 2. Zinc accumulation in root was dose-dependent and plants treated with high doses (600 and 1000 mg n-ZnO/kg respectively) had nearly 8.4- and 12-fold Zn contents in roots compared to control. Similar dose-dependent Zn accumulation was also observed in tomato shoots but to a lesser magnitude compared to roots. Highest Zn content of shoot was obtained in plants treated with both 1000 and 600 mg n-ZnO/kg concentrations and average accumulation was approximately 12-fold greater than Zn content in control. Zn accumulation in plants, especially from ZnO NPs has always been observed to be dose-dependent and this has been reported by several authors lately. For instance, Hernandez-Viezcas et al. (2013) reported a dose-dependent Zn accumulation in soybean treated with several concentrations of ZnO nanoparticles. Also, Zhao et al. (2012) and Mukherjee et al. (2013) have observed several fold-increase in Zn uptake by corn and green pea plants, grown with 800 mg/kg ZnO NP and 500 mg/kg ZnO NP, respectively. The high uptake and relative accumulation, despite the low soil Zn background content (Table S2), could be linked to great dissolution of the ZnO NPs in the slightly acidic soil. This possibly offered high phytoavailability of Zn to the plant in acidic soil. García-Gómez et al. (2017) reported that ZnO NPs dissolve faster in acidic soil due to high specific area of the nanoparticles released the ions in soil prior to accumulation by tomato plants. Higher shoot accumulation of Zn in plants exposed to ZnO

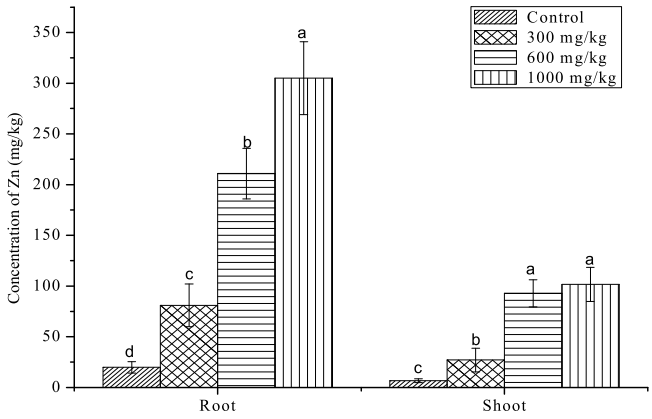


Fig. 2. Content of Zn in root and shoot tissues of *Solanum lycopersicum* L. after 90-day exposure to n-ZnO. Error bar represents standard deviation and means separated by Turkey test at $P < 0.05$.

NPs have also been reported by Liu et al. (2015) and Zhao et al. (2013), though soil medium was of alkaline nature (pH 8.2 and 7.6, respectively).

The increase in shoot Zn contents as compared to control is an indication that n-ZnO were readily phytoavailable to tomato in slightly acidic soil, though the translocation factor (defined as the ratio of Zn concentration in shoot to root) were low (0.34, 0.43 and 0.33 respectively for 300 mg /kg, 600 mg/kg and 1000 mg/kg n-ZnO treatments). The observed low Zn translocation to shoots could be due to the ability of n-ZnO to effectively bind to root tissues (Jahan et al., 2018), or possibly due to exclusion strategy by tomato to avoid Zn toxicity. Ogunkunle et al. (2013) reported a restricted translocation of Zn to shoots of tomato, possibly due to its ability to sequester/detoxify excess Zn in the vacuoles of root cells of the plant. Several other studies have observed similar low translocation of Zn from ZnO nanoparticles to shoot of plants (Zhao et al., 2012; Mukherjee et al., 2013; Jahan et al., 2018) also observed similar low translocation of ZnO NPs to shoot of corn and green pea plants, respectively.

3.3. H_2O_2 generation, lipid peroxidation and enzyme activity in tomato roots

Reactive oxygen species (ROS) are normal byproducts of plants that are undergoing natural cell functions, but increased production of ROS may result from both abiotic and biotic stress in plants (Foyer and Shigeoka, 2011). The scenario where there is overproduction of the ROS induces lipid peroxidation, which further promotes oxidative stress. In this study, compared to control, concentration-dependent increase in H_2O_2 content was observed in roots as n-ZnO concentration increased (Fig. 3a). Nano-ZnO treatment (300, 600 and 1000 mg/kg) generated approximately 3-, 10- and 18-fold increase of H_2O_2 content in roots which indicates potential oxidative stress. The pronounced H_2O_2 generation must have been pronounced by aging duration of the n-ZnO in the soil, as aging can greatly affect toxicity of nanomaterials in soil due to process of adsorption and dissolution speed (Reddy et al., 2016). In relation to ZnO NPs, dissolution can vary from 1 h in soils that are acidic to 30 days in calcareous soil (Wang et al., 2013), and for this study, aging was 48 h which allowed enough dissolution of n-ZnO in the soil.

