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Dose-Dependent Impacts of Nano-Sized Ceria (CeO_2) on Seed Germination, Early Growth and Physiological Parameters of Marigold Seedling

Sedighe Jahani[1], Sara Saadatmand[1], Malihe Jahani*[1], Homa Mahmoodzadeh[2], Ramazan Ali Khavari-Nejad[1]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

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*Corresponding author's e-mail: malihe.jahani2009@gmail.com

Abstract

Marigold is widely used as an ornamental-medicinal plant. Interaction between nanoparticles (NPs) and biological systems is one of the most promising areas of research in modern nanoscience and technology. Researchers have reported the uptake of cerium oxide or ceria (CeO_2) NPs by plants. The aim of this investigate was to determine the impacts of nanoceria on seed germination, growth and biochemical characteristics of 9-day-old seedling of marigold (*Calendula officinalis* L.). Seeds were germinated in Petri dishes containing eight various dosages of nanoceria (0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 g/L). After 9 days, early growth and biochemical parameters were measured. Results showed that seed germination, fresh and dry weights of seedling, and lengths of radicle, plumule, and seedling stimulated at 0.05 and/or 0.1 g/L of nanoceria but retarded at higher dosages (after 0.2 or 0.4 g/L) of NPs. The contents of H_2O_2, malondialdehyde (MDA) and lipoxygenase (LOX) activity incremented after 0.2 g/L of nanoceria. The activities of antioxidant enzymes and protein content were incremented at higher dosages of nanoceria. Also, the activity of phenylalanine ammonialyase (PAL), phenol content, and antioxidant capacity stimulated at 0.8 to 3.2 g/L of nanoceria. The proline content improved at 0.2-3.2 g/L of nanoceria. Altogether, the results confirmed the inducive oxidative stress of nanoceria that was accompanied by plant defense system include antioxidant enzymes, phenolic compounds and compatible osmolytes such as proline. These results showed that nanoceria at the low dosages (0.05 and/or 0.1 g/L) caused a positive induction on marigold germination and seedling growth but by increasing in its dosage (more than 0.2 g/L), the results was reversed and showed toxicity that forced the plant to activate its defense systems.

Keywords: *Calendula officinalis* L., Germination percentage and rate, Nanotoxicity, Plant defense system, Radicle and plumule length.

INTRODUCTION

Calendula officinalis L. (marigold) is as an ornamental-medicinal plant of the Asteraceae family, which originated in the Mediterranean and West Asian regions (Jalali and Naderi, 2019; Soroori *et al*., 2021). This plant utilize in urban gardens and landscapes (Nazdar *et al*., 2017; Vojodi Mehrabani *et al*., 2017; Zarghami Moghadam *et al*., 2021). The essential oils of this plant have several therapeutic activities, such as anti-inflammatory, anti-tumorogenic, anti-bacterial and wound healing properties (Ak *et al.*, 2021).

Cerium (Ce) is a rare earth element and abundant in the earth's crust that can be found in nature as cerium (III) or cerium (IV). Widespread applications of cerium oxide (ceria) nanoparticles (CeO_2 NPs) resulted in their toxic impacts on both human health and environment (Dahle and Arai, 2015; Hussain *et al*., 2019). It was estimated that around 1,255 tons of nanoceria would be utilized in diesel fuel added substances every year by 2020, of which basically 6% could be delivered into the environment, which has raised a few worries (Priester *et al.,* 2012). The physicochemical properties of nanoceria such as surface charge, particle size, shape as well as particle aggregation and reactivity specify its impacts on the environment (Baalousha *et al.,* 2012; Prakash *et al.,* 2021).

Ceria NPs could be absorbed in plants by the root or leaf and induce various responses depend on the exposure dosage and plant species. For example, low dosages (0.01-0.125 g/L) of nanoceria have positive influences in terms of growth and yield in tomato and rice (Wang *et al.*, 2012; Rico *et al.*, 2013a). Whereas, higher dosages of nanoceria (4 g/L) adversely affect the plant physiology, metabolism, and yield such as root elongation in alfalfa and tomato (López-Moreno *et al.*, 2010). In other study, high dosage of nanoceria (2 g/L) caused to the decrement of growth and photosynthetic pigments and the increment of anthocyanin in bean (Salehi *et al*., 2019).

