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Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants

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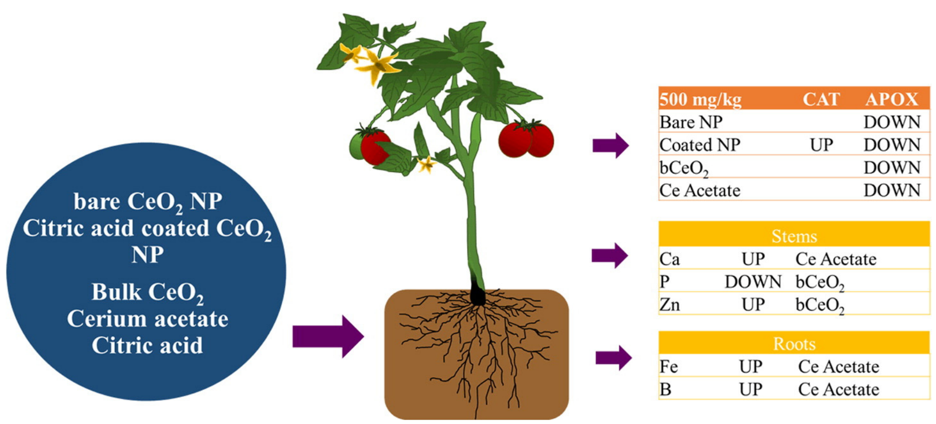
HIGHLIGHTS

- At 500 mg/kg, coated and bare NPs increased stem length by 13 and 9%, respectively.
- Coated NPs at 500 mg/kg increased CAT activity in leaves.
- Neither bare nor coated NP affected the homeostasis of nutrient elements in tissues.
- At 250 mg/kg, coated NPs increased total chlorophyll, chlo-a, and chlo-b.

GRAPHICAL ABSTRACT



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ABSTRACT

Little is known about the physiological and biochemical responses of plants exposed to surface modified nanomaterials. In this study, tomato (*Solanum lycopersicum* L.) plants were cultivated for 210 days in potting soil amended with uncoated and citric acid coated cerium oxide nanoparticles ($nCeO_2$, CA + $nCeO_2$) bulk cerium oxide ($bCeO_2$), and cerium acetate (CeAc). Millipore water (MPW), and citric acid (CA) were used as controls. Physiological and biochemical parameters were measured. At 500 mg/kg, both the uncoated and CA + $nCeO_2$ increased shoot length by ~9 and ~13%, respectively, while $bCeO_2$ and CeAc decreased shoot length by ~48 and ~26%, respectively, compared with MPW ($p \leq 0.05$). Total chlorophyll, chlo-*a*, and chlo-*b* were significantly increased by CA + $nCeO_2$ at 250 mg/kg, but reduced by $bCeO_2$ at 62.5 mg/kg, compared with MPW. At 250 and 500 mg/kg, $nCeO_2$ increased Ce in roots by 10 and 7 times, compared to CA + $nCeO_2$, but none of the treatments affected the Ce concentration in above ground tissues. Neither $nCeO_2$ nor CA + $nCeO_2$ affected the homeostasis of nutrient elements in roots, stems, and leaves or catalase and ascorbate peroxidase in leaves. CeAc at 62.5 and 125 mg/kg increased B (81%) and Fe (174%) in roots, while at 250 and 500 mg/kg, increased Ca in stems (84% and 86%, respectively). On the other hand, $bCeO_2$ at 62.5 increased Zn (152%) but reduced P (80%) in stems. Only $nCeO_2$ at 62.5 mg/kg produced higher total number of tomatoes, compared with control and the rest of the treatments. The surface coating reduced Ce uptake by roots but did not affect its translocation to the

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aboveground organs. In addition, there was no clear effect of surface coating on fruit production. To our knowledge, this is the first study comparing the effects of coated and uncoated $nCeO_2$ on tomato plants.

1. Introduction

Cerium oxide nanoparticles (NPs) or nanoceria ($nCeO_2$) are among the top 10 nanomaterials produced worldwide (Keller and Lazareva, 2014). Similar to the bulk cerium, these nanoparticles (NPs) are mainly used in the automotive industry as catalysts or in electronics and optics. Keller and Lazareva (2014) estimated that in 2010, the global production of $nCeO_2$ reached 10,000 tons of which 100 ended in air, 300 in water and 1400 in soil. Engineered nanomaterials (ENMs) including $nCeO_2$, have several applications; however, the uncoated forms tend to aggregate and overgrow, which limit their performance. To improve their stability, ENMs are surface capped with several materials (Niu and Li, 2014). Citric acid (CA) is a common coating agent due to its stability and availability (Masui et al., 2002; Chanteau et al., 2009; Liu et al., 2012). However, coating molecules change the surface chemistry and interaction of ENMs with the environment (Chanteau et al., 2009).

Previous studies have shown that $nCeO_2$ have the potential to alter the physiology and biochemistry of plants. However, there is a lack of uniformity in the reported results and none of the parameters seem to be affected in the same manner when there are variations in species, growth media, and treatment concentration. Lopez-Moreno et al. (2010a) exposed $nCeO_2$ to several seeds in liquid medium and found that at 2000 mg/L, $nCeO_2$ reduced the germination of tomato (*Solanum lycopersicum*), corn (*Zea mays*), and cucumber (*Cucumis sativus*). Lopez-Moreno et al. (2010a) also reported an increase in cucumber and corn root seedling elongation but a reduction in alfalfa and tomato root length. On the other hand, Ma et al. (2010) reported that at 2000 mg/L, $nCeO_2$ reduced the root elongation in lettuce but not in tomato, radish (*Raphanus sativus*), wheat (*Triticum aestivum*), cabbage (*Brassica oleracea*), cucumber, and rape (*Brassica napus* L.).

