JCHR (2022) 12(2), 237-246

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Investigation of Seed Germination, Early Growth and Physio-Biochemical Parameters of Canola Seedling Exposed to Co_3O_4 Engineered Nanoparticles

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

(Received: 18 January 2020 Accepted: 19 December 2020)

KEYWORDS

Antioxidant capacity;
Canola seedling;
Nano-sized cobalt oxide;
Oxidative stress;
Plumule and radicle growth;
Seed germination

ABSTRACT: The incessant use of nanoparticles (NPs) may pose serious threats on ecosystem and plants are at maximum risk of their delivery into the environment. The goal of this research was to explore the influence of nano-sized Co_3O_4 on seed germination, early growth and physio-biochemical parameters of 6-day-old seedling of canola. Seeds were sprouted in Petri plates involving eight various dosages of nano-sized Co_3O_4 (0-4 g L^{-1}) for 6 days. Germination and early growth parameters (fresh and dry weights of seedling and lengths of radicle and seedling) stimulated at 0.05 and/or 0.1 g L^{-1} of nano-sized Co_3O_4 but retarded after 0.1 g L^{-1} NPs. However, the length of plumule retarded after 0.25 g L^{-1} NPs. The antioxidant capacity and H_2O_2 content raised at higher dosages of nano-sized Co_3O_4. The activity of antioxidant enzymes were enhanced by nano-sized Co_3O_4 treatment but were repressed at higher dosages. The activity of phenylalanine ammonialyase and phenol content incremented at 0.5 and 1 g L^{-1} of nano-sized Co_3O_4 but decremented at higher dosages. The content of malondialdehyde and lipoxygenase activity heightened after 0.1 g L^{-1} of nano-sized Co_3O_4. Altogether, the results confirmed the inducive oxidative stress of nano-sized Co_3O_4 that was accompanied by plant defense system including enzymes, phenolic compounds and compatible osmolytes such as proline. However, high dosages of the NPs caused toxic impacts on physio-biochemical traits of canola seedling as an oilseed crop.

INTRODUCTION

Nanotechnology is at the front line of science and innovation, which has been progressing rapidly and has meaningful utilizations in human life [1-5]. The expanding utilization of NPs can prompt their delivery into the climate and their impacts on biological system [6]. The infiltration of NPs into the cell wall of plants may lead to destructive influence and alternation in the genetic and morpho-physiological properties of plants. The reaction of plants to NPs according to the plant species type and their development stages, and additionally the NPs nature is dissimilar [8].

Cobalt (Co) is a magnetic element with similar properties to nickel and iron [9]. Co can be a pollutant in soils because of agricultural additives or metal refineries [10]. In plants, Co has a crucial role in several processes such as photosynthesis, respiration, and cell growth [11]. Two salts

[*]Corresponding author: malihe.jahani63@gmail.com (M.Jahani)
DOI: 10.22034/jchr.2020.1891185.1092

of Co are utilized in industry on a large scale; water insoluble Co oxides (CoO, Co_2O_3, Co_3O_4) and water soluble cobalt chloride ($CoCl_2.6H_2O$) [12]. Cobalt oxide is utilized in different fields, for instance, catalysis, electrochromic, electrical and other opto-electronic devices and sensors [13, 14].

The growth and development of plants begin from seed germination followed by elongation of radicle and plumule egress [15]. The NPs, depending on their doses, have stimulative and deleterious impacts on seed germination, root elongation and biomass in plants [16]. In radish, cucumber and lettuce seeds, CuO- and NiO-NPs caused to decline of sprouting in seeds through infiltrating to seed surfaces and delivering free metal ions close to seeds [17]. Other research demonstrated the ameliorative impacts of TiO_2- and SiO_2-NPs on sprouting in seeds and growth of *Glycine max* seedlings [18]. Additionally, sprouting in seeds and growth of root of *Cucurbita pepo* were influenced by ZnO NPs [19].

Oxidative stress and reactive oxygen species (ROS) production is the critical biomarker of NPs toxicity and is measured by direct ROS quantification or antioxidant systems [20, 21]. It was reported ceria-NPs treatments increased H_2O_2, heat shock protein 70, catalase and ascorbate peroxidase protecting the plants from the oxidative status in corn [22].

Canola is a crucial source of vegetable oil in the world [23]. Due to need to understand of mechanisms of NPs on plant metabolism, it is crucial more studying on interaction of NPs with plants. To the best of author's knowledge, few researches have undertaken the impact of nano-sized cobalt oxide on plants. So, the current research was undertaken in order to explore the influence of nano-sized Co_3O_4 on seed germination, early growth and physio-biochemical properties of *B. napus* seedling.

MATERIALS AND METHODS

Nano-sized Co_3O_4 analysis

Nano-sized Co_3O_4 was bought from the corporation of Nanomaterials Pioneers (Mashhad, Iran). As indicated by the information gave by the corporation, the NPs were spherical, dark brown in color with surface area of 30-80 m^2 g^{-1}, 6.11 g cm^{3-1} true density and 99.5% purity. The TEM and XRD images were shown in Supplementary Figures 1 and 2. The XRD image showed a pure cubic phase structure and the TEM image showed the NPs size was less than 50 nm.

