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Foliar Application of Selenium Affects Nitrate Accumulation and Morpho-physiochemical Responses of Garden Cress Plants

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ABSTRACT

Consumption of vegetables with high nitrate content threaten human health. Garden cress is a hyperaccumulator of nitrate and quickly accumulates a large amount of nitrate. The objective of the current study was to investigate the effects of foliar application of selenium (Se) on the morphological and physiochemical traits of garden cress plants. Treatments included three levels of sodium selenite (1, 2, and 4 mg L^{-1}) and three levels of green synthesized Se nanoparticles (NPs) (1, 2, and 4 mg L^{-1}). Most nutrient treatments, especially 1 mg L^{-1} Se NPs, significantly increased plant height, number of leaves, fresh and dry weights, chlorophyll a, total chlorophyll, and nitrate reductase activity of garden cress plants. The foliar application of Se, especially 1 mg L^{-1} Se NPs, caused a significant decrease in the level of nitrate accumulation. Under different treatments of sodium selenite and green synthesized Se NPs on garden cress plants, the concentration of Se was increased, and concentrations of zinc and phosphorus were decreased. This research highlights the implications of Se for improving the quality and quantity of garden cress plants.

Introduction

Garden cress (*Lepidium sativum* L.) is a fast-growing and edible plant consumed by human beings, typically as a garnish or leaf vegetable (Malar et al., 2014). This annual plant can reach a height of 60 cm, with many branches on the upper part. It contains significant amounts of iron, calcium, folic acid, vitamin A and C (Malar et al., 2014). Recently, garden cress has gained more interest from consumers and producers. It can be a good choice for salads with its peppery taste and health-promoting substances such as glucotropaeolin, a glucosinolate compound, and the precursor of benzyl isothiocyanate and sterols (Zhan et al., 2009). However, Garden cress is a hyperaccumulator of nitrate and quickly accumulates a large amount of nitrate (2500–5000 mg/kg FW) in its stems and leaves (Colla et al., 2018). Nitrate, as one of the most important nitrogen sources for plant growth and development, is widely used in vegetable production (Bo et al., 2018). The excessive use of nitrate fertilizers to guarantee yield, increases the risk of nitrate accumulation. In the human body, approximately 80% of the daily intake of nitrate uptake from vegetables (Santamaria, 2006). Studies have indicated that consumption of vegetables with high nitrate content threaten human health, besides leading to methemoglobinemia, ingested nitrate could be converted to nitrite, a toxic carcinogen, causing cancers and methemoglobinemia (Prasad and Chetty, 2008).

Selenium (Se) is an essential trace element with multiple roles in animals and humans (Schrauzer, 2009). There is also plenty of evidence that Se promotes plant growth (Pilon-Smits et al., 2009). Although Se is not considered essential for the plants, biofortification of edible plants with Se is a valuable technique for increased consumption of Se by humans and animals through the food chain (Ramos et al., 2011). Garden cress is a Se-accumulating edible

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vegetable (Elguera et al., 2013). In addition, it was observed that the external application of Se reduces nitrate concentration in plants by increasing the activity of enzymes involved in nitrogen metabolism and increasing photosynthetic capacity (Bo et al., 2018). Therefore, the external application of Se can increase the concentration of Se and reduce the nitrate content in crops.

Soil application of Se can lead to low Se recovery rates in edible portions of crops or excessive Se accumulation in the soil, with long-term application of Se fertilizer becoming toxic for nearby ecosystems (Brown and Shrift, 1982). Hence, an effective method of fortifying food crops with Se is foliar application. It was found that foliar application of Se is the most effective strategy to increase Se uptake in most arable crops (Ros et al., 2016). Nano-fertilizers, due to their small size, high surface-to-volume ratio, and unique optical properties, are more effective than conventional fertilizers for foliar spraying (DeRosa et al., 2010). In addition, when the individual particle is converted to a nanoparticle, then its entropy will be changed. It causes enhancement in Gibbs free energy, which is directly proportional to entropy. Due to an increase in the Gibbs free energy, the particle's movement will also increase, and that increases the absorption of nano-fertilizer in the plant cell (Mochizuki et al., 2009).

