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Research article

Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L)

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ABSTRACT

The effects of nCeO$_2$ on food quality are not well known yet. This research was performed to determine the impact of nCeO$_2$ on radish (*Raphanus sativus* L.). Plants were cultivated to full maturity in potting soil treated with nCeO$_2$ at concentrations of 0, 62.5, 125, 250, and 500 mg/kg. Germination, growth, photosynthesis, ionome, and antioxidants were evaluated at different growth stages. Results showed that at 500 mg/kg, nCeO$_2$ significantly retarded seed germination but did not reduce the number of germinated seeds. None of the treatments affected gas exchange, photosynthesis, growth, phenols, flavonoids, and nutrients' accumulation in tubers and leaves of adult plants. However, tubers' antioxidant capacity, expressed as FRAP, ABTS$^{\bullet-}$ and DPPH, increased by 30%, 32%, and 85%, respectively, in plants treated with 250 mg nCeO$_2$ kg^{-1} soil. In addition, cerium accumulation in tubers of plants treated with 250 and 500 mg/kg reached 72 and 142 mg/kg d wt, respectively. This suggests that nCeO$_2$ could improve the radical scavenging potency of radish but it might introduce nCeO$_2$ into the food chain with unknown consequences.

1. Introduction

The rapid expansion of nanotechnology has triggered the production of large amounts of metal-based engineered nanoparticles (ENPs) (Casals et al., 2008; Roco, 2011; Keller et al., 2013). This has raised concerns about the environmental buildup of ENPs, such as cerium dioxide (nCeO$_2$), and their impact on plants. nCeO$_2$ are massively produced and widely used (Piccino et al., 2012). In 2010, an estimate of the global material flow for nCeO$_2$ indicated that 8200 tons ended in landfill, 1400 in soil, 100 in air, and 300 in water (Keller et al., 2013). Currently, the environmental impacts of nCeO$_2$ are still not well understood. Thus, they are still included among the 14 engineered nanomaterials in the list of priority to be evaluated for human health and environmental safety effects (OECD, 2010).

Previous reports have shown that nCeO$_2$ reduce seed germination in corn (*Zea mays* L.), tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) (Lopez-Moreno et al., 2010a). But contrasting results have been reported for nCeO$_2$ effects on plant growth. Lopez-Moreno et al. reported that nCeO$_2$ increased root elongation in cucumber and corn but reduced root length in alfalfa and tomato. Radish (*Raphanus sativus*) is a worldwide consumed plant. Radish tubers have been known to have very high antioxidant and free radical scavenging activity, due to the high content of polyphenols (Lugasi et al., 1998). In addition, flavonoids found in radish extract have shown high radical scavenging potency (Takaya et al. 2003). Only one report has described the effects of nCeO$_2$ on the growth of radish (*R. sativus* L.) (Ma et al., 2010). Ma et al. (2010)

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explored the effects of nanoparticles containing rare earth elements on root growth of several plant species. They found that nCeO$_2$ at 2000 mg L^{-1} did not affect root elongation in radish seedlings. It has been reported that in most cases nCeO$_2$ do not undergo biotransformation in vegetative and reproductive organs of plants like soybean (Lopez-Moreno et al., 2010b; Priester et al., 2012; Hernandez-Viezcas et al., 2013) and vegetative organs of cucumber (Zhang et al., 2012). This indicates nCeO$_2$ can enter into the food chain through plants.

A few studies have described the potential mechanisms of plant toxicity of nCeO$_2$. It seems that mixed valence states of nCeO$_2$ give them biological antioxidant capacity due to oxygen vacancies at the surface (Korsvik et al., 2007). This property allows nCeO$_2$ to quench reactive oxygen species (Niu et al., 2011), inducing cellular resistance to exogenous sources of oxidative stress (Xia et al., 2008). However, if cerium phosphate is formed in the surface, the redox cycling between Ce^{3+} and Ce^{4+} is altered; thus, the antioxidant capacity of nCeO$_2$ is reduced (Xue et al., 2012).

The interaction of nCeO$_2$ with plants depends on several factors such as organic matter content and surface coating. Zhao et al. (2012a) cultivated corn in organic matter-enriched and non-enriched soil treated with pristine and alginate coated nCeO$_2$. Higher Ce uptake was reported in corn roots treated with pristine nCeO$_2$ in organic soil, but the Ce translocation from roots to shoots was longer in non-enriched soil (Zhao et al., 2012a). nCeO$_2$ treatments increased H_2O_2 in corn; however, heath shock protein 70, catalase, and ascorbate peroxidase activities also increased, protecting the plants from the oxidative stress (Zhao et al., 2012b). In a more recent study, Rico et al. (2013a) found that 62.5 and 125 mg L^{-1} of nCeO$_2$ reduced the H_2O_2 generation in both roots and shoots of rice seedlings; while at 500 mg L^{-1}, nCeO$_2$ modified stress enzyme activities and the concentration of ascorbate and free thiols, damaging membranes and altering photosynthesis.