NPs were suspended in double-distilled water (ddH_2O) to acquire 0, 0.05, 0.1, 0.25, 0.5, 1, 2 and 4 g L^{-1} dosages and homogenized via sonication (Bandelin Sonorex, Faraz-Teb Tajhiz, Iran) for a half-hour prior to utilization.

Seed germination

The seeds of canola cultivar Zarfam were bought from Khorasan Razavi Agricultural and Natural Resources Research Center, Mashhad, Iran. Seeds were selected visually for uniform size and healthy aspect to minimize errors in sprouting of seed. The seeds were sterilized utilizing NaOCl solution (10%) for 5 min and rinsed three times with ddH_2O. The 25 seeds were placed in a 9 cm Petri plate with a one piece of sterilized filter paper (Whatman No. 1) and afterward 7 mL of each dosage treatment (distilled water (dH_2O) for control) was added. Finally, the Petri plates were placed in a germinator (light intensity 18 µmol photon m^{-2} s^{-1}; 25°C, 16h light/8h dark). The number of sprouted seeds was noted daily for 6 days (seeds were regarded as sprouted that their rootlet showed at least 1 mm) and germination percentage (GP) and germination rate (GR) were calculated [24, 25].

Also, growth parameters involving lengths of seedling (plantlet), plumule (shootlet) and radicle (rootlet), FW and DW of seedling were measured (Sartorius digital scale TE214S) (Figure 1) and the remained seedlings were frozen in liquid nitrogen for additional assays.

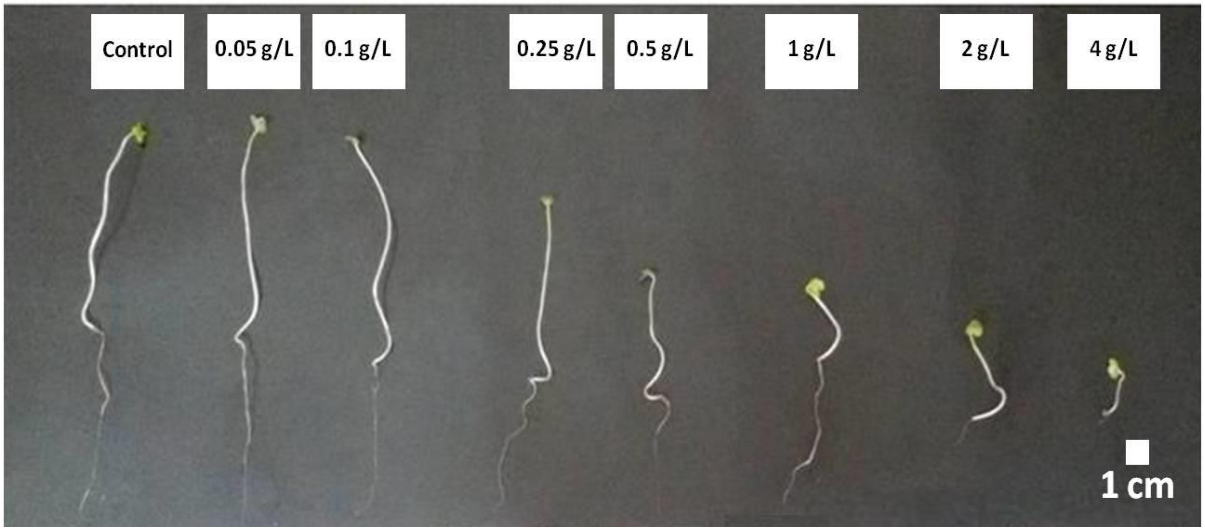

Figure 1. Influence of dosages of nano-sizes Co_3O_4 on elongation of 6-day-old seedling of canola.

Physio-biochemical analysis in 6-day-old seedling

The content of malondialdehyde was measured by absorbance at 532 nm [26]. The contents of other aldehydes (propanal, butanal, hexanal, heptanal and propanol dimethyl acetal) were calculated at 455 nm and the absorbance of other non-specific pigments was read at 600 nm and was deducted from this amount [27]. Lipoxygenase (EC 1.13.11.12) activity was measured with linoleic acid as a substrate [28]. Antioxidant capacity of methanolic extract was carried out using stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the absorbance was recorded at 517 nm [29]. The content of H_2O_2 as μM g^{-1} FW was calculated at 390 nm [30].

The enzymes activities of CAT [31], SOD [32], APX [33], GPOX [34], PPO [35] and PAL [36] were estimated by a spectrophotometric method at 240, 560, 290, 436, 430, and 290 nm, respectively. Moreover, total protein was assayed at 595 nm [37]. The proline content was determined by absorbance at 520 nm [38]. Additionally, total phenols content was assayed using Folin-Ciocalteu reagent at 765 nm [39].

The cobalt content of seedling was measured using ICP-OES (SPECTRO ARCOS, 76004555, Germany). Briefly, dried samples (70°C for 48 h) were digested in 5 mL HNO_3 for 24 h and heated at 90°C for one hour. After cooling, 1 mL H_2O_2 (30% v/v) was added and the samples were placed on heat block (LMS-1002, LabTech, Korea) until drying the mixture. Afterwards, the samples were removed from the heat block cooled down and the volume made up to 25 mL with dH_2O [40].