Seed germination as the primary stage of the plant developmental cycle plays an important role in assessing plant toxicity of NPs (Szőllősi *et al*., 2020). Few studies have examined the influences of nanoceria on seed germination. Tumburu *et al.* (2015) found the promoted germination in *Arabidopsis thaliana* exposed to up to 0.5 g/L nano-scale ceria. Ma *et al.* (2010) reported that higher dosages of nano-scale ceria decremented seed sprouting in cucumber, corn and tomato. In other study, reduction of radicle length in lettuce, ryegrass, and tomato exposed to 0.5-1 g/L nano-scale ceria was reported (Anderson *et al*., 2016). Also, diminution of radicle length in barley treated with 0.5 g/L nano-scale ceria was documented (Mattiello *et al*., 2015).

Adverse environmental conditions such as NPs toxicity and theirs harmful influences on plants growth could be associated with the generation of reactive oxygen species (ROS) in the plant system that results into oxidative stress (Rao and Shekhawat, 2016; Jahani *et al*., 2019b). As, in rice roots, nanoceria heightened membrane lipoperoxidation, ion leakage and H_2O_2 accumulation (Rico *et al.*, 2013b). ROS may assault the cell membrane phospholipids, inflicting lipoperoxidation and ion leakage. Plants may respond to oxidative stress by non-enzymatic- and enzymatic-ROS scavenging systems (Jahani *et al*., 2018). For instance, the increment of electrolyte leakage, lipoproxidation, and H_2O_2 as well as the increment of antioxidant enzymes activities in tomato seedlings imposed by nano-scale ceria was reported (Singh *et al*., 2019). Also, Zhao *et al.* (2012) showed that catalase (CAT) and ascorbate peroxidase (APX) might help plants defend against nano-CeO_2. In other study, Salehi *et al*. (2019) reported that the incremented CAT and peroxidase activity in bean seedlings exposed to nano-scale ceria.

Considering the key role of seed germination stage in phytotoxicity assay of NPs as well as the importance of investigating the dose-dependent impacts of NPs on seed germination, there is a need to comprehend the mechanisms of beneficial and toxic impacts of NPs under seed germination stage. To the best of author's knowledge, there is no study on seed germination and early growth seedling of marigold imposed by nanoceria. Therefore, the present study's experiments were

were undertaken to evaluate the dose-dependent impacts of nanoceria on seed germination, early growth and physiological properties of marigold seedling.

MATERIALS AND METHODS

Ceria NPs properties and preparation of NPs suspensions

Nanoceria was purchased from Iranian Nanomaterials Pioneers company (Mashhad, Iran). According to the data provided by the manufacturer, the NPs were spherical, light yellow color, 30-50 m^2/g surface area, 7.13 g/cm^3 true density and 99.9% purity. The particles properties (including TEM and XRD images) were presented in Figs.1 and 2. The XRD image showed a pure cubic phase structure and the TEM image showed the NPs size was between 10-30 nm.

NPs were suspended in double-distilled water to obtain 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 g/L dosages and homogenized by sonication (Bandelin Sonorex, Faraz-Teb Tajhiz, Iran) for 30 min before utilizing.

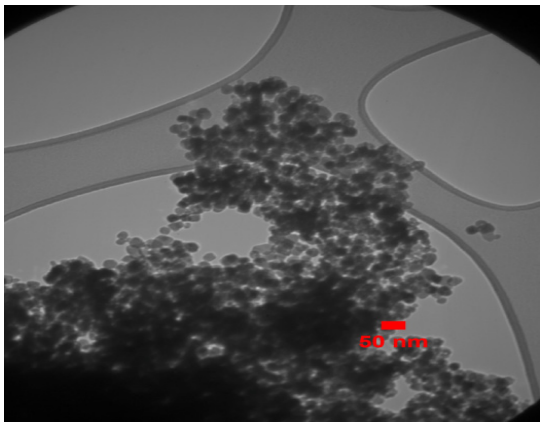

Fig. 1. Image of TEM (scale bar: 50 nm) of nano-ceria.

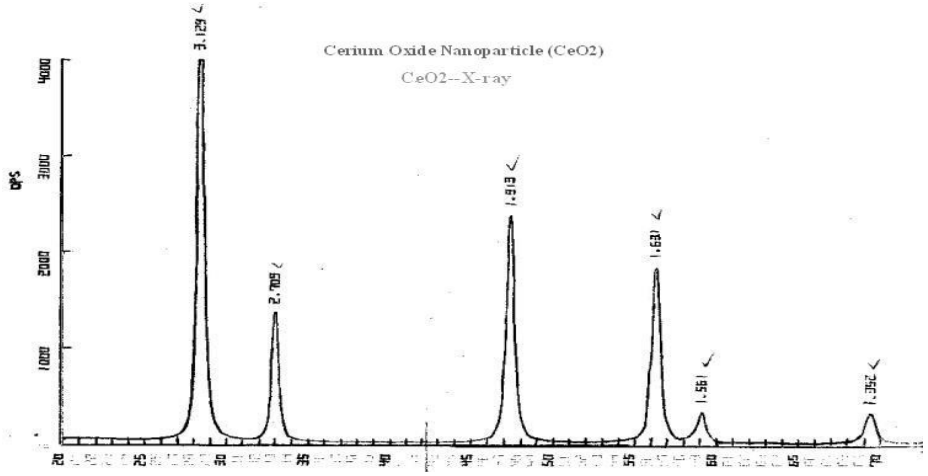

Fig. 2. Image of XRD of nano-ceria.