To the best of our knowledge, there is no information regarding Se NPs on the growth and physiology of garden cress plants. The present research aimed to evaluate the effects of sodium selenite and Se NPs on nitrate reductase activity, reduction of nitrate accumulation, morphological and physiochemical characteristics of garden cress plant.

Materials and Methods

Synthesis and characterization of green Se NPs

To produce green Se NPs, rosemary extract was used. About 20 g dry matter of the plant was added to 500 mL distilled water. The resulting mixture was then placed at a temperature of 90 °C for 30 min on a heater stirrer. After 24 h, the obtained solution was passed through a fine filter, and the final solution was prepared.

To synthesis the green Se NPs, 1.738 g of sodium selenite salt was dissolved in 500 mL of water and was stirred for 10 min using an electric stirrer. Then, 80 mL of sodium selenite salt was poured into 20 mL rosemary extract, and the concoction was placed in a hot water bath at 60 °C for 15 min. The shape and size of the Se NPs were determined using scanning electron microscopy (SEM) (CamScan Mv2300 SEM CANADA). The chemical identity of the Se NPs was verified using powder XRD.

Plant material and nutrient treatments

The present study was conducted in the research greenhouse of the Agriculture and Natural Resources Faculty of Arak University during the period from 2019 to 2020. The seeds of local garden cress accession were grown on pots containing garden soil and cocopeat (50:50 ratio). Four garden cress young plants were grown on each of the pots. All plants received similar cultural practices such as irrigation and fertilization. To accumulate nitrate in the aerial parts of the garden cress, a total of 6 g of ammonium nitrate fertilizer was added to each 10 L pot in three stages (before planting, 2- and 4-leaf stages). The experiment was conducted based on a completely randomized design, with seven treatments (1, 2, and 4 mg L^{-1} sodium selenite, 1, 2, and 4 mg L^{-1} green synthesized Se NPs and control) in seven replications. The nutrient solutions were sprayed till it runoff (about 15 mL) on each plant at two times (in stages 4- and 6-leaf stages). Forty-five days after the transplantation, the plants were removed from the cultivation pots, and the morphological and physiochemical parameters were measured. Plants were grown in the greenhouse with a 10 h light/14 h dark photoperiod and a temperature range of 30/20 °C during day/night.

Morphological characteristics

At the end of the experiment, the plants were removed from the pots, and the fresh weight of the aerial parts was immediately measured using a digital weighing scale; then, to measure their dry weight, the samples were kept in an oven at 70 °C for 48 h. Other measured traits were the plant height and leaf number.

Physicochemical parameters

Chlorophyll contents

Approximately 0.5 g of the leaf samples were used for chlorophyll extraction using acetone (80% v/v). Chlorophyll contents were assayed using a UV–visible spectrophotometer (Cary Win UV 100; Varian, Sydney, Australia) according to Lichtenthaler (1987), and their contents were expressed as mg g^{-1} FW leaf.

The activity of nitrate reductase

The activity of nitrate reductase in the leaves of garden cress was spectrophotometrically determined as described by Hageman and Reed (1980). Approximately 1 g of the leaf samples was homogenized with 25 mmol L^{-1} potassium phosphate buffer. The sample extract was centrifuged at 10000 rpm for 30 min at 4 °C. The supernatant referred to as 'crude enzyme' was used for enzyme assay. The assay mixture for calculating nitrate reductase activity contained 0.5 mL phosphate buffer (pH 7.5), 0.2 mL potassium nitrate solution (0.1 mmol L^{-1}), 0.4 mL NADH (2 mmol L^{-1}), and 0.2 mL of extract. After incubation for 15 min at 30°C, 1 mL 1% sulphanilamide and 1 mL NEDH were added to stop the reaction. The absorbance measured at 540 nm was used to calculate nitrate reductase activity. The specific activity of the enzyme is defined as μM N g^{-1} FW.

