Nano-Alumina Effects on *Lepidium draba*: Morphological Properties, H_2O_2 Scavenging Enzymes Activity, Content of Sulforaphane and Flavonoid, and Bioaccumulation of Aluminum

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Abstract

Nanoparticles have broad applications across various industries, making it likely that they will enter the environment and potentially impact living organisms in unexpected ways. Alumina nanoparticles, being widely used, were the focus of this study to assess their effects on the *Lepidium draba* plant. Seven-day-old seedlings were grown in Murashige and Skoog basal medium containing alumina particles at concentrations of 0, 25, 50, 250, 500, and 1000 mg/L, and both Nano and bulk forms were compared. The results showed a decrease in seed germination rate and root and shoot elongation with increasing particle concentrations, with the effect being more pronounced for nanoparticles at higher concentrations. While sulforaphane content decreased similarly for both Nano- and bulk-treated seedlings, flavonoid content increased with increasing particle concentrations, but this effect was more significant with nanoparticle treatment. While peroxidase activity significantly increased only in the presence of nanoparticles, catalase activity increased in seedlings treated with both particles, with a more significant increase in the presence of nanoparticles. Based on the bioaccumulation data, aluminium was absorbed by the roots and transported to the shoots in both forms, with greater accumulation in the nanoparticle treatment. Overall, it may be concluded that oxidative stress caused by the absorption of these particles is responsible for the more pronounced decrease in plant growth in the presence of nanoparticles compared to bulk forms.

Keywords: bioavailability, catalase, peroxidase, nanoparticle, Antioxidant system

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Introduction

Today, nanoparticles (NPs), materials with dimensions less than 100 nm, are produced from various bulk materials (Kargozar and Mozafari, 2018). These particles, with unique properties, have broad applications in various industries,

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including the agricultural sector and biomedicine (Baruah and Dutta, 2009; Behra and Krug, 2008; Handy et al., 2008). Via several different pathways, nanotechnology is able to increase agricultural productivity, which results in the enhancement of food security (Lowry et al., 2019; White and Gardea-Torresdey, 2018). It has been proposed that different nano-enabled strategies can be used to improve crop production. Also, these strategies are able to meet global demands for feed, fuel, and food while maintaining sustainable agriculture (Kah et al., 2019; Lowry et al., 2019). It was reported that using commercial nanoscale CuO in the form of a foliar application was able to suppress fungal infection and significantly enhance watermelon yield (Lowry et al., 2019; White and Gardea-Torresdey, 2018). It is also noted that nanomaterials and nanotechnology can strongly increase the efficiency of both nutrient and pesticide usage, leading to reduced environmental damage and decreased embodied energy losses (Lowry et al., 2019). However, it can be expected that these particles will find a way to enter the environment (Behra and Krug, 2008; Handy et al., 2008; Perreault et al., 2014). In this regard, a significant number of studies have focused on the potential effects of nanoparticles on different organisms (Exbrayat et al., 2015; Mukherjee et al., 2016; Nair, 2016; Sajid et al., 2015). Compared to all organisms, plants are directly exposed to different ecosystems, so a large number of scientific studies have focused on the phytotoxicity effects of NPs on them (Jiang et al., 2009). For example, Pagano et al. (2017) proved that nanomaterials can significantly change the physiological and molecular responses of zucchini. Xinghui and coworkers evaluated the effects of aluminum oxide nanoparticles on the growth and development of tobacco (*Nicotiana tabacum*). They reported a notable decrease in root length and biomass of treated seedlings as the concentration of nano alumina increased in the media (Xinghui et al., 2023). However, the data revealed that NPs affected plants depending not only on their size, doses, and composition but also on the plant species (Khan et al., 2021).

In recent years, some special properties of aluminum, such as good thermal conductivity, suitable plasticity, high stability, and stiffness, have made it a suitable material for use in industrial applications (Amirkhanlou and Ji, 2020). According to a recent report, alumina nanoparticles are among the most valuable nanoparticles in the world, ranking as the second market leader for nano-sized materials (Khan et al., 2017).

It is known that aluminum, as well as nano-alumina treatment, leads to the induction of reactive oxygen species (ROS) such as superoxide anion ($O^{\cdot}_2$) and hydrogen peroxide (H_2O_2) in plants (Divyapriya et al., 2022). Plants are equipped with two defensive systems: enzymatic antioxidants, such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and glutathione reductase, and non-enzymatic antioxidants, such as ascorbate, glutathione, flavonoids, and tocopherols (Rajput et al., 2021). However, it has been reported that the activity of CAT, SOD, and ascorbate peroxidase (APX) increases in plants that undergo aluminum treatment (Liu et al., 2018). Increased activity of CAT and SOD was also reported in wheat seedlings grown in the presence of alumina nanoparticles (Riahi-Madvar et al., 2012).