Statistical analysis

The experiment was conducted in a completely randomised design. All of the experiments were performed at least four independent repetitions. Statistical analyses were performed using ANOVA by SPSS v. 22 software (Armonk, NY: IBM Corp, 2013) and were expressed as the mean values ± SD. Duncan's test was utilized to compare the means at 5% probability level.

RESULTS

Seed germination and early growth parameters

In *B. napus* seeds, GP and GR were calculated at various dosages of nano-sized Co_3O_4 (Table 1). GP was increased at 0.05 and 0.1 g L^{-1} of nano-sized Co_3O_4 and decreased in 0.25-4 g L^{-1} of NPs. GR remained steady up to 0.1 g L^{-1} nano-sized Co_3O_4 but declined due to higher dosages.

Also, some early growth and biomass parameters e.g. length of radicle, plumule and seedling, FW and DW of seedling were measured in *B. napus* (Table 1). Length of radicle and seedling of *B. napus* were increased at the low dosages (0.05 and 0.1 g L^{-1}) of nano-sized Co_3O_4 and were decreased after 0.1 g L^{-1}; however, length of plumule was decreased after 0.25 g L^{-1}.

Seedling FW and DW increased at 0.05 and 0.1 g L^{-1} of nano-sized Co_3O_4 but started to decline at 0.25 g L^{-1} of nano-sized Co_3O_4 and maximum reduction occurred at 4 g L^{-1} of treatment.

Table 1. Influence of various dosages of nano-sized Co_3O_4 on GP, GR, lengths of radicle, plumule, and seedling, and FW and DW of 6-day-old seedling of *B. napus*. Data represent mean ± SD (n=4). Dissimilar letters express meaningful differences (Duncan's test; P≤0.05).

Co_3O_4 NPs (g L^{-1})	GP (%)	GR (number/day)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)	Seedling FW (g)	Seedling DW (g)
0	88.0±2.4^{b}	15.8±0.7^{a}	9.0±0.3^{b}	5.6±0.2^{bc}	14.6±0.5^{b}	0.085±0.004^{b}	0.0051±0.0002^{b}
0.05	97.7±2.2^{a}	16.0±0.6^{a}	9.9±0.4^{a}	6.2±0.3^{a}	16.1±0.6^{a}	0.094±0.004^{a}	0.0055±0.0002^{a}
0.1	96.2±2.1^{a}	15.9±0.8^{a}	10.2±0.4^{a}	5.8±0.3^{ab}	16.1±0.7^{a}	0.090±0.004^{a}	0.0052±0.0002^{ab}
0.25	82.2±3.4^{c}	14.7±0.6^{b}	8.3±0.6^{c}	5.2±0.4^{c}	13.5±0.9^{c}	0.078±0.003^{c}	0.0046±0.0003^{c}
0.5	76.2±4.6^{d}	14.5±0.8^{b}	6.3±0.4^{d}	4.3±0.3^{d}	10.6±0.8^{d}	0.063±0.003^{d}	0.0042±0.0002^{d}
1	61.5±4.5^{e}	13.4±0.9^{c}	5.1±0.6^{e}	4.0±0.2^{d}	9.1±0.8^{e}	0.058±0.003^{e}	0.0033±0.0002^{e}
2	50.0±2.2^{f}	11.3±0.5^{d}	4.5±0.4^{e}	2.7±0.2^{e}	7.3±0.6^{f}	0.036±0.003^{f}	0.0032±0.0003^{e}
4	45.0±5.1^{f}	10.4±0.8^{d}	2.5±0.4^{f}	2.0±0.19^{f}	4.6±0.6^{g}	0.033±0.003^{f}	0.0024±0.0001^{f}

Oxidative indexes, proline, DPPH, phenol and cobalt contents in 6-day-old seedling

Effects of nano-sized Co_3O_4 on lipid peroxidation, H_2O_2 and lipoxygenase activity contents are displayed in Table 2. Results showed that by increasing the dosage of nano-sized Co_3O_4, MDA and other aldehydes enhanced and reached the maximum at 2 and 4 g L^{-1}. H_2O_2 content increased meaningfully after 0.1 g L^{-1} of cobalt oxide NPs applied and reached a peak at 4 g L^{-1} (~2.6 fold of control). Also, nano-sized Co_3O_4 treatment improved LOX activity at 0.25-4 g L^{-1} and the maximum increase was at 4 g L^{-1}.

Compared to the control, the amount of proline was incremented after 0.1 g L^{-1} of nano-sized Co_3O_4 and received to maximum level at 2 g L^{-1} but decremented in 4 g L^{-1} (Table 2).

Also, DPPH in the control and treated seedlings were shown in Table 2. Notable differences of antioxidant capacity were detected in treated seedlings at 0.5 and 1 g L^{-1} of nano-sized Co_3O_4 compared to the control.

The amount of total phenols of seedling under different dosages of nano-sized Co_3O_4 displayed no remarkable variation at 0.05, 0.1 and 0.25 g L^{-1}. Its level enhanced at 0.5 and 1 g L^{-1} (Table 2).