Nitrate concentration

The amount of nitrate in the leaves of the garden cress was determined according to Cataldo et al. (1975). About 1 g of the leaf samples was grounded using a mortar and pestle and then suspended in 10 mL of distilled water. The sample was boiled for 30 min at 100 °C in a water bath. After cooling down with tap water, the extracts were filtered and then diluted with distilled water to a final volume of 20 mL. The 5% salicylic acid-concentrated sulfuric acid (0.4 mL) was added to 0.1 mL exact and then incubated at room temperature in the dark. After 20 min, the assay mixture was further diluted with 9.5 mL of 2.0 mol L^{-1} NaOH solution and shaken until yellow color appeared. The absorption monitored at 430 nm was used to calculate nitrate concentrations on its standard curve. The concentration of nitrate was represented as μg g^{-1} DW.

Leaf mineral contents

Concentrations of phosphorus (P), zinc (Zn), and Se in the leaves of the garden cress were measured. Phosphorus was determined using a spectrophotometer. The sample extracts were analyzed for Zn and Se using an atomic absorption spectrophotometer (Varian, 220). In this study, nitrogen, P, potassium, iron, Zn, and Se in the leaves of garden cress were measured, but in many treatments and repetitions, no concentration was detected for nitrogen, potassium, and iron, so only three elements of P, Zn, and Se that were fully detected were examined.

Statistical analysis

The data were analyzed using the GLM procedure of SAS software (Version 9.1), and significant differences were tested at P ≤ 0.05 using Duncan's multiple ranges.

Results

Synthesis and characterization of green Se NPs

The green synthesis of Se NPs was carried out via Se ions reduction during their exposure to rosemary extract. The electron microscopy images showed that Se NPs are quasi-spherical, and most of them are about 118 nm to 220 nm (Fig. 1). In addition, the SEM image of Se NPs reveals the presence of some large particles, which can be attributed to aggregation or overlapping of smaller particles (Fig. 1). In addition, the crystal structure of the Se NPs was recorded and confirmed by the XRD spectrum (Fig. 2).