This result contrasted with reports of Mukherjee et al. (2013) in green pea where there was no significant overproduction of H_2O_2 by the roots exposed to 0–500 mg/kg ZnO nanoparticles. Conversely, previous studies have reported that zinc induced the production of free radicals in plants (Jain et al., 2010; Hosseini and Poorakbar, 2013). Oxidative stress which is usually caused by the imbalance in accumulation and removal of H_2O_2 and other ROS molecules was measured by assessing the level of lipid peroxidation. Lipid peroxidation, which is the most prominent symptom of oxidative stress in plants (Gill and Tuteja, 2010) was measured as MDA content. In root tissue, MDA content showed patterns like H_2O_2 contents that were generated due to n-ZnO treatment (Fig. 3a). As shown in the figure, the MDA content in the roots of the plants treated with 300, 600 and 1000 mg n-ZnO/kg was significantly and progressively higher ($P < 0.05$), compared to control, which concurs with overproduction of H_2O_2in roots at same n-ZnO concentrations. The observed lipid peroxidation in tomato roots may be linked to increased Zn levels in the roots, and most studies also reported enhanced lipid peroxidation in roots of plants exposed to ZnO NPs (Kumari et al., 2011).

The control of ROS levels and tolerance level in plants exposed to abiotic stress is normally linked plants' potential to efficiently up-regulate antioxidant enzymes to mob up excess ROS in plants' cells and tissues (Ogunkunle et al., 2018). Antioxidant enzyme activity is highly variable in plants depending on species-type, concentration of stressor, length of exposure and soil pH (Peng et al., 2015). Antioxidant enzymes such as APX and CAT are known useful components of defense system in plants (Shen et al., 2010), as they primarily involved in detoxifying H_2O_2 into H_2O and O_2 (Shen et al., 2010). The APX is a major enzyme in the ascorbate-glutathione cycle that involves oxidation–reduction reactions in controlling ROS levels in plants (Lin et al., 2009; Rico et al., 2013). In this study, the activity of APX, CAT and SOD in tomato roots at 90-day

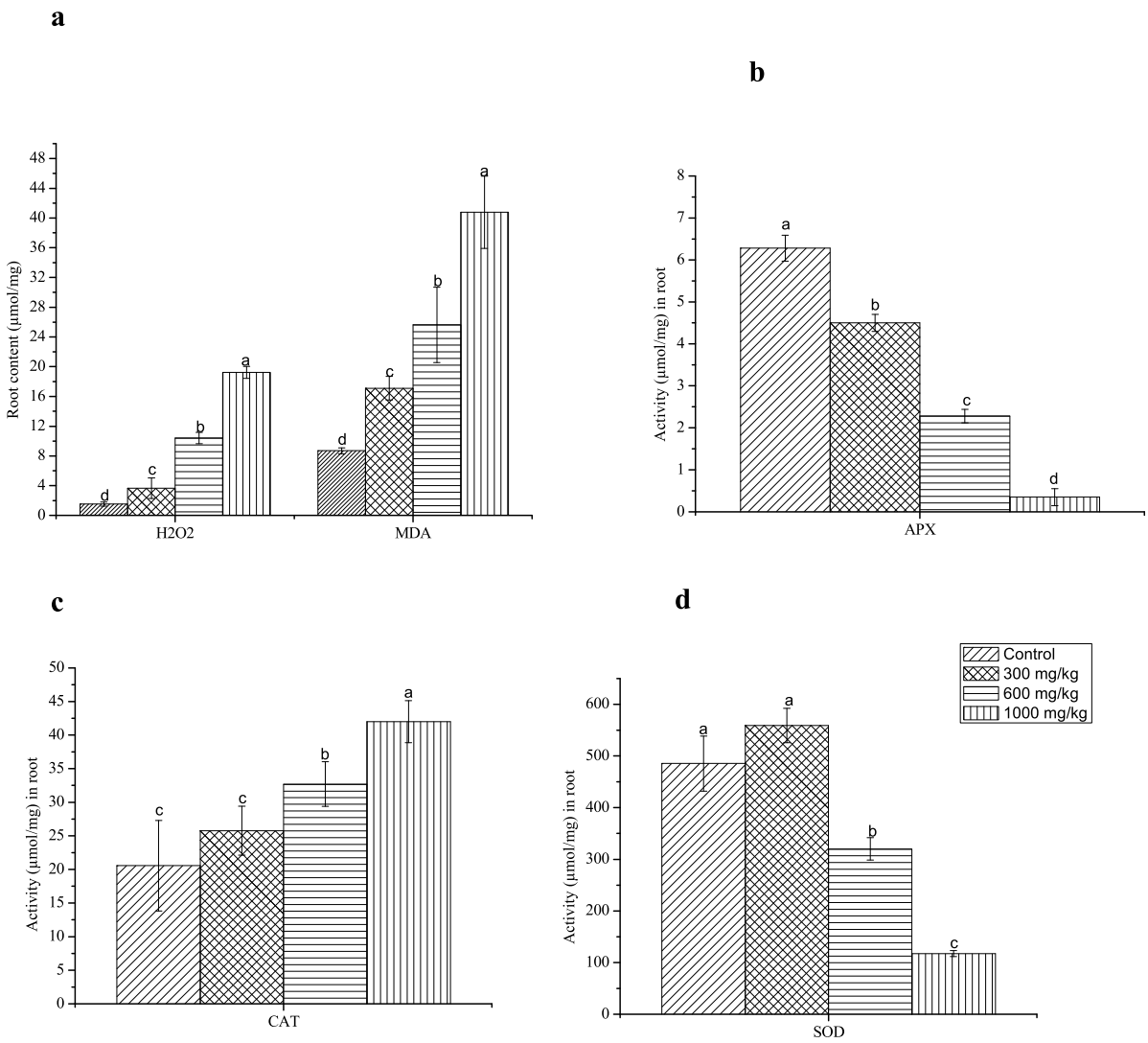


Fig. 3. Effect of n-ZnO treatment on (a) H_2O_2, activity of (b) APX, (c) CAT and(d) SOD in roots of *Solanum lycopersicum* L. after 90-day exposure. Error bar represents standard deviation and means separated by Turkey test at $P < 0.05$.