Experimental design, seed treatment and measurement of seed germination and early growth indices

This research was conducted as a completely randomized design with four replications in the Plant Research Laboratory of Mashhad Azad University. Marigold 'Gitana Orange' seeds were obtained from Hem Zaden B.V. Company (Netherlands). The 25 seeds were surface sterilized (10% sodium hypochlorite solution for 5 min) and were placed onto a one piece of sterilized filter

paper in a Petri dish. Thereafter, 7 mL of each dosage treatment of nano-scale ceria (0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 g/L) was added. In control sample, treatment solution was replaced by distilled water. The number of germinated seeds was noted daily for 9 days. Seeds were considered as germinated when their radicle was at least 1 mm long.

Germination percentage (GP) and germination rate (GR) were also computed from the following formulas proposed by Wu *et al.* (2011) and Maguire (1962), respectively.

$$GP = 100 \times GN / SN$$

- GN is the total number of germinated seed; SN is the total number of seeds tested.

$$GR = (a/1) + (b-a/2) + (c-b/3) +...+ (n-n-1/N)$$

- Where a, b, c … n are numbers of germinated seeds after 1, 2, 3, … N days from the start of Imbibition.

After 9 days, growth indices including lengths of seedling (Fig. 3), shootlet (plumule) and rootlet (radicle) and fresh and dry weights of seedling (with Sartorius digital scale TE214S) were estimated. The remained samples were frozen in liquid nitrogen and stored at -80°C for additional assessments.

Fig. 3. Impact of various dosages of nano-scale ceria on elongation of 9-day-old seedling of marigold.

Physio-biochemical assessments in 9-day-old seedling

Evaluation of Ce content in 9-day-old seedling

The quantity of Ce in seedling was examined utilizing ICP-OES (SPECTRO ARCOS, 76004555, Germany). Shortly, dried samples were digested in 5 mL HNO_3 for 24 h and warmed at 90°C for 1 h. After cooling, 1 mL H_2O_2 (30% v/v) was added and the samples were positioned on heater (LMS-1002, LabTech, Korea) until drying the blend. A while later, the samples were isolated from the cooled heater and the volume reached up to 25 mL with dH_2O (Li *et al*., 2014; Zhang *et al*., 2015).

Estimation of oxidative indexes (MDA, other aldehydes, lipoxygenase enzyme (LOX), and H_2O_2) in 9-day-old seedling

The level of membrane damage was examined by measuring malondialdehyde (MDA) as final

product of membrane lipoperoxidation at 532 and 600 nm and an absorption coefficient of 1.55×105 M^{-1} cm^{-1} (Heath and Packer, 1969). Other aldehydes contents were estimated according to Meirs *et al.* (1992). To compute the quantity of other aldehydes, an absorption coefficient of 0.457×105 M^{-1} cm^{-1} was utilized.

The enzyme activity of LOX was examined based on the method described by Doderer *et al.* (1992). First, linoleic acid substrate solution was prepared. The activity was expressed as produced hydroperoxide per min (unit) at 25°C per mg protein utilizing an extinction coefficient of 25,000 M^{-1} cm^{-1}.

The quantity of H_2O_2 was assessed as illustrated by Alexieva *et al.* (2001). The reaction between iodide (KI) and hydrogen peroxide was determined with a spectrophotometer at 390 nm.

Assessment of enzymes activities and protein content in 9-day-old seedling

The spectrophotometric assessment of CAT activity is performed following the method of Aebi (1984). The activity was estimated based on an extinction coefficient 40 mM^{-1} cm^{-1} for H_2O_2 at 240 nm and expressed as µmol of H_2O_2 min^{-1} mg^{-1} protein.

The Giannopolitis and Ries (1977) method was utilized to estimate superoxide dismutase (SOD) activity by evaluating its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT). One unit of enzyme is the quantity of SOD that inhibits the rate of NBT formation by 50% at 560 nm.

The activity of APX was estimated by recording the decrement in optical density due to ascorbic acid at 290 nm. One unit of enzyme determines the quantity needed to decompose 1 µmol of ascorbate min^{-1} (Nakano and Asada, 1981).