Lepidium draba (*L. draba*), a weed plant from the Brassicaceae family, can uptake several heavy metals such as Cu, Fe, Zn, Cd, and Ni (Chehregani and Malayeri, 2007). This plant has very valuable secondary metabolites called glucosinolates, which contain sulfur and nitrogen (Đulović et al., 2021). These metabolites are released after cell injury or pathogen attack. After being exposed to myrosinase (β-thioglucoside glucohydrolase, EX 3.2.3.1) (Bhat and Vyas, 2019), they are hydrolyzed to produce glucose and an unstable intermediate molecule called aglycone. Depending on environmental conditions, aglycone can be converted to nitriles, isothiocyanates, and thiocyanates (Shakour et al., 2022). *Lepidium draba* (*L. draba*), which contains two major types of glucosinolates (Jamshidi Goharrizi et al., 2020), has been recognized as a suitable plant for extracting glucoraphanin, which, after hydrolysis, produces an isothiocyanate called sulforaphane (SFN). SFN has attracted interest from researchers due to its pharmaceutical properties, such as its

potential in cancer treatment as a remedy for asthma and allergies, and for reducing microbial activity(Mangla et al., 2021). This plant, as a member of the Brassica family, may be introduced as a suitable source of peroxidase. Furthermore, a peroxidase gene from this plant has been sequenced and submitted to GenBank (AIJ01351), and it has been recombinant produced in a prokaryotic expression system and stabilized on a Zn-MOF nanostructure.

The objectives of the present study were to evaluate the effects of different concentrations of nano- and bulk alumina on growth parameters (including seed germination percentage and root and shoot lengths) and phytochemical contents (such as SFN, flavonoid, chlorophyll, and carotenoid). Furthermore, the responses of the treated seedlings to nano and bulk alumina were analyzed by assaying some antioxidant enzyme activities, including CAT and POD. Finally, bioaccumulation and bioavailability of aluminum were measured in the root and shoot.

Materials and Methods

Chemicals

Nano alumina was purchased from PlasmaChem GmbH. The characteristics, as reported by the commercial agent, were as follows: average diameter: 40 nm, surface area >40 (m^2/g), and purity: 99.9%. The sulforaphane standard and acetonitrile were HPLC grade and were obtained from Sigma Chemical Company. All other chemicals used were of analytical grade and were purchased from Merck.

Plant Growth and Treatment

Seeds of the *Lepidium draba* (L. draba) plant were gathered from native-grown plants (Kerman, Iran). They were disinfected by being soaked in a 2% sodium hypochlorite (NaClO) solution for 15 minutes. Thereafter, the disinfected seeds were washed with distilled water. The culture media were prepared as described recently by Riahi-Madvar et al. (2012). The MS (Murashige and Skoog, 1962) basal medium containing 1.0% agar and different concentrations of nano and bulk alumina (0, 25, 50, 250, 500, and 1000 mg/L) was used to cultivate the *Lepidium draba* (*L. draba*) seeds in Petri dishes (30 seeds per Petri dish and 3 Petri dishes for each concentration). Nano and bulk alumina were suspended in deionized water (pH = 7) and then, after vigorous shaking, the solutions were added to autoclaved agar media. The seeds were germinated, and the seedlings were grown in a germinator with a photoperiod of 8 h dark and 16 h light at 28 ± 2 °C with a relative humidity of 60-65% for seven days.

Measurement of Morphological Properties

Seed Germination Percentage

To measure the seed germination rate, the germinated seeds (minimum rootlet length of around 1 mm) were counted and recorded each day. The percentage of seeds germinated was reported on the fourth day.

Root and Shoot Lengths

The 7-day-old seedlings (25) were randomly harvested from the Petri dishes, and the roots were washed with distilled water. The lengths of the roots and shoots were measured using a ruler and were expressed in millimeters (mm).

Determination and Quantification of SFN

The extraction and determination of SFN were performed as reported by Liang et al. (2006). Acetonitrile/H_2O (65/35, v/v) was used as the mobile phase in the HPLC apparatus (ZORBAX SB-C18 column, Agilent 1100 series, USA). In comparison with the reference standard of SFN (Sigma-Aldrich) retention time, the SFN peak was recognized at 254 nm.