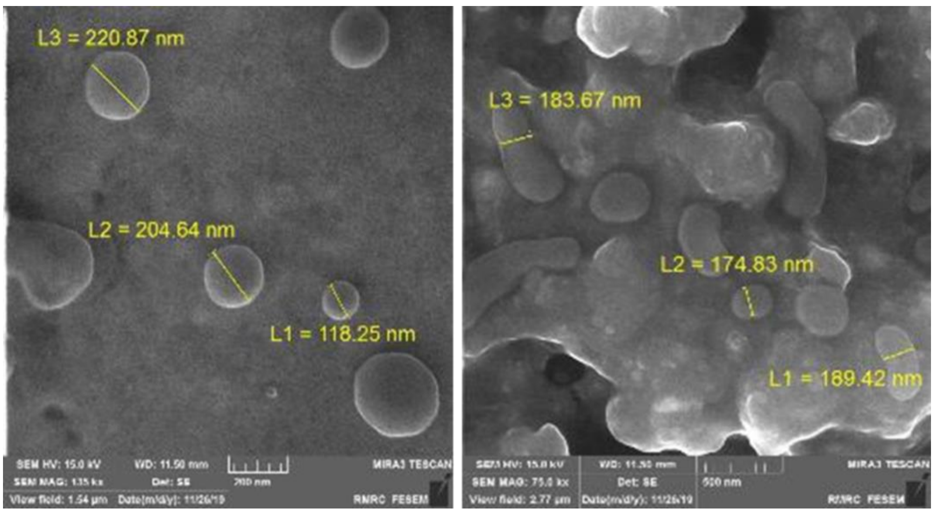

Fig. 1. SEM analysis of green synthesized Se nanoparticles

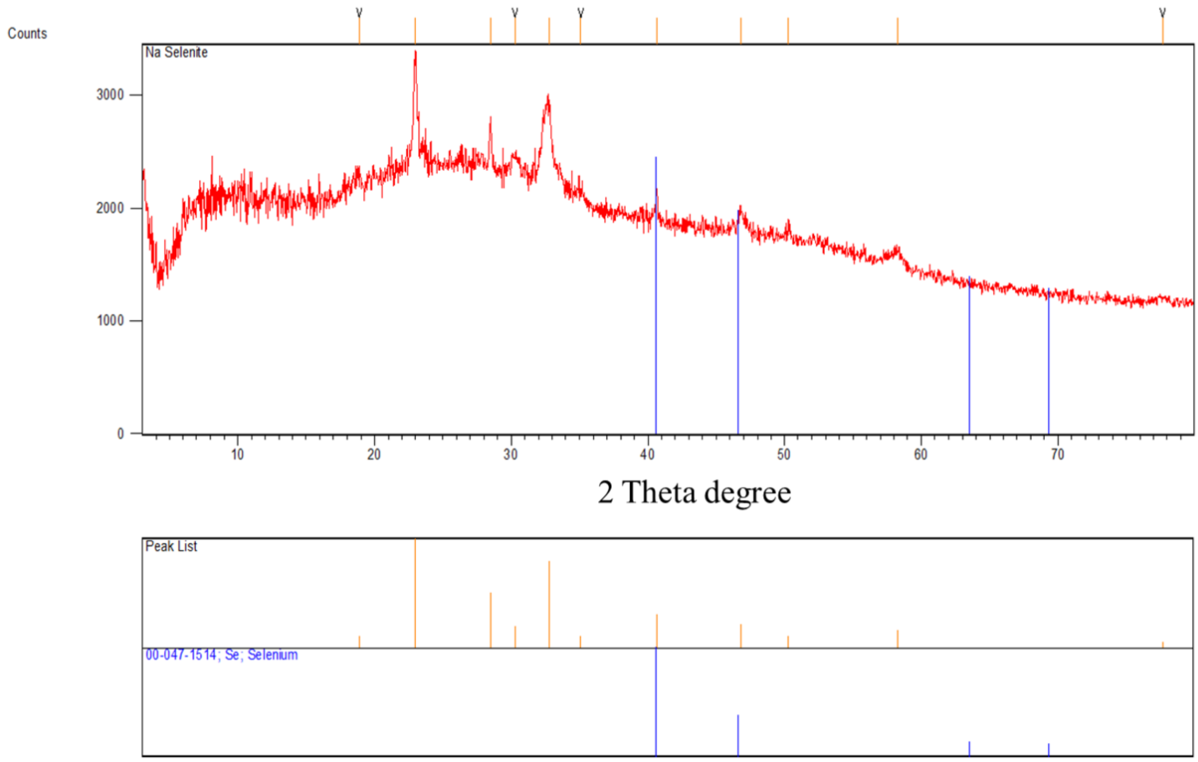

Fig. 2. XRD analysis of green synthesized Se NPs

Morphological parameters

Based on the obtained results, the various concentrations of sodium selenite and green synthesized Se NPs significantly affected the plant height, leaf number, fresh weight, and dry weight of garden cress plants (Table 1).

The highest plant height (17.01 cm) was found in plants treated with 1 mg L^{-1} Se NPs. Nevertheless, no significant difference was found between this treatment and most other nutrient treatments regarding this morphological index. Untreated control plants showed the lowest plant height (14.68 cm), although no significant difference was found between the control and 4 mg L^{-1} Se NPs treatment regarding this trait (Table 1).

The maximum leaf number (9.50) was observed in plants of 1 mg L^{-1} Se NPs treatment, although this treatment did not show a significant difference with the most nutrient treatments, whereas the lowest ones (8.34) were obtained with the 4 mg L^{-1} Se NPs treatment. However, no significant difference was found between this treatment and the control treatment regarding leaf number (Table 1).