exposure was determined and presented in Fig. 3b. As shown in the Figure, increasing concentrations of n-ZnO resulted in significant decreasing ($P < 0.05$) APX activity. Specifically, APX activity at 1000 mg n-ZnO/kg) was reduced by ∼6-fold, and 600 mg n-ZnO/kg concentration reduced APX activity by ∼1.5-fold in roots, compared to control. The decreasing APX activity in relation to increasing doses of the n-ZnO corresponds with the incremental accumulation of H_2O_2 in the roots. This observation could possibly be related the low content of APX enzyme in roots which may possibly be unable to combat the stress imposed by Zn toxicity to the root tissue. It has been documented that ascorbate enzymes are majorly localized in plants' chloroplasts but may also present in cytoplasm and other cellular organelles (Malar et al., 2014). In the other hand, the decrease of APX activity may likely be linked to the simultaneous increase in the H_2O_2 generation and lipid peroxidation. Mukherjee et al. (2013) reported the dose-dependent reduction of APX in green pea plants exposed to 0–500 mg/kg ZnO NP and likewise, Zhao et al. (2013) also reported decrease in APX activity in corn plants grown in organic soil treated with 400 mg/kg of ZnO NPs.

In similar pattern to APX activity, activity of SOD in tomato roots also increased significantly at >300 mg n-ZnO/kg treatments compared to control (Fig. 3c). It must be noted that low n-ZnO dose (300 mg/kg) did not influence the SOD activity in relation to control, though oxidative stress was observed at that concentration (see Fig. 3b). The reduced activity at elevated n-ZnO concentrations was unexpected as activation of SOD in response to metal toxicity is commonly observed and believed to efficient in reducing oxidative stress in plants. A plausible explanation posited for this observation may be the fact that the major ROS induced by the nano-ZnO in tomato roots could majorly be the generation of H_2O_2 and not

Table 1
Effect of n-ZnO on chlorophyll contents (mg/g fw) of *Solanum lycopersicum* L.

	Chl-a	Chl-b	T-Chl
	30-day exposure		
Control	597 ± 157^{a}	533 ± 138^{a}	1130 ± 221^{a}
300 mg n-ZnO/kg	387 ± 82^{b}	163 ± 41^{b}	550 ± 223^{b}
600 mg n-ZnO/kg	217 ± 65^{c}	103 ± 15^{b}	320 ± 56^{c}
1000 mg n-ZnO/kg	300 ± 20^{c}	190 ± 10^{b}	490 ± 26^{c}
	90-day exposure		
Control	607 ± 85^{a}	150 ± 51^{b}	750 ± 87^{ab}
300 mg n-ZnO/kg	657 ± 50^{a}	190 ± 43^{a}	847 ± 91^{a}
600 mg n-ZnO/kg	433 ± 35^{b}	227 ± 40^{a}	660 ± 75^{ab}
1000 mg n-ZnO/kg	263 ± 69^{c}	110 ± 10^{c}	367 ± 72^{c}

Note: Values are means ± SD. Mean with the same letter (s) along the same column are not statistically different at $p < 0.05$ by Turkey.

O^{2-} species that SOD naturally catalyzes. This may allude to the low SOD activity observed in the roots as the enzyme is considered a powerful stress enzyme that acts on superoxide free radicals (Alscher and Erturk, 2002).

CAT activity in roots showed no significant change ($P > 0.05$) at 300 mg nZnO/kg but significant increase of ∼45% and ∼110% was observed at 600 mg/kg and 1000 mg/kg respectively, compared to control (Fig. 3c). CAT is involved in defense system of plants against accumulation of ROS by breaking down H_2O_2 to H_2O and O_2. It is also important to note that the CAT activity followed the pattern of H_2O_2 generation in the roots (see Fig. 3a). Hernandez-Viezcas et al. (2013) reported an increase in CAT activity in roots of velvet mesquite (*Prosopis juliflora-velutina*) that was grown in soil exposed to ZnO NPs at 4000 mg/kg. In this study, enhanced CAT activity that was observed may have covered for the decrease in APX activity in the H_2O_2 scavenging process, though its activity alone could not alleviate the oxidative stress induced by the nano-ZnO in the roots.

3.4. Contents of chlorophyll, H_2O_2, MDA and antioxidant enzyme activity in tomato leaves

Germination reduction and growth alterations are reported to be the most toxic effects of metal nanoparticles in plants (Song et al., 2012). In the present study, the nano-zinc oxide significantly affected the chlorophyll contents at early stage of the growth (Table 1). Chl-a, -b and T-Chl at 30 days were all significantly reduced compared to control for all n-ZnO-treatments. The treatments caused reduction of Chl-a, b and T-Chl by at least 54.3%, 99.6% and 105.4%, respectively at 30-day exposure. This observation was also reported in Wang et al. (2018) and Mukherjee et al. (2013) where decreased chlorophyll content was significant at the early growing stage of tomato and green pea exposed to nanoparticulate ZnO, respectively. Wang et al. (2016b) also reported about 50% Chl-a reduction in 300 mg/l ZnO NPs-treated *Arabidopsis thaliana*.