The guaiacol peroxidase (GPX) activity was determined in accordance with the technique of Mac-Adam *et al.* (1992). Enzyme activity was assessed as the increment in absorbance at 436 nm (for three minutes) min^{-1} mg^{-1} protein.

Polyphenol oxidase (PPO) activity was estimated by a spectrophotometric method as described by Raymond *et al.* (1993). The activity was expressed as change in absorbance at 430 nm (for 4 minutes) min^{-1} mg^{-1} protein.

The phenylalanine ammonia-lyase (PAL) activity was determined by determining the rate of cinnamic acid production. The reaction mixture was made by mixing extraction buffer (Tris-HCl 500 µM with pH 8) with 6 µM phenylalanine and 100 µL enzyme extract. One unit of enzyme activity was expressed as the amount of PAL that produced 1 µmol of cinnamic acid within 1 min and was expressed as µmol cinnamic acid min^{-1} mg^{-1} protein (Beaudoin-Eagan and Thorpe, 1985).

Total protein was examined spectrophotometrically at 590 nm with bovine serum albumin as the standard utilizing the dye-binding method of Bradford (1976).

Assessment of DPPH activity, phenol and proline contents in 9-day-old seedling

The DPPH (2,2-diphenyl-1-picrilhydrazil) was determined accordance with Blois (1958). Shortly, 200 µL methanol was mixed with 1 mL 500 µM DPPH. After 30 min, absorbance was recorded at 517 nm. DPPH content was assessed utilizing the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

The quantity of total phenol was evaluated utilizing Folin-Ciocalteu reagent at 765 nm as described by Singleton and Rossi (1965). Methanolic extract (100 µL) was mixed with 2.5 mL Folin-Ciocalteu reagent and 2 mL sodium carbonate solution 7.5%. The mixtures were allowed standing for 1 h before its absorbance. Gallic acid was utilized as a standard for the calibration curve.

Proline content was evaluated by ninhydrin reagent spectrophotometrically at A_{520} nm accordance with the technique described by Bates *et al*. (1973).

Statistical analysis

All of the experiments were conducted in a completely randomized design with four independent repetitions. Statistical analyses were carried out utilizing ANOVA by SPSS v.22 software and were expressed as the mean values ± standard deviations (SD). Duncan's test was utilized to compare the means at 5% probability level.

RESULTS

Seed germination and seedling cerium accumulation

Impact of nanoceria on cerium content of seedling, and GP (%) and GR of seed are displayed in Table 1. The results of Ce accumulation in marigold seedling exposed to nanoceria showed an obvious dose-dependent impact and maximum level (118.20 mg/kg DW) was connected to 3.2 g/L. The GP % was incremented in 0.05 and 0.1 g/L of nanoceria, but GP % decremented with 0.4 to 3.2 g/L of NPs. The GR remained steady up to 0.4 g/L nanoceria, but decremented at higher dosages.

Table 1. Influence of different dosages of nanoceria on cerium accumulation, germination percentage (GP), and germination rate (GR) of 9-day-old seedling of marigold.

Nanoceria (g/L)	Cerium content of seedling (mg/kg DW)	Germination percentage of seed (%)	Germination rate of seed (number/day)
0	0.00±0.00^{f}	88.0±1.6^{b}	4.4±0.3^{ab}
0.05	9.46±0.80^{e}	92.7±3.0^{a}	4.5±0.2^{a}
0.1	13.39±0.63^{e}	94.2±1.7^{a}	4.5±0.1^{a}
0.2	29.49±1.70^{d}	86.0±1.8^{b}	4.2±0.2^{ab}
0.4	57.08±2.80^{c}	80.7±1.5^{c}	4.1±0.3^{b}
0.8	78.73±3.77^{b}	73.5±1.9^{d}	3.5±0.1^{c}
1.6	85.03±7.63^{b}	61.2±2.2^{e}	3.3±0.1^{c}
3.2	118.20±9.15^{a}	57.1±1.8^{f}	3.2±0.1^{c}

* Data represents mean ± SD of four replicates. Means with different letters are meaningful different at $P<0.05$ as determined by the Duncan test.

Seedling growth and biomass parameters in 9-day-old seedling

Early growth and biomass parameters e.g. length of radicle, plumule and seedling, fresh weight (FW) and dry weight (DW) of marigold seedling exposed to nanoceria are displayed in Table 2. Length of radicle and seedling of marigold were incremented at the low dosages (0.05 and 0.1 g/L) of nanoceria and were decremented after 0.2 g/L; however, length of plumule was decremented after 0.4 g/L. Seedling FW and DW incremented at 0.1 g/L of nanoceria but started to decline in higher dosages of nanoceria and maximum reduction occurred at 1.6 and 3.2 g/L of treatment.