Flavonoid Content Measurement

For the extraction of flavonoid, the fresh sample (0.1 g) was extracted in a solution (10 mL) containing glacial acetic acid and 95% ethyl alcohol (1:99, v/v). The obtained solution was centrifuged (4000 rpm, 10 min), and the supernatant was gently heated in a water bath at 80 °C for 10 minutes, and its absorbance at three wavelengths

(270, 300, and 330 nm) was measured by a UV-VIS spectrophotometer (Varian Cary 50, Australia). An extinction coefficient of 33000 M^{-1} cm^{-1} (Krizek et al., 1998) was used to calculate the flavonoid content cumulatively. The data were reported as mg/g fresh weight (FW).

Measurement of Chlorophyll and Carotenoid Content

Chlorophyll and carotenoid contents were measured based on the method described by Lichtenthaler (1987). The tissue (0.1 g) was homogenized in 85% acetone and then centrifuged (10000 rpm) at 4 °C for 15 minutes. The absorbance of the supernatant was measured at 646.8, 663.2, and 470 nm. The pigment content was thereafter calculated based on the formulas below:

$$\text{Chlorophyll a} = (12.25 \times OD_{663.2}\text{ nm} - 2.79 \times OD_{646.8}\text{ nm})$$

$$\text{Chlorophyll b} = (21.21 \times OD_{646.8}\text{ nm} - 5.1 \times OD_{663.2}\text{ nm})$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\text{Carotenoid} = [(1000 \times OD_{470}\text{ nm} - 1.8 \times \text{Chlorophyll a} - 85.02 \times \text{Chlorophyll b})/198]$$

Preparation of Enzyme Extract

Enzyme extraction was conducted by homogenizing fresh samples (0.5 g) in 50 mM potassium phosphate (pH = 7.0) buffer containing EDTA (1 mM) and 1% (w/v) polyvinylpyrrolidone (PVP). The solution was centrifuged for 20 minutes at 11000 rpm at 4 °C, and the supernatant (as a crude extract) was used for measuring enzyme activity.

CAT and POD Enzyme Activity

To measure catalase (CAT, EC 1.11.1.6) enzyme activity, the method of Chandlee et al. (1983) was used. For this purpose, 2.6 mL of potassium phosphate buffer (50 mM, pH 7.0), 0.4 mL H_2O_2 (15 mM), and 0.04 mL of enzyme extract were mixed, and absorbance was recorded at a wavelength of 240 nm. The H_2O_2 catalysis was measured by the reduction of absorbance at 240 nm. One unit of the enzyme was defined as the amount of the enzyme that decomposed 1 mM of H_2O_2 per minute. An extinction coefficient of 0.036 $mM^{-1}cm^{-1}$ was applied to calculate the activity of the enzyme (Dhindsa et al., 1981). The activity of the CAT enzyme was reported as enzyme units per milligram of total protein (U/mg protein).

The peroxidase (POD, EC 1.11.1.7) activity was measured as reported by Plewa et al. (1991). The assay mixture contained K_2HPO_4 buffer (50 mM, pH = 7.0), 1% H_2O_2, and 4% guaiacol. Guaiacol was oxidized to tetraguaiacol after the enzyme was added to the mixture, resulting in changes in absorbance at 470 nm, which were recorded for three minutes. The enzyme activity was calculated using the extinction coefficient for tetraguaiacol (26.6 mM^{-1} cm^{-1}) (Plewa et al., 1991). The activity unit was reported as U/mg protein.

Bioaccumulation and Bioavailability of Aluminum

To determine the uptake and accumulation of aluminum, the 7-day-old *Lepidium draba* (L. draba) seedlings were collected from their medium, and the stems and roots of the seedlings were separated, washed with distilled water, and dried at 80 ºC in an oven for 24 hours. The dried samples (0.2 g) were soaked in 5% HNO_3 for 24 hours. The resulting mixture was heated to release nitrogen monoxide and then diluted to reach a final volume of 10 mL. The bioaccumulation of aluminum in the roots and shoots was determined using Inductively Coupled Plasma (ICP) mass spectrometry (SpectrAA 220 Varian, Australia). Bioavailability was defined as the ratio of Al (L/kg) concentration in roots or shoots over the nano or bulk alumina concentration in each treatment (Lee et al., 2008). The bioavailability of nano or bulk alumina to the test plants was assessed by measuring the bioaccumulation factor, defined as the nano or bulk alumina concentrations in the plants (mg/kg dry tissue) divided by the nano or bulk alumina concentration in the growth media (mg/L media) (Lee et al., 2008).