The 90-day exposure effect of n-ZnO treatment on chlorophyll contents showed that the treatments did alter the contents of Chl-a, and T-Chl at ≥ 600 mg n-ZnO/kg. The significant reduction in contents of Chl-a and T-Chl at ≥ 600 mg n-ZnO/kg (especially 1000 mg/kg) may be an indication of the possibility of oxidative stress due to the treatment in the leaves. Chl-a is a known major photosynthetic pigment in plants that is more sensitive to photo-degradation than other pigments, and was posited as a better indicator of nanoparticles toxicity (Falco et al., 2011). Our finding was in variance with earlier report of Prasad et al. (2012) where 1000 mg/kg ZnO NPs increased chlorophyll contents in peanuts. However, Wang et al. (2016a,b) and Jahan et al. (2018) have reported that ZnO NPs reduced chlorophyll contents in red bean and arabidopsis whereas Falco et al. (2011) observed severe reduction in Chl-a content in plants exposed to ZnO NPs. Jahan et al. (2018) opined that reduction in chlorophyll contents could negatively affects plant's ability to quench oxidative stress. Contrarily to our report on chlorophyll contents, other studies have observed increased chlorophyll contents in several plants as responses to biologically synthesized ZnO NPs (Raliya et al., 2014, 2015). Hence, the fundamental mechanism that is responsible for the various effects of nanoparticles on photosynthetic pigments still needs proper study.

In contrast to H_2O_2 content in roots, the leaf tissue showed no significant difference ($P > 0.05$) in the production of H_2O_2 in the treatments compared to control at 90-day exposure (Fig. 4a). However, MDA content was significantly higher ($p < 0.05$) at 1000 mg n-ZnO/kg while contents were comparable in other treatments in relation to control (Fig. 4a). This apparently signifies that n-ZnO at low concentration possess low toxicity to tomato leaves but higher concentration (1000 mg/kg) induced oxidative stress. Furthermore, the absence of significant H_2O_2 content is an indication of abundance of other ROS species than the H_2O_2 in the leaves. The finding of significant MDA content at 1000 mg n-ZnO/kg was in consonance with the reduction in chlorophyll contents at the same treatment in the leaves, apparently confirming toxicity to the leaves.

Contrarily to roots, there was no significant effect of n-ZnO treatment on activity of APX except at the highest concentration (1000 mg/kg) where activity increased by 20% (Fig. 4b). Due to the fact that activity of antioxidant enzymes are mostly activated as counter-response to oxidative stress, the increased activity of APX at 1000 mg n-ZnO/kg could have been due to oxidative stress. Studies have shown that plant leaves (especially chloroplasts) are rich in ascorbate (Malar et al., 2014), hence the activation of APX in the tomato leaves to combat toxicity imposed by the high nano-zinc oxide