Table 2. Influence of different dosages of nanoceria on lengths of radicle, plumule, and seedling, and fresh weight (FW), and dry weight (DW) of 9-day-old seedling of marigold.

Nanoceria (g/L)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
0	3.28±0.10^{b}	5.06±0.26^{bc}	8.34±0.36^{b}	0.080±0.001^{bc}	0.0040±0.0002^{bc}
0.05	3.43±0.11^{a}	5.52±0.12^{a}	8.96±0.24^{a}	0.082±0.002^{ab}	0.0042±0.0001^{ab}
0.1	3.54±0.13^{a}	5.26±0.13^{ab}	8.80±0.27^{a}	0.085±0.003^{a}	0.0043±0.0001^{a}
0.2	3.20±0.10^{b}	4.90±0.19^{c}	8.11±0.29^{bc}	0.077±0.003^{cd}	0.0039±0.0001^{c}
0.4	2.91±0.11^{c}	4.78±0.31^{c}	7.70±0.42^{c}	0.074±0.002^{d}	0.0039±0.0001^{c}
0.8	2.75±0.09^{d}	4.26±0.15^{d}	7.02±0.25^{d}	0.062±0.002^{e}	0.0035±0.0001^{d}
1.6	2.04±0.07^{e}	4.18±0.18^{d}	6.23±0.25^{e}	0.059±0.001^{ef}	0.0034±0.0001^{de}
3.2	1.91±0.06^{e}	3.44±0.14^{e}	5.36±0.21^{f}	0.056±0.002^{f}	0.0032±0.0001^{e}

* Data represents mean ± SD of four replicates. Means with different letters are meaningful different at P<0.05 as determined by the Duncan test.

Oxidative indicators contents in 9-day-old seedling

Impact of nanoceria on oxidative markers contents are displayed in Table 3. Results showed that by raising the dosage of nanoceria, H_2O_2 and other aldehydes raised and reached the maximum at 0.8-3.2 g/L. Also, MDA content incremented after 0.2 g/L of nanoceria and reached a peak at 3.2 g/L. Furthermore, nanoceria treatment incremented LOX activity at 0.4-3.2 g/L.

Table 3. Influence of different dosages of nanoceria on contents of H_2O_2, MDA, and other aldehydes, and LOX activity of 9-day-old seedling of marigold.

Nanoceria (g/L)	H_2O_2 content (μM/g F.W.)	MDA content (μM/g F.W.)	Other aldehydes content (μM/g F.W.)	LOX activity (Unit/mg protein)
0	1.93±0.21^{c}	0.40±0.02^{c}	0.80±0.06^{b}	0.048±0.003^{c}
0.05	1.87±0.11^{c}	0.41±0.02^{c}	0.82±0.04^{b}	0.049±0.002^{c}
0.1	1.83±0.08^{c}	0.41±0.02^{c}	0.82±0.05^{b}	0.048±0.003^{c}
0.2	2.05±0.16^{c}	0.44±0.03^{c}	0.85±0.06^{b}	0.052±0.005^{c}
0.4	2.60±0.19^{b}	0.59±0.03^{b}	0.89±0.07^{b}	0.068±0.003^{b}
0.8	2.69±0.17^{ab}	0.61±0.02^{b}	1.06±0.09^{a}	0.069±0.005^{b}
1.6	2.82±0.17^{ab}	0.62±0.02^{b}	1.14±0.10^{a}	0.080±0.004^{a}
3.2	2.87±0.18^{a}	0.73±0.03^{a}	1.09±0.04^{a}	0.081±0.005^{a}

* Data represent mean ± SD of four replicates. Means with different letters are meaningful different at P<0.05 as determined by the Duncan test.

Antioxidant enzymes activities in 9-day-old seedling

Changes enzymatic antioxidants activities including CAT, SOD, APX, GPX, and PPO in marigold seedling imposed by nanoceria are shown in Table 4. The activities of CAT and GPX remained steady up to 0.4 g/L nanoceria but improved at higher dosages.

Compared with control treatment, the activities of SOD and APX did not show any change up to 0.2 g/L of nanoceria but incremented at higher dosages and their maximum activities were observed when 1.6 and 3.2 g/L nanoceria were applied. Furthermore, the PPO activity was almost in steady state at applied dosages and showed an increment at 1.6 and 3.2 g/L of nanoceria.

Table 4. Influence of different dosages of nanoceria on the activities of CAT, SOD, APX, GPX, and PPO of 9-day-old seedling of marigold.

