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Toxicity assessment of metal oxide nano-pollutants on tomato (*Solanum lycopersicon*): A study on growth dynamics and plant cell death☆

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ABSTRACT

The present study for the first time demonstrated the interactions of metal oxide (MO) nano-pollutants (CuO and Al_2O_3-NPs) with tissues and cellular DNA of tomato plants grown in soil sand: silt: clay (667:190:143) and Hoagland-hydroponic system and assessed the hazardous effects of NPs on cell physiology and biochemistry. Results of SEM equipped with EDX revealed attachment of variably shaped CuO-NPs (18 nm) and Al_2O_3-NPs (21 nm) on roots, and internalization followed by translocation in plants by ICP-MS and TEM. Significant variations in foliage surface area, chlorophyll, proteins, LPO, and antioxidant enzymes were recorded. Roots and shoots accumulated 225.8 ± 8.9 and 70.5 ± 4 μgAl g^{-1} DW, whereas Cu accumulation was 341.6 ± 14.3 (roots) and 146.9 ± 8.1 μg g^{-1} DW (shoots) which was significant ($p \leq 0.0005$) as compared to control. The total soluble protein content in roots, shoots, and leaves collected from Al_2O_3-NPs treated plants increased by 120, 80, and 132%, respectively while in CuO-NPs treatments, the increase was 68 (roots), 36 (shoots), and 86% (leaves) over control. The level of antioxidant enzymes in plant tissues was significantly ($p \leq 0.05$) higher at 2000 μg ml^{-1} of MONPs over control. A dose-dependent increase in reactive oxygen species (ROS), biphasic change of lower and higher fluorescence in mitochondria due to dissipation of mitochondrial membrane potential ($\Delta\Psi m$) and membrane defects using propidium iodide were observed. Comparatively, CuO-NPs induced higher toxicity than Al_2O_3-NPs. Perceptible changes in proteins (amide-I & II), cellulose, glucose, galactose and other carbohydrates were observed under FT-IR. The binding studies with TmDNA showed fluorescence quenching of EtBr-TmDNA and acridine orange-TmDNA complex only by CuO-NPs with -ΔG and +ΔH and +ΔS values. However, Al_2O_3-NPs induced lesser change in TmDNA conformation. Conclusively, the results are novel in better demonstrating the mechanistic basis of nano-phyto-toxicity and are important which could be used to develop strategies for safe disposal of Al_2O_3-NPs and CuO-NPs.

1. Introduction

Recent developments in nanotechnology and widespread applications of manufactured nanoparticles (NPs) have revolutionized the field of science and technology. The smaller size provides high reactivity which makes NPs suitable for use in biomedical, pharmaceuticals, electronics, defense, aerospace industries and agriculture fields (Ocsoy et al., 2013; Vittori Antisari et al., 2015; Rizwan et al., 2016). Among the MONPs, Al_2O_3-NPs and CuO-NPs are used in explosives, alloys, sensors, drug delivery, personal care products, catalysts, gas sensor, semiconductor devices, batteries, microelectronics, antimicrobial coatings, textiles, and food containers (Siddiqui et al., 2013; Rajeshwari et al., 2015; Ahmed et al., 2018). Due to ever increasing applications, the production of MONPs is likely to increase from 0.27 million tons (2012) to 1.663 million tons by 2020 (The Global Market for Metal Oxide Nanoparticles to 2020). Of these, 8–28%, 0.4–7.0%, and 0.1–1.5% are likely to enter into soils, water bodies and atmosphere, respectively (Keller et al., 2013; Rajput et al., 2018). Furthermore, the uncontrolled disposal of nanoparticles may also trigger their accumulation in the environment (Ma et al., 2010; Rastogi et al., 2017). The accumulation of NPs has however, caused human health problems (Siddiqui et al., 2013;

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Dwivedi et al., 2018). For instance, oxidative stress mediated toxicity of CuO-NPs is reported in diverse human cell lines (Siddiqui et al., 2013). Also, the Al_2O_3-NPs have been found to- (i) decrease protein expression, (ii) induce toxicity to microvascular endothelium, and (iii) alter the properties of blood-brain barrier in mammalian systems (Chen et al., 2008).

Apart from humans, plants have also been reported to accumulate NPs in different organs (Faisal et al., 2013; López-Moreno et al., 2016) and via food chains NPs affect human health (Yang et al., 2017). For example, the highest content of Al has been detected in roots of *Allium cepa*, *Lepidium sativum, Zea mays,* and *Kalanchoe daigremontiana* when grown with Al_2O_3-NPs (Asztemborska et al., 2015). Similar accumulation of CuO-NPs has been reported in *Cucumis sativus* (Kim et al., 2013). However, the CuO and Al_2O_3-NPs were found phytotoxic against maize and rice which varied with varying concentrations of each NP (Yang et al., 2015) Mechanistically, the NPs (e.g. CuO-NPs) destruct DNA causing mutagenic DNA lesions and consequently inhibit plant growth (Atha et al., 2012). Apart from these, the NPs following interaction with plants, generates ROS which in turn alters lipid peroxidation, DNA/membrane structure, and metabolism of plants (Du et al., 2017; García-Gómez et al., 2017). Additionally, ROS serves as important signaling molecules during apoptotic development (Faisal et al., 2013). To counter this, plants have evolved enzymatic defense system (García-Gómez et al., 2017). Despite efforts, the reports on systematic and finer details on phyto-toxic impact of nano-pollutants are limited.