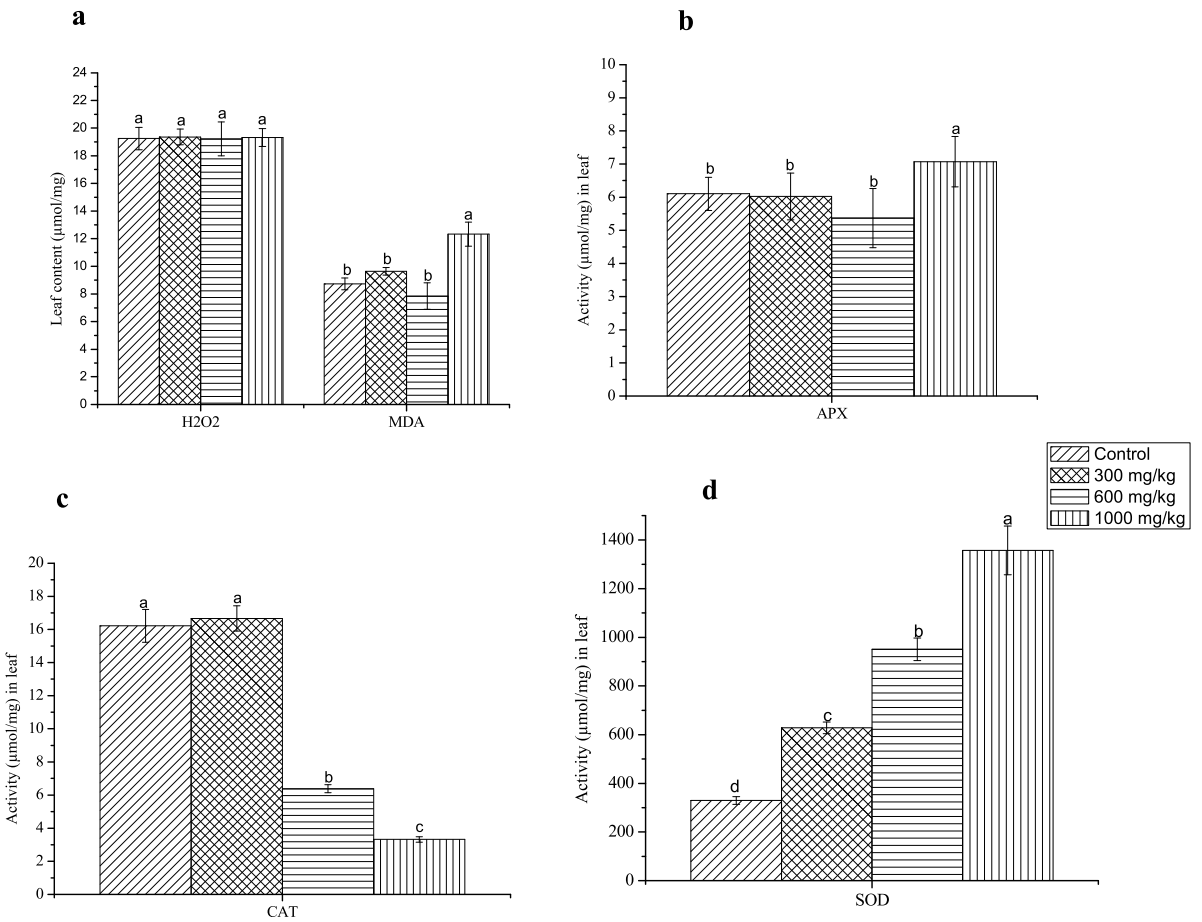


Fig. 4. Effect of n-ZnO treatment on (a) H_2O_2, activity of (b) APX, (c) CAT and (d) SOD in leaves of *Solanum lycopersicum* L. after 90-day exposure. Error bar represents standard deviation and means separated by Turkey test at $P < 0.05$.

concentration. However, a decrease in APX activity of 40 to 79% was observed in tomato leaves exposed to ZnO NP for 30–90 days in acidic soil but at a lower concentration of 225 mg/kg (García-Gómez et al., 2017). CAT activity in the leaves was reduced by high concentrations of n-ZnO (600 and 1000 mg/kg) whereas at low n-ZnO (300 mg/kg), CAT activity was not affected significantly as compared to control (Fig. 4c). Reduction in CAT activity was 63.6% and 81.8% for 600 and 1000 mg n-ZnO/kg treatments, respectively. García-Gómez et al. (2017) have also reported decreased CAT activity in tomato leaves exposed to 225 mg/kg ZnO NP for 90 days in acidic soil. The SOD activity was significantly induced at ≥300 mg n-ZnO/kg compared to control (Fig. 4d). The highly activated SOD in tomato leaves did not correspond to generated H_2O_2 content, and the lipid peroxidation. In relation to this result, it is imperative to note that H_2O_2 is among several ROS species (such as superoxide radicals, peroxide radicals, hydroxyl radicals, and singlet oxygen) that are involved in the induction of oxidative stress in plants. Similar discrepancies on the effects of ZnO NPs on enzymatic activity and oxidative stress have been reported in the literature (Du et al., 2017; Hernandez-Viezcas et al., 2011; Wang et al., 2016b). This alluded to the general belief that biochemical parameters, particularly those that are related to enzymes, are species-dependent, tissue-dependent, exposure duration and environmental conditions (García-Gómez et al., 2017). However, the increase in the activity SOD at elevated n-ZnO concentration may have resulted in enhanced ROS scavenge and low oxidative stress in the leaves because effectiveness of ROS scavenging in plants is noted to be greatly enhanced when activities of SOD and APX complement each other (Lu et al., 2010). Up-regulated SOD activity has also been reported in tomato exposed to ZnO nanoparticles (Wang et al., 2018).

3.5. Level of non-enzymatic antioxidants in tomato fruits

The contents of selected bioactive compounds (total phenol, flavonoids, β-carotene, lycopene and ascorbic acid) in tomato fruits after 90-day exposure to n-ZnO are presented in Table 2. All the non-enzymatic antioxidants except ascorbic acid were influenced negatively by all concentrations. The contents of total phenol and flavonoid in the mature green fruits were reduced by at least 16.4% and 4.8%, respectively compared to control, following the n-ZnO treatment (Table 2). Furthermore,

Table 2
Content of selected bioactive compounds in fruits of *Solanum lycopersicum* after 90-day exposure to n-ZnO.

Treatment (mg nZnO/kg)	Total phenols (mg GAE/100 g fw)	Flavonoids (mg EQ/100 g fw)	β-carotene (mg/100 g fw)	Lycopene	Ascorbic acid
Control	2.41 ± 0.01^{a}	6.39 ± 0.01^{a}	2.32 ± 0.02^{a}	2.58 ± 0.00^{a}	1.02 ± 0.00^{c}
300	2.07 ± 0.01^{b}	6.10 ± 0.00^{b}	2.13 ± 0.01^{b}	2.42 ± 0.06^{b}	1.06 ± 0.01^{a}
600	2.01 ± 0.01^{c}	6.01 ± 0.01^{c}	2.13 ± 0.01^{bc}	2.21 ± 0.00^{c}	1.04 ± 0.01^{b}
1000	2.00 ± 0.00^{c}	6.00 ± 0.01^{c}	2.10 ± 0.00^{c}	2.17 ± 0.01^{c}	1.02 ± 0.01^{c}

Values with the same letter (s) along the same column are not statistically different at $p < 0.05$ by Turkey.