Nanoceria (g/L)	CAT activity (Unit/mg protein)	SOD activity (Unit/mg protein)	APX activity (Unit/mg protein)	GPX activity (Unit/mg protein)	PPO activity (Unit/mg protein)
0	1.57±0.19^{b}	2.11±0.19^{c}	4.19±0.34^{c}	2.01±0.34^{c}	3.17±0.42^{b}
0.05	1.62±0.16^{b}	2.21±0.24^{c}	4.24±0.26^{c}	2.11±0.27^{c}	3.16±0.34^{b}
0.1	1.68±0.13^{b}	2.12±0.16^{c}	4.31±0.30^{c}	2.14±0.33^{c}	3.26±0.38^{b}
0.2	1.73±0.13^{b}	2.27±0.23^{c}	4.43±0.20^{c}	2.42±0.30^{c}	3.39±0.30^{b}
0.4	1.79±0.22^{b}	2.79±0.10^{b}	5.18±0.16^{b}	2.45±0.37^{c}	3.46±0.32^{b}
0.8	2.30±0.15^{a}	2.92±0.27^{b}	5.25±0.22^{b}	3.69±0.20^{b}	3.69±0.54^{b}
1.6	2.40±0.12^{a}	3.29±0.19^{a}	5.30±0.26^{ab}	4.89±0.22^{a}	5.42±0.26^{a}
3.2	2.40±0.20^{a}	3.47±0.26^{a}	5.70±0.37^{a}	5.21±0.34^{a}	5.73±0.15^{a}

* Data represents mean ± SD of four replicates. Means with different letters are meaningful different at P<0.05 as determined by the Duncan test.

Proline, protein, phenol contents, antioxidant capacity (DPPH), and PAL activity in 9-day-old seedling

The content of proline remained steady up to 0.1 g/L nanoceria but improved at higher dosages (Table 5). In comparison of control, protein content of seedling did not a lot of change by application of 0.05 to 0.4 g/L of nanoceria and decremented in higher dosages (Table 5). The content of total phenol compound of seedling under various dosages of nanoceria displayed no remarkable variation at 0.05 to 0.4 g/L. Its level enhanced at 0.8 g/L and its maximum level observed at 1.6 and 3.2 g/L (Table 5). Also, the free radical-scavenging activities (DPPH %) in the control and treated seedling were shown in Table 5. DPPH did not changed in low dosage but its level incremented at 0.8 to 3.2 g/L of nanoceria.Furthermore, the activity of PAL exhibited a meaningful increment at 0.8-3.2 g/L nanoceria (Table 5).

Table 5. Influence of different dosages of nanoceria on the proline, protein, and phenol contents, antioxidant capacity (DPPH), and PAL activity in 9-day-old seedling of marigold.

Nanoceria (g/L)	Proline content (µmol/g F.W.)	Protein content (mg/g F.W.)	Phenol content (mg/g D.W.)	Antioxidant capacity (%)	PAL activity (Unit/mg protein)
0	0.31±0.02^{c}	0.75±0.02^{a}	5.12±0.61^{c}	75.10±2.58^{b}	2.36±0.22^{c}
0.05	0.32±0.04^{c}	0.73±0.02^{a}	5.45±0.38^{c}	77.13±2.07^{b}	2.42±0.16^{c}
0.1	0.30±0.02^{c}	0.76±0.01^{a}	5.38±0.51^{c}	77.79±2.42^{b}	2.47±0.15^{c}
0.2	0.34±0.03^{b}	0.77±0.03^{a}	5.56±0.43^{c}	76.29±1.73^{b}	2.58±0.22^{c}
0.4	0.48±0.03^{b}	0.77±0.03^{a}	5.62±0.58^{c}	77.55±2.66^{b}	2.58±0.13^{c}
0.8	0.49±0.02^{b}	0.64±0.01^{b}	6.87±0.28^{b}	89.80±2.08^{a}	3.16±0.10^{b}
1.6	0.49±0.01^{b}	0.61±0.01^{b}	7.75±0.47^{a}	90.38±1.73^{a}	3.24±0.17^{ab}
3.2	0.59±0.04^{a}	0.60±0.03^{b}	7.87±0.18^{a}	90.97±2.91^{a}	3.44±0.11^{a}

*Data represents mean ± SD of four replicates. Means with different letters are meaningful different at P<0.05 as determined by the Duncan test.