A complete assessment of the effects of $nCeO_2$ on plants is difficult due to the lack of studies covering the entire life cycle. A review of current literature reported that by 2014, only 30 studies covered the effects of ENMs over the full life cycle of plants (Gardea-Torresdey et al., 2014). Of those, only five were about $nCeO_2$. Wang et al. (2012) exposed tomato in potting soil to consecutive applications of $nCeO_2$ suspension at 10 mg/L. These researchers reported no effects on plant growth and production; however, high Ce content was found in the fruit. Morales et al. (2013) reported that at 250 mg/kg, $nCeO_2$ decreased biomass and caused conformational changes in the macromolecular composition of cilantro. Rico et al. (2013a, 2014) reported changes in essential elements and other nutritional components in rice (*Oryza sativa*) and wheat (*T. aestivum*) grains. Zhao et al. (2014) reported 31.3% reduction in cucumber fruit production under exposure to 800 mg $nCeO_2$/kg; Corral-Diaz et al. (2014) also exposed $nCeO_2$ (500 mg/kg) to radish and reported no effects in production but changes on the antioxidant power of radish tubers. Rico et al. (2015) reported that $nCeO_2$ increased plant biomass in *Hordeum vulgare*, but inhibited grain formation in plants exposed to 500 mg/kg.

Several reports have also shown that $nCeO_2$ affect the activity of stress enzymes. Zhao et al. (2012b) reported that catalase (CAT) and ascorbate peroxidase (APOX) activities increased up to day 15 in shoots of corn seedlings exposed to $nCeO_2$ at 800 mg/kg soil. Rico et al. (2013b) found a decrease in CAT activity, yet an increase in APOX activity in rice roots exposed to 500 mg $nCeO_2$/kg soil. Majumdar et al. (2014) reported a decrease in APOX in kidney bean leaves of plants exposed for 15 days to 250 and 500 mg $nCeO_2$/kg.

A few studies have shown the effects of surface coating on the interaction of ENMs with plants. Zhao et al. (2012a) reported that the uptake of Ce by corn plants exposed to alginate coated $nCeO_2$ was driven by the soil organic matter. In a more recent study, Trujillo-Reyes et al. (2013) found that the Ce uptake by radish was significantly lower in plants exposed to citric acid coated $nCeO_2$, compared to uncoated NPs. Continuous increments in the applications of coated CeO_2 NPs increase the chances for their build up in the environment, which could result in unpredicted effects on crop plants. In addition, Hernandez-Viezcas et al. (2013) have shown that $nCeO_2$ taken up by crop plants are stored without changes in plant organs. Tomatoes are berry-type fruits widely consumed in raw form. Thus, they could become a carrier of $nCeO_2$ into the food chain.

In this research, effects of Ce compounds/NPs on the growth, fruit production, uptake of Ce and essential elements, as well as chlorophyll content and the activity of CAT and APOX enzymes were measured in fully developed tomato plants.

2. Materials and methods

2.1. Preparation of nanoparticle suspensions and other treatments

Uncoated CeO_2 NPs ($nCeO_2$) (10 nm, Meliorum Technologies, Rochester, NY) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to a previous characterization (Keller et al., 2010), these $nCeO_2$ have primary size of 8 ± 1 nm, particle size of 231 ± 16 nm in DI water, surface area of 93.8 $m^2\ g^{-1}$, and 95.14% purity. Citric acid coated CeO_2 NPs (CA + $nCeO_2$, 1:2 ratio) were prepared and characterized according to Trujillo-Reyes et al. (2013). Enough particles were suspended in an 8:2 v/v water:ethanol solution to reach a 0.001 M concentration. Nanoparticles were sonicated (Crest Ultrasonics, Trenton, NJ) in a water bath for 60 min at 20 °C with a sonication intensity of 180 watts. Another 8:2 v/v water:ethanol solution was prepared with enough citric acid to reach a concentration of 0.002 M. The reaction was adjusted to pH 7–8 with a 3 M NaOH solution. Both solutions were mixed and maintained in reflux for 3 h. At last, ethanol evaporated, and the coated NPs were oven dried at 65 °C for 24 h. Suspensions/solutions of NPs or compounds including $nCeO_2$, CA + $nCeO_2$, bulk CeO_2 ($bCeO_2$), cerium acetate (CeAc), and citric acid (CA) were prepared with MPW in order to add to each pot 0, 62.5, 125, 250 and 500 mg/kg of the respective compound. Each pot was irrigated with 450 mL of the corresponding suspension/solution. These concentrations were selected after Rico et al. (2013b). The calculations were done according to the amount of potting soil used per pot (~450 g). Suspensions were stirred and sonicated for 30 min to avoid aggregation before homogeneous mixing with the soil.

2.2. Seed germination and plant growth

Seeds of tomato (*S. lycopersicum*), Roma variety, were purchased from Del Norte Seed & Feed (Vinton, TX). Seeds were placed in a beaker with MPW and stirred for 3 h until hydrated. One thousand, six hundred and eighty grams of Miracle-Gro® organic potting mix were separated, put in a glass container, and mixed with the Ce treatments. A brief description of the Miracle-Gro® is shown in Table S9 of the Supplementary data. Four hundred and twenty grams of the Ce amended soil and control soil were placed in each pot, creating four replicates per treatment, except the MPW control that had 16 replicates, four for each Ce