provitamin contents (β-carotene and lycopene, respectively) in the tomato fruits reduced by at least 8.9% and 6.6% in comparison to control. Contrarily to all non-enzymatic antioxidants, ascorbic acid, which is one of the main water-soluble antioxidants in tomato was increased significantly ($P < 0.05$) by nano-ZnO treated at 300 and 600 mg/kg concentrations whereas, at the high concentration (1000 mg n-ZnO/kg) the content reduced significantly ($P < 0.05$). It is important to note that the contents of all the non-enzymatic antioxidants were relatively low in the tomato fruits. This is connected to the fact that the fruits were collected when still green and firm but matured. It has been posited that the levels of antioxidants (such as ascorbic acid, lycopene and β-carotene) increase gradually as ripening proceeds in tomato fruits (Brandt et al., 2006; Radzevicius et al., 2012; Viskelis et al., 2007). The results indicate that the application of n-ZnO has a negative effect on the total phenols, flavonoid, β-carotene and lycopene contents in the green tomato fruits. This possibly suggests that the n-ZnO-treatment has an inhibitory effect on the induction of these antioxidant compounds in the tomato fruits. This was contrary to the reported stimulatory effect of copper nanoparticles on total phenols, β-carotene and ascorbic acid in tomato according to Hernández-Fuentes et al. (2017).

Conclusion

Summarily, the results of the study showed the concentration-dependent uptake of Zn from n-ZnO-amended soil and modulation of antioxidant enzyme activities in the root of tomato. The $\geq$300 mg n-ZnO/kg treatment increased the H_2O_2 content and lipid peroxidation in roots, and reduced the activities of APX and SOD whereas CAT activity was up-regulated at same n-ZnO treatments. Significant lipid peroxidation in the leaves occurred only at the highest dose of n-ZnO (1000 mg n-ZnO/kg), possibly due to low generation of H_2O_2 at lower n-ZnO doses. The observed low oxidative stress in the leaves of tomato at $\leq$600 mg n-ZnO/kg may be due to the absence of significant toxic effect of n-ZnO which was apparent in the results of chlorophyll contents. Though, there was low oxidative stress in the leaves, the green fruits of tomato presented reduced contents of non-enzymatic antioxidants like total phenols, flavonoids β-carotene and lycopene. This may possibly imply that antioxidant contents of tomato fruits may be susceptive to n-ZnO toxicity, even at low doses.

Conflict of interest

The authors declare no conflict of interest in this study

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2019.100325.