DISCUSSION

NPs interact with plants causing morpho-physiological, structural and ultrastructural changes, depending on the properties of NPs (Hussain *et al*., 2019; Jahani *et al*., 2021). The effectiveness of NPs is described with their chemical composition, surface covering, size, reactivity, and dose (Zia-ur-Rehman *et al*., 2018). Nanoceria are one of the most produced metal oxide-NPs worldwide, accounting for about 10,000 tons/year globally (Piccinno *et al*., 2012). In this research investigated a complete evaluation of the impact of nanoceria on seed germination, early growth and some physiological processes of marigold seedling.

The results of present research showed that GP and GR were improved at 0.05 and/or 0.1 g/L of nanoceria. It is presumed that NPs pass through the seed coat and induce an ameliorative influence on the mechanism of seed sprouting (Shinde *et al*., 2020; Szőllősi *et al*., 2020). Also, it was reported that nano-scale ceria via accelerate in water uptake and increment of α-amylase activity caused to increment in seed sprouting (Khan *et al*., 2021). In the present study, it seems that low dosages of nanoceria via facilitating water uptake (Faraji and Sepehri, 2020; Khan *et al*., 2021) cause to increment of germination indices of marigold seeds. In addition, all early growth indices (lengths of radicle, plumule and seedling and fresh and dry weights of seedling) were promoted by 0.05 and/or 0.1 g/L of nanoceria. In the present study, it seems that the ameliorative influence could be because of the intensified water uptake (Nile *et al*., 2022) and cell division (Mahakham *et al*., 2017; Nile *et al*., 2022) by treated seeds. Similarly, the improved impact in seed sprouting and early growth indices of canola seedling imposed by low doses (0.05 and 0.1 g/L) of nano-cobalt oxide was reported (Jahani *et al*., 2022).

On the other hand, in the current study, a meaningful diminish in both seed germination (GP and GR) and early growth indices was observed with incrementing the dosage of nanoceria. Here, it may be due to NPs accumulation, ROS overgeneration, and induction of oxidative stress as well as disturbance of biochemical activities of seed sprouting process. Previous studies have shown that high dosages of metal oxide-NPs can inhibit seed sprouting and consequent seedling growth for various reasons such as inhibition of starch hydrolysis in the endosperm, prevention of release of stored energy, damage to embryos via accumulation of NPs in the respiratory tract and disorder of biochemical activities (Faisal *et al*., 2016; Szőllősi *et al*., 2020).

Plant growth begins from the seed sprouting with signs of radicle elongation and plumule appearance. So, evaluating the plant growth trends in relation to NPs is a key issue (Szőllősi *et al*., 2020). The reported data from different studies suggested the influence of NPs on seed sprouting was dosage-dependent (Zia-ur-Rehman *et al*., 2018; Szőllősi et al., 2020). Low dosages of nano-SiO_2 enhanced seed sprouting of tomato (Siddiqui and Al-Whaibi, 2014). In other study, low dosages of nanoceria enhanced root biomass in kidney bean (Majumdar *et al*., 2015). In Arabidopsis seeds under 0.4-4 g/L nano-Fe_3O_4 had no any impact on sprouting (Lee *et al.*, 2010). Also, nano-SiO_2 could increment radicle length of Arabidopsis at 0.4 g/L (Lee *et al*., 2010) and nanoceria ameliorated the radicle growth of cucumber and corn, indeed in spite of the fact that the GR diminished (López-Moreno *et al*., 2010). Wu *et al.* (2012) reported that nano-sized Co_3O_4 solution had no any inhibitory impact on cucumber seed sprouting and even promoted radicle length of radish under high levels. In other study, high doses (0.25-4 g/L) of nano-Co_3O_4 diminished seed sprouting and early growth indices of canola seedling (Jahani *et al*., 2022).

In the present study, high dosages of nanoceria (more than 0.2 g/L) caused lipoperoxidation by rising of oxidative indices involving the heightened H_2O_2, MDA and other aldehydes contents as well as the elevated LOX activity in marigold seedlings. Here, the meaningful intensification of lipoperoxidation in marigold seedling despite of activated defense mechanisms could be due to the intemperate ROS levels and the raised LOX activity. Similarly, nanoceria (0.4-0.8 g/kg) and

nano-CuO treatments raised H_2O_2 content in maize and mung bean, respectively (Zhao *et al.*, 2012; Nair *et al.*, 2014). Also, in rice roots, nanoceria (0.125 g/L) raised lipoperoxidation; though H_2O_2 production was elevated at 0.5 g/L NPs (Rico *et al*., 2013b). The intemperate quantities of ROS can cause oxidative damage in plants but ROS may be scavenged via antioxidant defense systems (Zia-ur-Rehman *et al*., 2018; Hussain *et al*., 2019). The ultimate result of oxidative damage might be evaluated by peroxidation reactions products like MDA as a marker of lipoperoxidation (Zia-ur-Rehman *et al*., 2018).