Statistical Analysis

Experiments were performed in fully randomized designs. Three replicates were used for data analyses. The data were analyzed using the SPSS statistical software (Release 16.0.0). One-way

ANOVA with a Duncan test was used to evaluate significant differences ($P \leq 0.05$) in the parameters. The results were presented as mean values ± Standard Deviation (SD).

Results

As seen in Table 1, for all treatments, the germination rate decreased in the presence of both types of alumina compared with the control samples. The germination rates decreased and reached their lowest levels at the highest concentration of nano and bulk alumina (1000 mg/L). A reduction of 1.1-fold and 1.2-fold was calculated for the germination rate under 1000 mg/L nano and bulk alumina, respectively, in comparison with the control condition. The shoot and root lengths of the treated seedlings were substantially decreased compared with the control samples. Decrements of 1.2-fold and 1.38-fold in shoot length and 1.16-fold and 1.13-fold in root length were calculated under 1000 mg/L nano and bulk alumina compared to the control sample, respectively (Table 1).

SFN Content

As observed in Fig. IA, the SFN contents were approximately the same as those under the control condition in the seedlings exposed to 25, 500, and 1000 mg/L alumina nanoparticles. However, its content decreased by 1.01-fold in the seedlings treated with 50 and 250 mg/L of nano alumina compared to the control condition. Also, a reduction of 1.01-fold was seen in the SFN content in the seedlings exposed to 25, 50, and 250 mg/L bulk alumina compared to the control condition (Fig. IB).

Flavonoid Content

The flavonoid content gradually increased in the treated seedlings with both nano and bulk alumina compared to the control condition. The greatest flavonoid content was observed at the highest doses, showing 1.2-fold and 1.15-fold increases for nano- and bulk-treated seedlings, respectively (Fig. II).

Chlorophyll and Carotenoid Contents

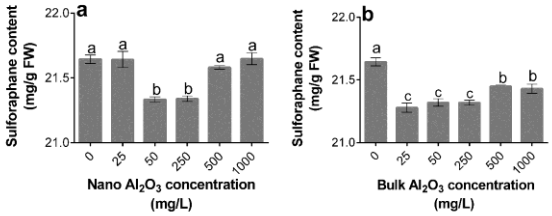

Fig. I. SFN content in nano-alumina (A) and bulk alumina (B) treated seedlings. Signs with different letters are significantly different at p≤0.05, according to Duncan's multiple range tests. The bars represent SD

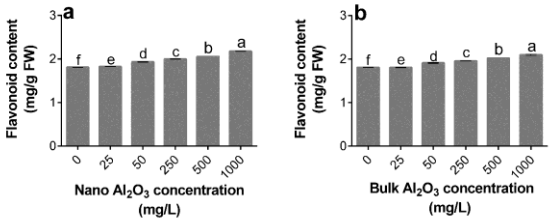

Fig.2. Flavonoid content in nano-alumina (A) and bulk-alumina (B) treated seedlings. Different letters at the top of each column indicate significant difference at P≤ 0.05 according to Duncan's Multiple Range Test.

The contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were drastically reduced in the treated seedlings compared with the control group in a dose-dependent manner with increasing concentrations of both nano and bulk alumina in the media (Tables 2 and 3). The chlorophyll and carotenoid contents decreased and reached their lowest levels at the highest concentration of nano and bulk alumina (1000 mg/L). Under these conditions, the total chlorophyll and carotenoid contents decreased by 1.83-fold and 1.39-fold in nano-treated seedlings and by 2.49-fold and 1.6-fold in bulk-treated seedlings (Tables 2 and 3).