Tomato (*Lycopersicon esculentum*), one of the most cultivated food crops is a rich source of β-carotene, flavonoids, lycopene, and vitamins. Due to these, it is consumed by humans globally (Ahmed et al., 2017a, 2017b). In this study, tomato was chosen as a model plant to assess the phyto-toxicity of two MONPs (Al_2O_3 and CuO) due to its huge requirement in human dietary systems. Also, tomato responds well to other NPs such as $CoFe_2O_4$-NPs (López-Moreno et al., 2016), ZnO-NPs (de la Rosa et al., 2013) and Ag (Karami Mehrian et al., 2015). The CuO-NPs and Al_2O_3-NPs selected in this study were used to assess nano-phyto-toxicity due largely to their high demand in various industries. However, to the best of our information, no systematic study on the effects of Al_2O_3 and CuO-NPs on tomato grown in soil system and hydroponics is available. Therefore, the present experiments were designed to investigate (i) changes in length and biomass (ii) attachment and internalization/localization of NPs (iii) changes in photosynthetic pigments and total soluble proteins (iv) effects on membrane lipid peroxidation and antioxidant enzymes (v) ROS production root cell death, changes in $\Delta\Psi m$, and (vi) interaction of Al_2O_3 and CuO-NPs with leaf DNA and changes in root biomolecules using tomato as a model plant.

2. Material and methods

2.1. Characterization of nanoparticles

CuO-NPs and Al_2O_3-NPs purchased from Sisco Research Laboratories (Mumbai, India) were characterized for their primary and secondary sizes by standard techniques (Ali et al., 2015; Saleem et al., 2017) (See supplementary methods).

2.2. Hydroponics and soil experiments

Sterilized tomato seeds (Shahid et al., 2018) were placed on soft agar plates of ¼ strength of modified Hoagland's solution for 5 days in dark at 25 °C. Seedlings were transferred to 180 ml cups containing 140 ml of modified ½ strength of Hoagland's solution and grown in a growth chamber maintained at photons flux density of 340 μmol m^{-2} s^{-1}, 25/20 °C D/N temperature, 60/70% D/N RH and 14/10 h D/N photoperiod. Plants received a replacement of ½ strength Hoagland's solution daily at early growth stage and 1X Hoagland's solution during exposure period. After 10 days, plants were divided into four groups and three replications randomly. All plants except untreated control received 20, 200, and 2000 μg ml^{-1} of CuO and Al_2O_3-NPs for 20 days and the exposure media was refreshed after every three days. The air supply was controlled through air pumps which was sufficient to uniformly disperse the CuO and Al_2O_3-NPs in solution without causing any damage to growing plant roots.

To assess the effect of CuO and Al_2O_3-NPs on tomato in soils, 150 g of soil (sand: silt: clay 667:190:143, pH 7.2, water holding capacity 0.71 ml g^{-1}, electric conductivity 0.97 mv/cm^2, organic carbon 0.4%, cation exchange capacity 11.7 cmol kg^{-1}, anion exchange capacity 5.1 cmol kg^{-1}, Kjeldahl N 0.75 g kg^{-1}, Olsen P 16 mg kg^{-1}, available K 25.04 kg ha^{-1}, organic matter 6.2 g kg^{-1}) was sieved using 2 mm mesh and sterilized by autoclaving and then thoroughly mixed with 0–2000 mg g^{-1} soil each of CuO and Al_2O_3-NPs. The SEM-EDX analysis of soil mixed with NPs was done to confirm the presence and extent of NPs aggregation compared to primary particle size. Five seeds per plastic pot (6.8 × 8 cm) were planted 1 cm below soil-NPs mixture. After 10 days of growth, thinning was done and one plant/pot was maintained. The plants were irrigated with sterile water throughout the experiment on every alternate day depending upon the magnitude of soil dryness. A small hole of approx. 2 mm diameter was punched at the bottom center of plastic pots to prevent water logging. After 40 days of growth, length and biomass of plants were measured. Fresh biomass from each set of experiment was also frozen until further analysis.

2.3. Surface attachment of CuO and Al_2O_3-NPs on roots by SEM analysis

Detached roots of tomato grown in the absence and presence of NPs in hydroponics were cut 3 cm distant from root tip and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde prepared in 0.1 M sodium phosphate buffer (pH 7.2) for 2 h at room temperature and at 4 °C for overnight with intermittent vortexing. Samples processed using the method of Shahid et al. (2018) were visualized under JSM 6510LV SEM (JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. The attachment of NPs onto root surfaces was detected by Oxford Instruments INCAx-sight EDAX spectrometer equipped SEM followed by mapping of elements.

2.4. Internalization and translocation of CuO and Al_2O_3-NPs in tomato plant

Roots and shoots collected from treated and control plants were dried at 60 °C for 24 h and finely powdered using mortar and pestle. The samples (0.5 g) were then digested with 10 ml concentrated HNO_3 for 2 h and filtered through 0.45μ filter. The concentrations of CuO and Al_2O_3-NPs internalized into filtered plant tissues were determined by Inductively Coupled Plasma (ICP) Mass Spectrometry (Perkin Elmer ELAN DRC-e, USA). After the uptake of NPs in roots, the translocation of NPs in various compartments of root cells was determined by ultrastructure analysis of roots treated with 2000 μg ml^{-1} each of CuO and Al_2O_3-NPs using TEM. Small root tips (2 × 2 × 2 mm in size) from untreated and treated groups were fixed in primary fixative mentioned for SEM analysis and were further processed (Ahmed et al., 2017a, 2017b). The processed sample grids were visualized under JEOL 2100F TEM (JEOL, USA).