References

Aebi, H., 1974. Catalases. In: Bergmeyer, HU. (Ed.), Methods of Enzymatic Analysis 2. Academic Press, New York, pp. 673–684.
Alscher, R.G., Erturk, N.L., 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Environ. Exp. Botany 53, 1331–1341.
Beyer, W.F., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161, 559–566.
Brandt, S., Pek, Z., Barna, E., 2006. Lycopene content and colour of ripening tomatoes as affected by environmental conditions. J. Sci. Food Agric. 86, 568–572.
Cai, R., Van, G.M., Aw, P.K., Itoh, K., 2006. Solar-driven self-cleaning coating for a painted surface. Chemosphere 9, 829–835.
Chithrani, B.D., Ghazani, A.A., Chan, W.C., 2006. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett. 6, 662–668.
Du, W.C., Sun, Y.Y., Ji, R., Zhu, J.G., Wu, J.C., Guo, H.Y., 2011. Tio_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. J. Environ. Monit. 13, 822–828.
Du, W., Tan, W., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Guo, H., 2017. Interaction of metal oxide nanoparticles with higher terrestrial plants: physiological and biochemical aspects. Plant Physiol. Biochem. 110, 210–225.
Falco, W.F., Botero, E.R., Falcao, E.A., Santiago, E.F., Bagnato, V.S., Caires, A.R.L., 2011. In vivo observation of chlorophyll fluorescence quenching induced by gold nanoparticles. J. Photochem. Photobiol. A 225, 65–71.
Fedorenko, V.F., Buklagin, D.S., Golubev, I.G., Nemenushchaya, L.A., 2015. Review of Russian nanoagents for crops treatment. Nanotechnol. Russ. 10 (3–4), 318–324.
Foyer, C.H., Shigeoka, S., 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol. 155, 93–100.
García-Gómez, C., Obrador, A., González, D., Babín, M., Fernández, M.D., 2017. Comparative effect of ZnO NPs, ZnO bulk and ZnSO4 in the antioxidant defences of two plant species growing in two agricultural soils under greenhouse conditions. Sci. Total Environ. 589, 11–24.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stresstolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Hernández-Fuentes, A.D., López-Vargas, E.R., Pinedo-Espinoza, J.M., Campos-Montiel, R.G., Valdés-Reyna, J., Juárez-Maldonado, A., 2017. Postharvest behavior of bioactive compounds in tomato fruits treated with Cu nanoparticles and NaCl stress. Appl. Sci. 7, 980. http://dx.doi.org/10.3390/app7100980.
Hernandez-Viezcas, J.A., Castillo-Michel, H., Andrews, J.C., Cotte, M., Rico, C., Peralta-Videa, J.R., Ge, Y., Priester, J.H., Holden, P.A., Gardea-Torresdey, J.L., 2013. In Situ synchrotron X-ray fluorescence mapping and speciation of CeO2 and ZnO nanoparticles in soil cultivated soybean (glycine max). ACS Nano 7 (2), 1415–1423.
Hernandez-Viezcas, J.A., Castillo-Michel, H., Servin, A.D., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2011. Spectroscopic verification of zinc absorption and distribution in the desert plant *Prosopis juliflora*-velutina (velvet mesquite) treated with ZnO nanoparticles. Chem. Eng. J. 170, 346–352.
Hosseini, Z., Poorakbar, L., 2013. Zinc toxicity on antioxidative response in (Zea mays L.) at two different pH. J Stress Physiol. Biochem. 9 (1), 66–73.
Islam, E., Liu, D., Li, T., Yang, X., Jin, X., Mahmood, Q., Tian, S., Li, J., 2008. Effect of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of Elsholtzia argyi. J. Hazard. Mater. 154, 914–926.
ISO, 2012. ISO Guideline 11269-2: Soil Quality – Determination of the Effects of Pollutants on Soil Flora - Part 2: effects of Contaminated Soil on the Emergence and Early Growth of Higher Plants. International Organization for Standardization, Geneva, Switzerland.
Jahan, S., Alias, Y.B., Abubakar, A.F., Yussof, I., 2018. Toxicity evaluation of Zno and TiO2 nanomaterials in hydroponic red bean (*Vigna angularis*) plant: Physiology, biochemistry and kinetic transport. J. Environ. Sci. 1, http://dx.doi.org/10.1016/j.jes.2017.12.022.
Jain, R., Srivastava, S., Solomon, S., Shrivastava, A.K., Chandra, A., 2010. Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (*Saccharum* spp.). Acta Physiol. Plantarum 32, 979–986.
Kadar, A.A., Stevens, M.A., Albright-Holton, M., Morris, L., Algazi, M., 1977. Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. J. Amer. Soc. Hortic. Sci. 102, 724 –731.
Kulkarni, N., Muddapur, U., 2014. Biosynthesis of metal nanoparticles. J. Nanotechnol. 2 (1), 1–8.
Kumari, M., Sudheer, K.S., Pakrashi, S., Mukherjee, A., Chandrasekaran, N., 2011. Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium* cepa. J. Hazard. Mater. 190, 613–621.
Lee, D.H., Lee, C.B., 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci. 159, 75–85.
Li, H., 2000. Principles and Techniques of Plant Physiological Biochemical Experiment. Higher Education Press, Beijing, pp. 260–263.
Lin, C., Fugetsu, B., Su, Y., Watar, F., 2009. Studies on toxicity of multi-walled carbon nanotubes on Arabidopsis T87 suspension cells. J. Hazard. Mater. 170, 578–583.
Lin, D., Xing, B., 2007. Root uptake and phytotoxicity of ZnO nanoparticles. Environ. Sci. Technol. 42, 5580–5585.
Liu, Q., Chen, B., Wang, Q., Shi, X., Xiao, Z., Lin, J., Fang, X., 2009. Carbon nanotubes as molecular transporters for walled plant cells. Nano Lett. 9 (3), 1007–1010.
Liu, X., Wang, F., Shi, Z., Tong, R., Shi, X., 2015. Bioavailability of Zn in ZnO nanoparticle-spiked soil and the implications to maize plants. J. Nanoparticle Res. 17 (4), https://doi.org/10.1007/s11051-015-2989-2.
Lu, Y., Li, X., He, M., Zhao, X., Liu, Y., Cui, Y., Pan, Y., Tan, H., 2010. Seedlings growth and antioxidative enzyme activities in leaves under heavy metal stress differ between two desert plants: A perennial (*Peganum harmala*) and an annual (*Halogeton glomeratus*) grass. Acta Physiol. Plantarum 32, 583–590.
Ma, H., Bertsch, P.M., Glenn, T.C., Kabengi, N.J., Williams, P.L., 2009. Toxicity of manufactured zinc oxide nanoparticles in the nematode *Caenorhabditis elegans*. Environ. Toxicol. Chem. 28, 1324–1330.
Ma, H., Kabengi, N.J., Bertsch, P.M., Unrine, J.M., Glenn, T.C., Williams, P.L., 2011. Comparative phototoxicity of nanoparticulate and bulk ZnO to a free-living nematode *Caenorhabditis elegans*: the importance of illumination mode and primary particle size. Environ. Pollut. 159, 1473–1480.
Makeen, K., Babu, G.S., Lavanya, G.R., Abraham, G., 2007. Studies of chlorophyll by different methods in blackgram (*Vigna mungo* L.). Int. J. Agric. Res. 2, 651–654.
Malar, S., Vikram, S.S., Favas, P.J.C., Perumal, V., 2014. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Bot. Stud. 55, 54–64.
Manzo, S., Rocco, A., Carotenuto, R., De Luca, P.F., Miglietta, M., Rametta, G., Di Francia, G., 2011. Investigation of ZnO nanoparticles' ecotoxicological effects towards different soil organisms. Environ. Sci. Pollut. Res. 18, 756–763.
Mohammad, G., Hossein, S., Iraj, A., Hossein, N., Morteza, H., 2017. Synthesis of highly intercalated urea-clay nanocomposite via domestic montmorillonite as eco-friendly slow- release fertilizer. Arch. Agronomy Soil Sci. 63 (1), 84–95.
Mukherjee, A., Peralta-Videa, J.R., Bandyopadhyay, S., Rico, C.M., Zhao, L., Gardea-Torresdey, J.L., 2013. Physiological effects of nanoparticulate ZnO in green peas (*Pisum sativum* L.) cultivated in soil. Metallomics 6, 132–138.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Ogunkunle, C.O., Fatoba, P.O., Awotoye, O.O., Olorunmaiye, K.S., 2013. Root-shoot partitioning of copper, chromium and zinc in *Lycopersicon esculentum and Amaranthus hybridus grown* in cement-polluted soil. Environ. Exp. Biol. 11, 131–136.
Ogunkunle, C.O., Jimoh, M.A., Asogwa, N.T., Viswanathan, K., Vishwakarma, V., Fatoba, P.O., 2018. Effects of manufactured nano-copper on copper uptake, bioaccumulation and enzyme activities in cowpea grown on soil substrate. Ecotoxicol. Environ. Saf. 155, 86–93.
Peng, D., Shafi, M., Wang, Y., Li, S., Yan, W., Chen, J., Ye, Z., Liu, D., 2015. Effect of Zn stresses on physiology, growth, Zn accumulation, and chlorophyll of *Phyllostachys pubescens*. Environ. Sci. Pollut. Res. 22, 14983–14992.
Plibeam, D.J., 2015. Breeding crops for improved mineral nutrition under climate under change conditions. J. Exp. Bot. 66 (12), 3511–3521.
Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K.R., Sreeprasad, T.S., Sajanial, P.R., Pradeep, T., 2012. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J. Plant Nutr. 35, 905–927.
Radzevicius, A., Viskelis, P., Karkleliene, R., Viskelis, J., Bobinas, C., Dambrauskiene, E., Sakalauskiene, S., 2012. Tomato ripeness influence on fruit quality. World Acad. Sci. Eng. Technol. 6 (4), 585–588.
Raliya, R., Biswas, P., Tarafdar, J.C., 2015. Tio_2 nanoparticles biosynthesis and its physiological effect on mung bean (*Vigna radiate* L.). Biotechnology Reports 5, 22–26.
Raliya, R., Tarafdar, J.C., Singh, S., Gautam, R., Choudhary, K., Maurino, V.G., Saharan, V., 2014. Mgo nanoparticles biosynthesis and its effect on chlorophyll contents in the leaves of clusterbean (*Cyamopsis tetragonoloba* L.). Adv. Sci. Eng. Med. 6 (5), 538–545.
Reddy, P.V.L., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2016. Lessons learned: are engineered nanomaterials toxic to terrestrial plants? Sci. Total Environ. 568, 470–479.
Rico, C.M., Hong, J., Morales, M.I., Zhao, L., Barrios, A.C., Zhang, J.Y., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Effect of cerium oxide nanoparticles on rice: A study involving the antioxidant defense system and in vivo fluorescence imaging. Environ. Sci. Technol. 47, 5635–5642.
Sabir, S., Arshad, M., Chaudhari, S.K., 2014. Zin oxide nanoparticles for revolutionizing agriculture: synthesis and application. Sci. World J. 10, 1–8.
Scott, N., Chen, H., 2013. Nanoscale science and engineering for agriculture and food system. Ind. Biotechnol. 9 (1), 17–18.
Shen, C., Zhang, Q., Li, J., Bi, F., Yao, N., 2010. Induction of programmed cell death in Arabidopsis and rice by single-wall carbon nanotubes. Am. J. Bot. 97, 1602–1609.
Song, G., Gao, Y., Wu, H., Hou, W., Zhang, C., Ma, H., 2012. Physiological effect of anatase TiO2 nanoparticles on *Lemna minor*. Environ. Toxicol. Chem. 31, 2147–2152.
Stampoulis, D., Sinha, S.K., White, J.C., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. Technol. 43, 9472–9479.