CAT and POD Enzyme Activities

CAT activity greatly increased in all the treated seedlings with both nano and bulk alumina compared to the control samples. The CAT enzyme activity increased in a dose-dependent manner and reached its highest level at 1000 mg/L treatment. At this concentration, the activity of this enzyme increased 14.53-fold and 5.86-fold under nano and bulk alumina treatments,

Table 1
Germination percentage and lengths of shoot and root in *L. draba* seedlings treated with nano and bulk alumina

Al_2O_3 Concentration (mg/L)	Nano-Alumina			Bulk-Alumina		
	Germination (%)	Shoot length (mm)	Root length (mm)	Germination (%)	Shoot length (mm)	Root length (mm)
0	100±0.01^{a}	1.40±0.14^{a}	1.61±0.15^{a}	100±0.02^{a}	1.40±0.13^{a}	1.61±0.11^{a}
25	95±2.30^{b}	1.35±0.19^{a,b}	1.35±0.18^{b,c}	96.7±1.7^{a}	1.31±0.17^{a,b}	1.57±0.17^{a,b}
50	93.3±1.15^{b,c}	1.23±0.17^{b,c}	1.33±0.16^{b}	96.6±2.08^{a}	1.30±0.20^{a,b}	1.50±0.10^{a,b}
250	91.2±3.21^{c}	1.22±0.20^{b,c}	1.18±0.18^{c}	95.4±3^{a}	1.22±0.15^{b}	1.49±0.10^{a,b}
500	90.8±2.08^{c}	1.17±0.15^{c}	1.17±0.12^{c}	83.3±2.08^{b}	1.21±0.18^{b}	1.43±0.18^{b}
1000	90.6±2.08^{c}	1.16±0.15^{c}	1.16±0.15^{c}	83.1±2.8^{b}	1.20±0.17^{b}	1.42±0.19^{b}

Values in the same column followed by various letters are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test. Data are mean ± SD.

Table 2
Chlorophylls and carotenoid contents in *L. draba* seedlings treated with different concentrations of nano-alumina.

Nano alumina Concentration (mg/L)	Chlorophylla (mg/g FW)	Chlorophyllb (mg/g FW)	Total Chlorophyll (mg/g FW)	Carotenoid (mg/g FW)
0	1.66±0.01^{a}	0.94±0.03^{a}	2.61±0.04^{a}	0.368±0.007^{a}
25	1.42±0.005^{b}	0.64±0.001^{b}	2.07±0.006^{b}	0.367±0.001^{a,b}
50	1.28±0.003^{c}	0.54±0.001^{c}	1.83±0.005^{c}	0.364±0.0007^{b,c}
250	1.27±0.003^{c}	0.53±0.003^{c}	1.80±0.006^{c}	0.362±0.001^{c}
500	1.08±0.001^{d}	0.43±0.001^{d}	1.49±0.001^{d}	0.305±0.0008^{d}
1000	0.99±0.005^{e}	0.41±0.008^{d}	1.42±0.003^{e}	0.263±0.008^{e}

Values in the same column followed by various letters are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test. Data are mean ± SD.

Table 3
Chlorophyll and carotenoid contents in L. draba seedlings treated with different concentrations of bulk-alumina.

Bulk alumina Concentration (mg/L)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)
0	1.66±0.01^{a}	0.94±0.03^{a}	2.69±0.04^{a}	0.368±0.007^{a}
25	1.20±0.002^{b}	0.48±0.001^{b}	1.61±0.003^{b}	0.364±0.001^{b}
50	1.07±0.001^{c}	0.46±0.0005^{c}	1.54±0.002^{c}	0.321±0.0003^{c}
250	1.05±0.002^{c}	0.45±0.002^{c}	1.51±0.004^{c}	0.298±0.010^{d}
500	0.85±0.002^{d}	0.34±0.0005^{d}	1.19±0.002^{d}	0.253±0.001^{e}
1000	0.76±0.0008^{e}	0.32±0.001^{e}	1.08±0.002^{e}	0.229±0.0004^{f}

Values in the same column followed by various letters are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test. Data are mean ± SD.

respectively, compared to the control samples (Table 4).

POD activity in the presence of nano alumina concentrations increased up to 1000 mg/L gradually, such that at a concentration of 1000 mg/L of nano alumina, the POD activity increased 1.47-fold compared to the control sample (Table 4). As presented in Table 4, no significant differences in POD activity were observed between the bulk alumina-treated seedlings and the control samples.

Table 4
CAT and POD activity in *L. draba* seedlings treated with different concentrations of nano-alumina and bulk-alumina.