Studies have shown that nCeO$_2$ affect the radical scavenging ability of plant stress enzymes (Korsvik et al., 2007; Niu et al., 2011; Morales et al., 2013). In addition, Rico et al. (2013b) reported that nCeO$_2$ at 500 mg/kg reduced all antioxidants in rice grains but flavonoids. To the authors' knowledge the effects of nCeO$_2$ on antioxidant capacity and nutritional value of radish plants have yet to be reported. In the present study, radish plants were germinated and grown to full maturity in soil spiked with nCeO$_2$ at several concentrations. Effects on germination were evaluated at four and 12 days while agronomic parameters were evaluated at 12 and 40 days. nCeO$_2$ effects on nutrient accumulation and antioxidant capacity were determined through biochemical assays and spectroscopic determinations.

2. Materials and methods

2.1. Nanoparticle characteristics

Commercial nCeO$_2$ (Meliorum Technologies, Rochester, NY) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). As per Keller et al. (2010) and Rico et al. (2013a) "nCeO$_2$ used in this study were previously characterized as rod, with primary size of 8 ± 1 nm, particle size of 231 ± 16 nm in deionized water (DI), surface area of 93.8 m^2/g, and 95.14% purity."

2.2. Substrate

The substrate was prepared as previously described (Zhao et al., 2014). Seeds were sown "in Poly-Tainer general purpose pots containing 2.34 kg of a mixture (by volume) of 1:1:3 of Fabens (Texas, USA) loamy sand soil (3.7% clay, 12.2% silt, 84.1% sand, and 0.04% organic matter, pH 7.9), sand (Quikrete® Premium Play Sand, Atlanta, GA), and Sunshine Mix #4 (SunGro Hort., Bellevue, WA). nCeO$_2$ suspensions, previously sonicated for 30 min, were applied to the soil to have quadrupled pots with final concentrations of 0, 62.5, 125, 250, and 500 mg/kg soil." The seeds were sown 24 h after NPs application to soil.

2.3. Seed planting and greenhouse conditions

Radish (*R. sativus* L.) seeds, Champion variety (Del Norte Seed & Feed, Anthony, TX), were screened to have similar size units, immersed in NaClO al 4% for four min and rinsed six times with Millipore water (MPW, Milli-Q Advantage A10, 18.2 ± 0.7 M Ohm cm). Each pot was planted with 20 seeds at 0.5 cm depth and allocated in the greenhouse. Plants were grown until harvesting (40 days after planting) in a greenhouse under conditions previously described by Zhao et al. (2014) "30.7 ± 5.3 °C (mean ± standard deviation) during the day and 24.8 ± 2.9 °C during the night and light intensity of 18.0 ± 3.5 mol m^{-2} d^{-1}. Each pot was added with 100 mg of 15-15-15 (N–P–K) and plants were sprayed one time with Abamectin 2% (Lucid, Rotam North America, Miami, FL) for aphids control."

2.4. Seed germination and seedlings growth

Germination was recorded at four and 12 days after sowing and, at that stage, plants were thinned to have only eight plants per pot. The effects of nCeO$_2$ on seed germination were evaluated as previously described (Lopez-Moreno et al., 2010a). Equations (1)–(3) were used to calculate germination percentage (%G), relative germination (RG), and germination change (GC), respectively.

$$\%G = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \cdot 100 \quad (1)$$

$$RG = \frac{\%G \text{ in treatment}}{\%G \text{ in control}} \cdot 100 \quad (2)$$

$$GC = \%G \text{ of treatment} - \%G \text{ of controls} \quad (3)$$

At each evaluation period (four and 12 days), seedlings were first washed under the tap water stream, then with 2% HNO_3 solution, and subsequently rinsed three times with MPW. The seedlings length was measured from the root tip to the tip of the longest leaf. Root and stem length was measured from the main root apex to the crown and from the crown to the emission of cotyledonal leaves. The number of true leaves was also recorded. The seedlings were severed into root, stem, cotyledonal leaves, and true leaves. The pooled portions of 10 plant/replicates were freshly weighed, oven dried for 72 h at 70 °C (Fisher Scientific), and weighed to determine biomass production and percent of humidity (PH) by using equation (4).

$$PH = \frac{\text{Fresh weight} - \text{Dried weight}}{\text{Fresh weight}} \cdot 100 \quad (4)$$

2.5. Accumulation of cerium, micro and macroelements

Elements were determined in seedlings collected 12 days after sowing and in mature plants (40 days after sowing). Briefly, "0.1 g of dried sample was microwave-assisted acid digested in a microwave acceleration reaction system (CEM Mars$_x$, Mathews, NC) using plasma pure HNO_3 and 30% H_2O_2 in a 1:4 (v/v) ratio," as described by Lopez-Moreno et al. (2010a). Digests were adjusted to 25 mL