In plants exposed to stressful conditions, lipoxygenase–induced polyunsaturated fatty acid hydroperoxidation is another potential cause of ROS (Viswanath *et al*., 2020). In the current study, increment of LOX activity at high dosages of nanoceria may be as a consequence the stimulation of enzyme activity and involvement in lipid peroxidation. Similarly, the heightened activity of LOX in canola seedling imposed by high dosages (0.25-4 g/L) of nano-cobalt oxide was reported (Jahani *et al*., 2019a).

In the present study, the activities of antioxidant enzymes were incremented at high dosages of nanoceria. Here, the heightened antioxidant enzymes activities may be due to the activation of the plant's enzymatic antioxidant defense system against toxicity due to high dosages of nanoceria. Similarly, Rico *et al*. (2013a) showed that nanoceria changes the antioxidative enzyme activities in rice seedlings. Here, the CAT activity incremented at 0.8-3.2 g/L of nanoceria that could be correlated with the NPs accumulation in tissues and induction an oxidative stress.

In addition, here, SOD activity was elevated after 0.2 g/L of ceria-NPs. This enzyme indicates the conversion of $O_2^{\bullet-}$ to H_2O_2 and may change depend on the power and length of stress as well as on the organism's sensibility and development stage (Pulishchuk *et al.*, 2015). Here, a similar pattern was observed in the increment of APX activity that catalyzes H_2O_2 to H_2O that may preserve *C. officinalis* seedlings imposed by nanoceria. Similarly, the heightened activity of CAT and APX in wheat and pumpkin exposed to nano-scale ceria was recorded (Schwabe *et al*., 2015). Also, here, the activity of GPX enzyme was improved after high dosages of nanoceria and such this change has been reported in spinach under nano-TiO_2 (Lei *et al*., 2008). Moreover, in the current study, PPO activity raised after the application of nanoceria at 1.6 and 3.2 g/L. PPO which catalyzes phenols to quinones, is another antioxidant enzyme correlated with ROS deletion and metal detoxification (Hafizi and Nasr, 2018).

The results of present study showed the increment in both phenolic contents and antioxidant capacity under high dosages of nano-scale ceria. Here, the raised phenolic contents and antioxidant capacity in marigold seedlings may be correlated with the activation of the plant's non-enzymatic antioxidant defense system against toxicity due to high dosages of nano-scale ceria. In agreement with this research, the incremented phenol contents in lettuce seedlings exposed to nano-scale ceria was reported (Kalisz *et al*., 2021). Phenolic compounds are secondary metabolites, which have an important function against stress (Zia-ur-Rehman *et al*., 2018). On the other hand, in the current study, nano-scale ceria treatments (0.8-3.2 g/L) heightened PAL activity. Here, the raised PAL activity in marigold seedlings may be due to the nanotoxicity-induced plant defense. Non-oxidative deamination of L-phenylalanine to form *trans*-cinnamic acid catalyzes by PAL and the reaction is the primary step to produce phenylpropanoid and phenolic compound. In parallel with this research, the raised total phenol content as well as heightened activity of PAL in canola under nano-cobalt oxide was reported (Jahani *et al*., 2020).

In the current study, proline content incremented at high dosages (0.2-3.2 g/L) of nanoceria. Here, the raised proline content may be associated with the induction of the plant's defense system against nanotoxicity. Also, it was reported that the accumulated proline under NPs treatment could be associated with increment of proline synthesis or reduction of proline degradation in response

to nanotoxicity (Zia-ur-Rehman *et al*., 2018). Proline plays a serious role in protein preservation against denaturation as well as in ROS detoxification (Zia-ur-Rehman *et al*., 2018; Soroori *et al*., 2021). In agreement with present research, the heightened proline content in rapeseed imposed by nano-cobalt oxide was reported (Jahani *et al*., 2019a).

CONCLUSION

The impact of NPs on plants can play a critical part in the environment. Despite an increment at lower dosages (0.05 and/or 0.1 g/L), there was a meaningful diminish in seed germination and early growth indices of marigold seedling imposed by high dosages of nanoceria (more than 0.2 g/L). Furthermore, these data demonstrated that high dosages of nanoceria are able to induce oxidative stress and meaningful cytotoxic impacts despite the activation of antioxidant defense system. The toxicity mechanisms of nanoceria at high dosages may be related to ROS overgeneration and increment of LOX activity that induced oxidative stress and lipoperoxidation, which eventually led to decrement in both stages of seed germination and growth seedling. It is suggested that further researches are required to illuminate the impacts of nanoceria on seed germination and early growth indices of other plants.

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