Alumina concentration (mg/L)	Nano-Alumina		Bulk-Alumina	
	POD (U/mg protein)	CAT (U/mg protein)	POD (U/mg protein)	CAT (U/mg protein)
0	12.51±0.3^{d}	0.15±0.02^{e}	12.51±0.3^{a,b}	0.15±0.02^{c}
25	12.67±0.5^{d}	0.21±0.03^{d,e}	12.27±0.05^{b}	0.31±0^{b}
50	14.33±0.1^{c}	0.30±0.02^{c,d}	12.27±0.2^{b}	0.38±0.04^{b}
250	14.48±0.09^{c}	0.39±0.03^{c}	12.14±0.1^{b}	0.39±0.04^{b}
500	16.22±0.1^{b}	1.05±0.05^{b}	12.22±0.1^{b}	0.42±0.04^{b}
1000	18.42±0.1^{a}	2.18±0.1^{a}	12.76±0.1^{a}	0.88±0.1^{a}

Values in the same column followed by various letters are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test. Data are mean±SD.

Table 5
Bioaccumulation and bioavailability of nano alumina in root and shoot of *L. draba* seedlings after 7 days. Data show mean ± SD, n = 3 replicates**.**

Nano-Al2O3 Concentration (mg/L)	Root		Shoot	
	Bioaccumulation of Al (mg/kg)	Bioavailability of Al (L/kg)	Bioaccumulation of Al (mg/kg)	Bioavailability of Al (L/kg)
0	nd	-	nd	-
25	490±17.3^{e}	19.60±0.6^{a}	448.3±2.8^{d}	17.93±0.1^{a}
50	543.3±5.7^{d}	10.86±0.1^{b}	455±17.3^{d}	9.10±0.3^{b}
250	731.6±20.2^{c}	2.92±0.08^{c}	540±21.7^{c}	2.16±0.08^{c}
500	938.3±20.2^{b}	1.87±0.04^{d}	648.3±25.1^{b}	1.29±0.05^{d}
1000	1146.6±5.7^{a}	1.14±0.005^{d}	740±31.2^{a}	0.74±0.03^{e}

Values in the same column followed by various letters are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test. Data are mean ± SD. nd, not detected.

Table 6
Bioaccumulation and bioavailability of bulk alumina in root and shoot of *L. draba* seedlings after 7 days. Data show mean ± SD, n = 3 replicates.

Bulk-Al_2O_3 Concentration (mg/L)	Root		Shoot	
	Bioaccumulation of Al (mg/kg)	Bioavailability of Al (L/kg)	Bioaccumulation of Al (mg/kg)	Bioavailability of Al (L/kg)
0	nd	-	nd	-
25	396.6±15.2^{e}	15.86±0.6^{a}	331.6±27.5^{e}	13.26±1.1^{a}
50	500±25^{d}	10±0.5^{b}	441.6±23.6^{d}	8.83±0.4^{b}
250	686.6±23.09^{c}	2.74±0.09^{c}	488.3±24.6^{c}	1.95±0.09^{c}
500	880±26.4^{b}	1.76±0.05^{d}	638.3±20.2^{b}	1.27±0.04^{c}
1000	1041±7.6^{a}	1.04±0.007^{e}	738.3±12.5^{a}	0.7±0.01^{d}

Values in the same column followed by various letters are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test. Data are mean ± SD. nd, not detected.

Bioaccumulation and Bioavailability of Aluminum

As observed in Tables 5 and 6, the accumulation of Al was notable in the roots and shoots of the seedlings grown in media containing nano and bulk alumina. At all doses, the bioaccumulation of Al in the roots and shoots of seedlings treated with nano alumina was higher than in those treated with the corresponding doses of bulk alumina. Specifically, at 1000 mg/L of nano alumina, Al accumulation was approximately 10% higher in roots and 1% higher in shoots than at the corresponding concentration of bulk alumina. On the other hand, bioavailability, defined by the bioaccumulation factor, decreased with the increasing concentration of nano and bulk alumina in the medium. The bioavailability of Al in the roots and shoots decreased 17.19-fold and 24.22-fold for nano alumina and 15.25-fold and 18.94-fold for bulk alumina, respectively (Tables 5 and 6).

Discussion

In this study, to determine and compare the phytotoxicity effects of nano- and bulk alumina, several morphological and biochemical properties of *Lepidium draba* seedlings that were grown for 7 days in the presence of different concentrations of these particles were examined. As previously reported, seed germination and root elongation are useful tests for evaluating the phytotoxicity effects of pollution on plants(You et al., 2019). The phytotoxicity of these particles was examined by considering the rate of seed germination as well as root and shoot elongation. As observed in Table 1, these parameters were reduced by increasing the particle concentration in the media, which is in line with findings at 0.1, 0.5, and 1% Al2O3 nanoparticles (Hayes et al., 2020). They reported that seed germination, root growth, and biomass of lettuce decreased during treatment with aluminum oxide nanoparticles.