The results of Co accumulation in *B. napus* seedlings with nano-sized Co_3O_4 treatment showed a clear dose-dependent impact and maximum levels (~45 fold of the control) were related to 2 and 4 g L^{-1} (Table 2).

Table 2. Oxidative indexes, proline, DPPH, phenol and cobalt contents of 6-day-old seedling of *B. napus* under various dosages of nano-sized Co_3O_4. Data represent mean ± SD (n=4). Dissimilar letters express meaningful differences (Duncan's test;P≤0.05).

Co_3O_4 NPs (g L^{-1})	MDA content (µM g^{-1} FW)	Other aldehydes (µM g^{-1} FW)	H_2O_2 content (µM g^{-1} FW)	LOX activity (Unit/mg protein)	Proline content (µmol g^{-1} FW)	DPPH (%)	Phenol content (mg g^{-1} DW)	Seedling cobalt content (mg kg^{-1} DW)
0	0.61±0.05^{d}	1.1±0.06^{c}	0.34±0.02^{e}	0.019±0.002^{d}	0.98±0.05^{d}	71.39±2.85^{b}	1.53±0.08^{b}	8.77±0.53^{f}
0.05	0.62±0.04^{d}	1.12±0.09^{c}	0.32±0.02^{e}	0.018±0.002^{d}	0.97±0.07^{d}	73.64±2.17^{b}	1.57±0.07^{b}	42.79±3.05^{e}
0.1	0.59±0.05^{d}	1.09±0.05^{c}	0.35±0.02^{e}	0.019±0.002^{d}	0.98±0.06^{d}	73.67±2.57^{b}	1.60±0.06^{b}	78.03±5.21^{d}
0.25	0.77±0.06^{c}	1.17±0.06^{c}	0.46±0.03^{d}	0.024±0.002^{c}	1.16±0.04^{c}	72.62±3.30^{b}	1.56±0.07^{b}	132.40±10.61^{c}
0.5	0.80±0.02^{c}	1.46±0.08^{b}	0.54±0.04^{c}	0.026±0.003^{c}	1.18±0.05^{c}	83.80±2.46^{a}	1.86±0.10^{a}	225.41±9.62^{b}
1	1.02±0.07^{b}	1.49±0.03^{b}	0.70±0.05^{b}	0.033±0.002^{b}	1.33±0.08^{b}	85.94±3.15^{a}	1.98±0.12^{a}	241.66±22.87^{b}
2	1.19±0.09^{a}	1.67±0.07^{a}	0.73±0.02^{b}	0.036±0.004^{b}	1.44±0.10^{a}	67.68±1.69^{c}	1.38±0.06^{c}	378.61±19.38^{a}
4	1.22±0.04^{a}	1.69±0.1^{a}	0.84±0.03^{a}	0.044±0.003^{a}	0.85±0.04^{e}	64.98±1.36^{c}	1.30±0.09^{c}	391.77±25.93^{a}

Antioxidant enzymes activities and protein content in 6-day-old seedling

Alterations of enzymatic antioxidants activities (CAT, GPOX, APX, SOD, PPO, and PAL) and protein amount in *B. napus* seedlings treated with different dosages of nano-sized Co_3O_4 are shown in Table 3.

The activities of CAT and GPOX increased after 0.1 g L^{-1} application of nano-sized Co_3O_4 and showed maximum value at 0.25 and 0.5 g L^{-1} dosages.

By incrementing the dosage of nano-sized Co_3O_4, APX activity was meaningfully enhanced and reached the maximum at 1 and 2 g L^{-1}. Also, an enhancement in SOD activity was found at same dosages (1 and 2 g L^{-1}).

The activity of PPO exhibited meaningful increment at 1-2 g L^{-1} nano-sized Co_3O_4 and the activity of PAL incremented at higher dosages of nano-sized Co_3O_4 and maximum activity was observed at 0.5 and 1 g L^{-1} nano-sized Co_3O_4.

In comparison of control, amount of protein of seedling incremented notably at 0.25 and 0.5 g L^{-1} of nano-sized Co_3O_4 but declined at higher dosages.

Table 3. Influence of various dosages of nano-sized Co_3O_4 on enzymes activities and protein content of 6-day-old seedling of *B. napus*. Data represent mean ± SD (n=4). Dissimilar letters express meaningful differences (Duncan's test;P≤0.05).