USEPA, 1996. Ecological Effects Test Guidelines (OPPTS 850.4200). Seed Germination/Root Elongation Toxicity Test. http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-4200.pdf.

Viskelis, P., Jankauskiene, J., Bobinaite, R., 2007. Influence of ripeness on tomato fruit quality. Sodinink. Daržinink. 26 (4), 278–288.

Wang, X.P., Li, Q.Q., Pei, Z.M., Wang, S.C., 2018. Effects of zinc oxide nanoparticles on the growth, photosynthetic traits, and antioxidative enzymes in tomato plants. Biol. Plantarum https://doi.org/10.1007/s10535-018-0813-4.

Wang, F., Liu, X., Shi, Z., Tong, R., Adams, C.A., Shi, X., 2016a. Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants- a soil microcosm experiment. Chemosphere 147, 88–97.

Wang, P., Menzies, N.W., Lombi, E., McKenna, B.A., Johannessen, B., Glover, C.J., 2013. Fate of ZnO nanoparticles in soils and cowpea (Vigna unguiculata). Environ. Sci. Technol. 47, 13822–13830.

Wang, X., Yang, X., Chen, S., Li, Q., Wang, W., Hou, C., Gao, X., Wang, L., Wang, S., 2016b. Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in Arabidopsis. Front. Plant Sci. https://doi.org/10.3389/fpls.2015.01243.

Zhao, L., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Bandyopadhyay, S., Peng, B., Munoz, B., Keller, A.A., Gardea-Torresdey, J.L., 2013. ZnO nanoparticle fate in soil and zinc bioaccumulation in corn plants (Zea mays) influenced by alginate. Environ. Sci. Process. Impacts 15 (1), 260–266.

Zhao, L., Peralta-Videa, J.R., Ren, R., Varela-Ramirez, A., Li, C., Hernandez-Viezcas, J.A., Aguilera, R.J., Gardea-Torresdey, J.L., 2012. Transport of Zn in a sandy loam soil treated with ZnO NPs and uptake by corn plants: electron microprobe and confocal microscopy studies. Chem. Eng. J. 184, 1–8.