The seed coat protects the embryo from hazardous external factors (Radchuk and Borisjuk, 2014); therefore, as the seed germination rate decreased (at the highest concentration, it decreased by about 8 percent more in bulk-treated than in nano-treated ones), it can be suggested that these compounds can pass through the seed coat. This finding is consistent with that reported by Aminizadeh et al. (2016). They showed that the seed germination rate of *Lepidium draba* (*L. draba*) dramatically decreased when seeds were germinated in the presence of different concentrations of CuO and Fe3O4 nanoparticles (Aminizadeh et al., 2016). It also showed that cucumber seed germination rate decreased when exposed to Fe_3O_4 nanoparticles (Hayes et al., 2020). Nevertheless, the percentage of seed germination rate of wheat was the same as the control in treatment with Al_2O_3 nanoparticles (Riahi-Madvar et al., 2012).

According to the results, while root and shoot lengths of the nano-alumina-treated seedlings decreased by about 28% and 18%, respectively, in the bulk alumina-treated seedlings, these parameters decreased by about 12% and 15%, respectively. It was specified that nanoparticles of CeO_2, La_2O_3, and CuO were able to decrease the shoot and root lengths of tomato compared to untreated plants (Pagano et al., 2016).

Although numerous studies have shown that metal nanoparticles can negatively affect root elongation(Hayes et al., 2020; Panakkal et al., 2021), positive effects of some nanoparticles including Al_2O_3 (50 mg/L) and Fe_3O_4 (25 mg/L) have been reported on root elongation of wheat and *Lepidium draba*, respectively (Aminizadeh et al., 2016; Riahi-Madvar et al., 2012). Furthermore, the positive effect of Al2O3 nanoparticles on root elongation of *Arabidopsis thaliana* has also been reported (Akdemir, 2021). Also, in a study, it was specified that “All” nano, but not the “All” bulk treatment stimulated flower formation, shoot elongation, shoot biomass production, and K and N accumulation compared to control samples in soybean (Dimkpa et al., 2019). However, it has been reported that the positive or negative effects of nanoparticles are related to the nanoparticles' components, doses, treatment duration, and plant species (Aminizadeh et al., 2016; Dimkpa et al., 2019).

It was specified that damage to epidermal and cortical cells by zinc oxide nanoparticles in the roots of ryegrass can be the main reason for the decrease in root growth (Lin and Xing, 2008).

Another study proved that the ability of maize roots to uptake water can be disturbed by titanium dioxide nanoparticles because of the aggregates forming along the root cell walls, which ultimately blocks water uptake and thus leads to decreased root development (Avellan et al., 2021). On the other hand, several studies concerning Al-toxicity have explained the inhibition of root growth (Hajiboland et al., 2023) in terms of cell division and elongation . Some researchers have reported the reduction of mitotic activity in the Al-exposed roots of wheat and bean(Yadav et al., 2021). While the exact mechanism of inhibition of root and shoot elongation in the presence of nano and bulk alumina has not been clearly understood, it seems that accumulation of Al in the root and shoot is responsible for the reduction of treated-plant growth.

However, Chehregani et al. introduced *Lepidium draba* as an accumulator plant that uptakes and accumulates heavy metals such as Pb, Zn, and Fe (Chehregani and Malayeri, 2007). As the included data demonstrates, Al can also be absorbed in both nano and bulk alumina culture mediums by L. draba roots and transported to the stems and the leaves. Interestingly, the root and shoot of the treated seedlings with 1000 mg/L nano alumina were shown to accumulate approximately 10% and 1% more, respectively, then the plant treated with this concentration of the bulk form. These findings are in agreement with the absorption of Zn by *Arabidopsis thaliana* grown in the presence of nano-ZnO particles and larger ZnO particles (Jośko et al., 2021). The bioavailability of Al was reduced by increasing the nano and bulk particles in the media. These observations are consistent with the conclusions drawn from the bioavailability of Al in nano-alumina treated wheat seedlings (Riahi-Madvar et al., 2012). Reduction of bioavailability upon increasing nanoparticles in media may be due to agglomeration. Aggregation of NPs is a common behavior that occurs with an increase in their concentration in the culture medium (Shrestha et al., 2020).