Co_3O_4 NPs (g L^{-1})	CAT activity (Unit/mg protein)	GPOX activity (Unit/mg protein)	APX activity (Unit/mg protein)	SOD activity (Unit/mg protein)	PPO activity (Unit/mg protein)	PAL activity (Unit/mg protein)	Protein content (mg g^{-1} FW)
0	0.58±0.05^{b}	1.02±0.06^{b}	1.61±0.10^{c}	1.23±0.05^{c}	0.29±0.02^{c}	0.57±0.03^{b}	1.36±0.07^{c}
0.05	0.60±0.04^{b}	1.06±0.07^{b}	1.66±0.09^{c}	1.21±0.05^{c}	0.27±0.02^{c}	0.56±0.03^{b}	1.34±0.09^{c}
0.1	0.59±0.05^{b}	1.04±0.05^{b}	1.68±0.08^{c}	1.25±0.06^{c}	0.28±0.02^{c}	0.58±0.03^{b}	1.36±0.07^{c}
0.25	0.74±0.03^{a}	1.39±0.04^{a}	1.64±0.11^{c}	1.52±0.09^{b}	0.26±0.02^{c}	0.57±0.04^{b}	1.53±0.09^{b}
0.5	0.75±0.06^{a}	1.43±0.08^{a}	2.00±0.14^{b}	1.53±0.07^{b}	0.28±0.03^{c}	0.79±0.05^{a}	1.68±0.10^{a}
1	0.48±0.03^{c}	1.00±0.07^{b}	2.32±0.08^{a}	1.80±0.14^{a}	0.35±0.01^{b}	0.80±0.07^{a}	1.29±0.06^{c}
2	0.47±0.04^{c}	0.87±0.05^{c}	2.36±0.16^{a}	1.87±0.06^{a}	0.38±0.01^{a}	0.48±0.04^{c}	1.15±0.03^{d}
4	0.40±0.02^{d}	0.79±0.03^{c}	1.50±0.17^{c}	1.18±0.10^{c}	0.26±0.03^{c}	0.41±0.05^{c}	1.00±0.06^{e}

DISCUSSION

Aggregation of NPs in ecosystem may influence plants process and function [41]. Sprouting of seed ensures plant endurance and may alter species composition in the ecosystem [42]. The influence of NPs on sprouting of seed as a first step of plant growth and development is very important that is related to the dosage of these compounds [41].

Cobalt oxide NPs can cross plasmalemma and translocate in different tissues after absorption into the plants [43]. In this study, higher amounts of Co_3O_4 NPs than the control confirmed aggregation of these NPs in canola seedlings.

This study showed that GP was enhanced at 0.05 and 0.1 g L^{-1} of nano-sized Co_3O_4. It is possible that NPs infiltrate to the seed coat and apply an advantageous impact on the process of sprouting of seed [44]. In addition, all early growth parameters (fresh and dry weight of seedling and length of radicles, plumule and seedling) were stimulated by 0.05 and/or 0.1 g L^{-1} of nano-sized Co_3O_4, The positive responses could due to the amplified uptake of the water and cell division by treated seeds [45, 46]. By contrast, a decrease in both early growth and germination parameters was observed in seedling treated with high dosages of nano-sized Co_3O_4. Similarly, the decline of root length in *Allium cepa* [47] and *Solanum melongena* [48] exposed to nano-sized cobalt oxide was reported. Interestingly, it was reported that nano-sized Co_3O_4 did not retard the sprouting of cucumber seeds and even ameliorated rootlet length of radish at high dosages [17]. Also, in *Arabidopsis thaliana* seeds, treatment of Fe_3O_4 NPs did not cause any impact on sprouting in a dosage range of 0.4-4 g L^{-1} [16].

Oxidative stress and excessive formation of ROS (H_2O_2, $O_2^{\bullet-}$, $OOH^{\bullet}$ and $OH^{\bullet}$) is accepted as one of the leading mechanisms of NPs toxicity [20]. Nano-cobalt can deliver ions and due to fast generate of ROS at cell, higher than those induced by cobalt ions [49]. Here, evaluation of Co_3O_4 NPs-treated seedlings exhibited a conspicuous increment in H_2O_2 content after 0.1 g L^{-1}. Similarly, nano-alumina (50 g L^{-1}) and nano-sized CeO_2 (0.2-3.2 g L^{-1}) treatments increased H_2O_2 content in leaves of wheat and marigold, respectively [50, 51].

Moreover, the activities of antioxidant enzymes altered depend on NPs dosage. The CAT activity increased at 0.25 and 0.5 g L^{-1} of nano-sized Co_3O_4 that may be attributed to the accumulation of the NPs in tissues and induction an oxidative stress. Also, the activity of SOD was stimulated meaningfully, up to 2 g L^{-1} of NPs. This enzyme indicates the conversion of $O_2^{\bullet-}$ to H_2O_2 [52]. A similar pattern was observed in the increase of APX activity that catalyzes H_2O_2 to H_2O that may protect canola seedlings exposed to nano-sized Co_3O_4. The heightened SOD and CAT activity in the pumpkin and ryegrass imposed by nano-sized iron was demonstrated [53]. The activity of GPOX as a tensile enzyme was increased after cobalt oxide NPs treatment and such this alteration has been reported under cadmium treatment [54]. Moreover, the activity of PPO was enhanced after the usage of nano-sized Co_3O_4 at 1 and 2 g L^{-1}. PPO enzyme converts phenols into quinines and has related with ROS eliminate and detoxification of metal [55, 56].

The results showed similar trend and an increase of both phenolic contents and capacity for scavenging DPPH free radicals under cobalt oxide NPs. Phenolic compounds are a main group of plant defense system against oxidative status [57]. Therefore, the increase of phenol in canola seedlings may be related to the NPs-induced plant defense. In addition, the effect of cobalt oxide NPs on the activity of PAL is explored to evaluate resistance behavior of the plant. Conversion of L-phenylalanine to trans-cinnamic acid catalyzes by PAL and this reaction is the first step to produce phenylpropanoid [51]. However, as the dosage of NPs increased after 1 g L^{-1}, the PAL activity and phenol content tend to decline.

The final result of oxidative damage could be assessed by peroxidation reactions products like MDA as an indicator of lipoperoxidation [5, 20, and 58]. In the tested seedling, nano-sized Co_3O_4 generate lipoperoxidation by incrementing the MDA and other aldehydes contents. The reason for such a trend can be attributed to increment of ROS production which induce membrane destabilization resulting in the formation of peroxides. Similarly, the

increase of MDA was observed in strawberry exposed to 100 μmol L^{-1} nano selenium [59].

Increase of LOX that catalyzes peroxidation of unsaturated fatty acids of biomembranes after 0.1 g L^{-1} of nano-sized Co_3O_4 could be due to the stimulation of the enzyme activity and involving in lipoperoxidation.

Proline is an organic osmolite that plays a crucial role in modulating stress in plants [60]. The proline accumulated under nano-sized Co_3O_4 treatment which may be due to increment of proline synthesis or decline of proline degradation in response to high dosage of NPs.

CONCLUSIONS

Exposure to NPs can ameliorate sprouting of seed. There was a meaningful decrement in canola seed germination and early growth parameters imposed by nano-sized Co_3O_4, despite an increment at lower dosages. Moreover, these data exhibited that nano-sized Co_3O_4 could incite oxidative stress and notable cytotoxic impacts at high dosages in canola seedlings, despite the activation of antioxidant defense system. Consequently, this impact seems to be cause to injury at the plasmalemma level. The study of magnetic contamination with cobalt oxide NPs administered to canola seedlings led to beneficial results for environmental issues and identifying potential agricultural applications in crop improvement.

ACKNOWLEDGEMENTS

The authors thank the Department of Biology, Mashhad Branch, Islamic Azad University for supporting this project.

Conflict of interests

The authors declare no conflict of interest regarding this manuscript.

REFERENCES

1. Shafaghat A., Shafaghatlonbar M., 2018. Nanophytosynthesis and characterization of silver nano particles using *Chrysanthemum parthenium* extract as an eco-friendly method. J Chem Health Risks. 8(1), 85-94.

2. Feyz S.S., Khamchin Moghaddam F., 2019. The evaluation and comparison of single- and multi-walled carbon nanotubes in the removal of heavy metals from water. J Chem Health Risks. 9(4), 311-320.

3. Hasanabadi T., Lack S., Modhej A., Ghafurian H., Alavifazel M., Ardakani M.R., 2019. Feasibility study on reducing lead and cadmium absorption by alfalfa (*Medicago scutellata* L.) in a contaminated soil using nano-activated carbon and natural based nano-zeolite. Not Bot Horti Agrobo. 47(4), 1185-1193.

4. Vicas S.I., Cavalu S., Laslo V., Tocai M., Costea T.O., Moldovan L., 2019. Growth, photosynthetic pigments, phenolic, glucosinolates content and antioxidant capacity of broccoli sprouts in response to nanoselenium particles supply. Not Bot Horti Agrobo. 47(3), 821-828.

5. Hossain Z., Mustafa G., Komatsu S., 2015. Plant responses to nanoparticle stress. Int J Mol Sci. 16(11), 26644-26653.

6. Handy R.D., Owen R., Valsami-Jones E., 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges and future needs. Ecotoxicology. 17(5), 315-325.

7. Rai M., Ribeiro C., Mattoso L., Duran N. 2015. Nanotechnologies in Food and Agriculture, 1st ed., Springer: Switzerland. pp: 321-342.

8. Singh A., Singh N.B., Hussain I., Singh H., Singh S.C., 2015. Plant-nanoparticle interaction: an approach to improve agricultural practices and plant productivity. Int J Pharm Sci Invent. 4(8), 25-40.

9. Gal J., Hursthouse A., Tatner P., Stewart F., Welton R., 2008. Cobalt and secondary poisoning in the terrestrial food chain: data review and research gaps to support risk assessment. Environ Int. 34(6), 821-838.

10. Bakkaus E., Gouget B., Gallien J.P., Khodja H., Carrot F., Morel J.L., Collins R., 2005. Concentration and distribution of cobalt in higher plants: the use of micro-PIXE spectroscopy. Nucl Instrum Meth B. 231(1-4), 350-356.

11. Palit S., Sharma A., Talukder G., 1994. Effects of cobalt on plants. Bot Rev. 60(2), 149-181.

12. Chattopadhyay S., Dash S.K., Tripathy S., Das B., Mandal D., Pramanik P., Roy S., 2015. Toxicity of cobalt

oxide nanoparticles to normal cells; an *in vitro* and *in vivo* study. Chem Biol Interact. 226, 58-71.

13. Koosha E., Ramezani M., Niazi A., 2018. Determination of cobalt by air-assisted liquid-liquid microextraction, Toxicol Environ Chem. 100(3), 317-325.

14. Khalil A.T., Ovais M., Ullah I., Ali M., Shinwari Z.K., Maaza M., 2020. Physical properties, biological applications and biocompatibility studies on biosynthesized single phase cobalt oxide (Co_3O_4) nanoparticles via *Sageretia thea* (Osbeck.). Arab J Chem. 13(1), 606-619.