On the other hand, the chlorophyll and carotenoid contents were significantly reduced in the nano- and bulk-alumina treated seedlings. Our results are consistent with reports of disorders of the photosynthetic apparatus under Al stress in wheat (Filaček et al., 2022) and Citrus (Meng et al., 2021). Some researcher proved that under salinity stress (sodium toxicity), the reduction of chlorophyll occurs because of ROS formation and/or photoinhibition (Muhammad et al., 2021). Previous research proved that Al_2O_3-NPs induce oxidative stress in mung bean seedlings (Shabnam and Kim, 2018), and most likely, the decrease in root and shoot length as well as the chlorophyll and carotenoid contents may result from ROS formation upon treatment of seedlings with nano and bulk alumina.

In this study, to determine the probable mechanism of nano and bulk toxicity, the non-enzymatic antioxidant system including SFN content as a derivative of glucosinolates, glucoraphanin, and flavonoid content as well as the enzymatic antioxidant system including CAT and POD activities were examined.

It is reported that secondary metabolites are used by plants to eliminate the damaging effects of stress (Yeshi et al., 2022). It is also believed that glucosinolates and their derivatives play a defensive role in plants (Chhajed et al., 2020). According to the results, SFN content decreased under 50 and 250 mg/L nano and all doses of bulk alumina compared to the control condition. These results are inconsistent with those reported by Aminizadeh et al. (2016). They reported that SFN concentration in the seedlings and cell suspension of *Lepidium draba* was induced by CuO and Fe3O4 nanoparticles. They suggested that inducing oxidative stress (increasing ROS formation) upon exposure to NPs can be introduced as a possible mechanism for SFN induction.

In contrast to the SFN content, flavonoid content increased upon treatment of the seedlings with both particles. These results are completely similar to the findings of a previous study (Zhou et al., 2016). Flavonoids are phenolic metabolites that serve as ROS scavengers under stress conditions (Laoué et al., 2022). It is also specified that in *Brassica nigra* seedlings, flavonoid content increases in response to ZnO nanoparticles (Zafar et al., 2016).

The CAT (an essential enzyme for ROS detoxification during stressed conditions, which decomposes H_2O_2 to H_2O and O_2) activity increased in the seedlings that were treated with both forms of alumina in a manner that increased with these particles in the media. These findings align with recent studies by Riahi-Madvar et al. (2012) and Aminizadeh et al. (2016), which demonstrated increased CAT activity in *Triticum aestivum* seedlings treated with nano-alumina and in *Lepidium draba* seedlings exposed to nano Fe_3O_4 and nano CuO particles, respectively. Additionally, Nhan Le et al. (2015) also reported an increase in CAT activity in the leaves of cotton treated with CeO_2 nanoparticles (Nhan Le et al.), while an increase in CAT activity has also been reported in *Vaccinium corymbosum* L. in response to aluminium exposure (Cárcamo-Fincheira et al., 2021).It should be noted that the increase in CAT activity in nano-alumina treatment was higher than that found in bulk alumina treatment.

According to the results, the effects of nano-alumina were greater than bulk alumina on POD activity in treated seedlings. While no significant differences were detected in POD activity between bulk-alumina exposed plants and the control sample, its activity drastically increased in the nano-alumina treated seedlings. An increase in POD activity in *Lepidium draba* seedlings has been recently reported under treatment with Fe_SO_4 and $CuSO_4$ (Aminizadeh et al., 2014) and CuO and Fe_3O_4 nanoparticles (Aminizadeh et al., 2016). Furthermore, many studies have reported the enhancement of POD activity in some plant species, including triticale, maize, and tea under aluminum stress(Yan et al., 2022).

Conclusion

According to the results, the increase in flavonoid content and CAT and POD activities indicates that oxidative stress occurred upon treatment with these particles. Therefore, it can be deduced that the reduction in root and shoot lengths and the decreased contents of SFN, chlorophylls, and carotenoids are the results of oxidative damage under experimental conditions. Based on the presented results, Al accumulation in the roots and shoots of the nano-treated seedlings is greater than in the bulk-treated ones. This event may contribute to the higher ROS production in nano-treated seedlings, as indicated by the increased activity of CAT and POD and the greater reduction in root and shoot lengths compared to bulk-treated seedlings. However, further studies are needed to determine the exact mechanisms of these particles' effects on *Lepidium draba* plants.

Contribution:

Study conception and design: ARM, Performing the experiments: LS, Analysis and interpretation of data: LS, MM, LS, KJG, Drafting of manuscript: LS, KJG

Declaration

The authors declare that there is no conflict of interest.

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