15. Rajjou L., Duval M., Gallardo K., Catusse J., Bally J., Job C., Job D., 2012. Seed germination and vigor. Annu Rev Plant Biol. 63, 507-533.

16. Lee C.W., Mahendra S., Zodrow K., Li D., Tsai Y.C., Braam J., Alvarez P.J., 2010. Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. Environ Toxicol Chem. 29(3), 669-675.

17. Wu S.G., Huang L., Head J., Chen D.R., Kong I.C., Tang Y.J., 2012. Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on seed surfaces. J Pet Environ Biotechnol. 3(4), 126-130.

18. Lu C.M., Zhang C.Y., Wen J.Q., Wu G.R., Tao M.X., 2002. Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. Soybean Sci. 21, 168-171.

19. Stampoulis D., Sinha S.K., White J.C., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ Sci Technol. 43(24), 9473-9479.

20. Anjum N.A., Gill S.S., Duarte A.C., Pereira E., 2019. Oxidative Stress Biomarkers and Antioxidant Defense in Plants Exposed to Metallic Nanoparticles. In: Nanomaterials and Plant Potential, 1st ed., Husen A., Iqbal M., Eds. Springer: Switzerland. pp. 427-439.

21. Singh V.P., Singh S., Kumar J., Prasad S.M., 2015. Investigating the roles of ascorbate-glutathione cycle and thiol metabolism in arsenate tolerance in ridged luffa seedlings. Protoplasma. 252(5), 1217-1229.

22. Zhao L., Peng B., Hernandez-Viezcas J.A., Rico C., Sun Y., Peralta-Videa J.R., Tang X., Niu G., Jin L., Varela-Ramirez A., Zhang J.Y., Gardea-Torresdey J.L., 2012. Stress response and tolerance of *Zea mays* to CeO_2 nanoparticles: cross talk among H_2O_2, heat shock protein, and lipid peroxidation. ACS Nano. 6(11), 9615-9622.

23. Dolatabadi N., Toorchi M., Valizadeh M., Bandehagh A., 2019. The proteome response of salt-sensitive rapeseed (*Brassica napus* L.) genotype to salt stress. Not Bot Horti Agrobo. 47(1), 17-23.

24. Wu G.L., Ren G.H., Shi Z.H., 2011. Phytotoxic effects of a dominant weed *Ligularia virgaurea* on seed germination of *Bromus inermis* in an alpine meadow community. Plant Ecol Evol. 144(3), 275-280.

25. Maguire J.D., 1962. Speed of germination-aid selection and evaluation for seedling emergence and vigor. Crop Sci. 2, 176-177.

26. Heath R.L., Packer L., 1968. Photoperoxidation in isolated chloroplasts kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 125(1), 189-198.

27. Meir S., Philosoph-Hadas S., Aharoni N., 1992. Ethylene-increased accumulation of fluorescent lipid-peroxidation products detected during senescence of parsley by a newly developed method. J Am Soc Hortic Sci. 117(1), 128-132.

28. Doderer A., Kokkelink I., Van der veen S., Valk B.E., Schram A.W., Douma A.C., 1992. Purification and characterization of two lipoxygenase isoenzymes from germinating barley. Biochim Biophys Acta. 1120(1), 97-104.

29. Blois M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature. 181, 1199-1200.

30. Alexieva V., Sergiev I., Mapelli S., Karanov E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 24, 1337-1344.

31. Aebi H., 1984. Catalase *in vitro*. Methods Enzymol. 105, 121-126.

32. Giannopolitis C.N., Ries S.K., 1997. Superoxide dismutase. I. occurrence in higher plants. Plant Physiol. 59(2), 309-314.

33. Nakano Y., Asada K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867-880.

34. MacAdam J.W., Nelson C.J., Sharp R.E., 1992. Peroxidase activity in the leaf elongation zone of tall feseue. J Plant Physiol. 99(3), 872-878.

35. Raymond J., Rakariyatham N., Azanza J.L., 1993. Purification and some properties of polyphenoloxidase from sunflower seeds. Phytochemistry. 34(4), 927-931.

36. Beaudoin-Eagan L.D., Thorpe E., 1985. Tyrosine and phenyl alanine ammonialyase activities during shoot initiation in tobacco callus cultures. Plant Physiol. 78(3), 438-441.

37. Bradford M.M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 72(1-2), 248-254.

38. Bates L.S., Waldren R.P., Teare I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39(1), 205-207.

39. Singleton V.L., Rossi J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 16(3), 144-158.

40. Mousavi Kouhi S.M., Lahouti M., Ganjeali A., Entezari M.H., 2015. Comparative effects of ZnO nanoparticles, ZnO bulk particles, and Zn^{2+} on *Brassica napus* after long-term exposure: changes in growth, biochemical compounds, antioxidant enzyme activities, and Zn bioaccumulation. Water, Air, Soil Poll. 226(11), 392.

41. Tripathi D.K., Shweta., Singh S., Singh S., Pandey R., Singh V.P., Sharma N.C., Prasad S.M., Dubey N.K., Chauhan D.K., 2017. An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. Plant Physiol. Biochem. 110, 2-12.

42. Donohue K., Rubio de Casas R., Burghardt L., Kovach K., Willis C.G., 2010. Germination, postgermination adaptation, and species ecological ranges. Annu Rev Ecol Evol Syst. 41(1), 293-319.

43. Bossi E., Zanella D., Gornati R., Bernardini G., 2016. Cobalt oxide nanoparticles can enter inside the cells by crossing plasma membranes. Sci Rep. 6, 22254-22263.

44. Shinde S., Paralikar P., Ingle A.P., Rai M., 2020. Promotion of seed germination and seedling growth of *Zea mays* by magnesium hydroxide nanoparticles synthesized by the filtrate from *Aspergillus niger*. Arab J Chem. 13(1), 3172-3182.

45. Mahakham W., Sarmah A.K., Maensiri S., Theerakulpisut P., 2017. Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. Sci Rep. 7(1), 8263.

46. Nur Azura M.S., Zamri I., Rashid M.R., Mohd Shahrin G., Rafidah A.R., Mohammad Rejab I., Azima A., Suria M.S., Amyita W.U., 2017. Evaluation of nanoparticles for promoting seed germination and growth rate in MR263 and MR269 paddy seeds. J Trop Agric and Fd Sc. 45(1), 13-24.

47. Ghodake G., Seo Y.D., Lee D.S., 2011. Hazardous phytotoxic nature of cobalt and zinc oxide nanoparticles assessed using *Allium cepa*. J Hazard Mater. 186(1), 952-955.

48. Faisal M., Saquib Q., Alatar A.A., Al-Khedhairy A.A., Ahmed M., Ansari S.M., Alwathnani H.A., Dwivedi S., Musarrat J., Praveen S., 2016. Cobalt oxide nanoparticles aggravate DNA damage and cell death in eggplant via mitochondrial swelling and NO signaling pathway. Biol Res. 49, 20.

49. Mauro M., Crosera M., Pelin M., Florio C., Bellomo F., Adami G., Apostoli P., De Palma G., Bovenzi M., Campanini M., Filon FL., 2015. Cobalt oxide nanoparticles: behavior towards intact and impaired human skin and keratinocytes toxicity. Int J Environ Res Public Health. 12(7), 8263-8280.

50. Yanik F., Vardar F., 2018. Oxidative stress response to aluminum oxide (Al_2O_3) nanoparticles in *Triticum aestivum*. Biologia. 73, 129-135.

51. Jahani S., Saadatmand S., Mahmoodzadeh H., Khavari-Nejad R.A., 2018. Effects of cerium oxide nanoparticles on biochemical and oxidative parameters in marigold leaves. Toxicol Environ Chem. 100(8-10), 677-692.

52. Polishchuk S.D., Nazarova A.A., Kutskir M.V., Churilov D.G., Ivanycheva Y.N., Kiryshin V.A., Churilov G.I., 2015. Ecologic-biological effects of cobalt, cuprum, copper oxide nano-powders and humic acids on wheat seeds. Mod Appl Sci. 9(6), 354-364.

53. Wang H., Kou X., Pei Z., Xiao J.Q., Shan X., Xing B., 2011. Physiological effects of magnetite (Fe_3O_4)

nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. Nanotoxicology. 5(1), 30-42.

54. Gzyl J., Rymer K., Gwóźdź E.A., 2009. Differential response of antioxidant enzymes to cadmium stress in tolerant and sensitive cell line of cucumber (*Cucumis sativus* L.). Acta Biochim Pol. 56(4), 723-727.

55. Ma C., Liu H., Guo H., Musante C., Coskun S.H., Nelson B.C., White J.C., Xing B., Dhankher O.P., 2016. Defense mechanisms and nutrient displacement in *Arabidopsis thaliana* upon exposure to CeO_2 and In_2O_3 nanoparticles. Environ Sci Nano. 3(6), 1369-1379.

56. Jahani S., Saadatmand S., Mahmoodzadeh H., Khavari-Nejad R.A., 2019. Effect of foliar application of cerium oxide nanoparticles on growth, photosynthetic pigments, electrolyte leakage, compatible osmolytes and antioxidant enzymes activities of *Calendula officinalis* L.. Biologia. 74(9), 1063-1075.

57. Skórska E., Grzeszczuk M., Barańska M., Wójcik-Stopczyńska B., 2019. Long wave UV-B radiation and asahi SL modify flavonoid content and radical scavenging activity of *Zea mays* var. Saccharata leaves. Acta Biol Cracov Ser Bot. 61(1), 87-92.

58. Łukasik I., Goławska S., 2019. Biochemical markers of oxidative stress in triticale seedlings exposed to cereal aphids. Acta Biol Cracov Ser Bot. 61(2), 35-46.

59. Soleymanzadeh R., Iranbakhsh A., Habibi G., Oraghi Ardebili Z., 2020. Selenium nanoparticle protected strawberry against salt stress through modifications in salicylic acid, ion homeostasis, antioxidant machinery, and photosynthesis performance. Acta Biol Cracov Ser Bot. 62(1), 33-42.

60. Ashraf M., Foolad M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 59(2), 206-216.