The highest fresh weight (25.08 g) was obtained for 1 mg L^{-1} Se NPs treatment, and this effect was superior to that of the other treatments except for 4 mg L^{-1} sodium selenite. Furthermore, the lowest fresh weight (17.37 g) was found with 4 mg L^{-1} Se NPs treatment, although no significant difference was found between 4 mg L^{-1} Se NPs treatment and some other nutrient treatments (Table 1). The highest level of dry weight (2.13 g) of garden cress plants was observed in 1 mg L^{-1} Se NPs treatment, and the effect of this treatment was more considerable than the other nutrient treatments. In contrast, untreated control plants showed the lowest dry weight (1.46 g), although no significant difference was found between the control, 2 and 4 mg L^{-1} Se NPs treatments concerning this trait (Table 1).

Chlorophyll contents

Sodium selenite and green synthesized Se NPs treatments significantly affected the contents of chlorophyll a and total chlorophyll, whereas nutrient treatments had no significant effects on chlorophyll b content (Table 2).

Garden cress plants fed with the various concentrations of sodium selenite and green synthesized Se NPs had a higher content of chlorophyll a and total chlorophyll than untreated plants, where the effect of 1 mg L^{-1} Se NPs treatment on the content of chlorophyll a and total chlorophyll was higher than the other nutrient treatments (Table 2).

Table 1. Effect of foliar spray of sodium selenite and green synthesized Se NPs on growth parameters of garden cress plants

Nutrient treatments (mg L^{-1})	Plant height (cm)	Leaf number	Fresh weight (g)	Dry weight (g)
Control	14.68±0.51 b	8.45±0.77 bc	17.49±2.21 d	1.46±0.20 c
Sodium selenite (1)	16.68±0.82 a	9.08±0.62 ab	20.59±3.16 bcd	1.79±0.23 b
Sodium selenite (2)	16.80±0.70 a	9.25±0.67 a	21.09±3.53 bc	1.77±0.19 b
Sodium selenite (4)	16.92±0.41 a	9.37±0.69 a	22.92±4.09 ab	1.81±0.20 b
Se NPs (1)	17.01±0.86 a	9.50±0.38 a	25.08±1.50 a	2.13±0.22 a
Se NPs (2)	16.55±0.51 a	8.98±0.58 abc	18.28±3.40 cd	1.63±0.29 bc
Se NPs (4)	15.40±1.42 b	8.34±0.40 c	17.37±2.59 d	1.49±0.20 c
Significance	**	**	**	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). ** is significant at $P \leq 0.01$. Values are means of seven replicates $\pm$ SD

Table 2. Effect of foliar spray of sodium selenite and green synthesized Se NPs on chlorophyll contents of garden cress plants

Nutrient treatments (mg L^{-1})	Chlorophyll a	Chlorophyll b	total chlorophyll
		mg g^{-1} FW	
Control	1.31±0.18 d	0.54±0.24	1.85±0.32 d
Sodium selenite (1)	1.77±0.27 ab	0.67±0.18	2.44±0.18 abc
Sodium selenite (2)	1.86±0.32 ab	0.69±0.16	2.56±0.46 ab
Sodium selenite (4)	1.88±0.21 ab	0.70±0.17	2.59±0.27 ab
Se NPs (1)	1.98±0.15 a	0.72±0.21	2.70±0.29 a
Se NPs (2)	1.61±0.38 bc	0.63±0.35	2.24±0.59 bcd
Se NPs (4)	1.44±0.13 cd	0.59±0.15	2.04±0.18 cd
Significance	**	ns	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). ** and ns are significant at $P \leq 0.01$ and not significant, respectively. Values are means of seven replicates $\pm$ SD

Nitrate reductase activity

The various concentrations of sodium selenite and green synthesized Se NPs markedly increased nitrate reductase activity of garden cress plants. The maximum nitrate reductase activity (0.24 μMN g^{-1} FW) was found in 1 mg L^{-1} Se NPs treatment, and the effect of this treatment was superior to that of the other treatments (Fig. 3). In contrast, the minimum nitrate reductase activity (0.12 μMN g^{-1} FW) occurred in the leaves of the control treatment. However, no significant difference was found between the control and 4 mg L^{-1} Se NPs treatment regarding this physiological index (Fig. 3).

Nitrate concentration

The effect of sodium selenite and green synthesized Se NPs treatments on the nitrate concentration of the garden cress plants is presented in Figure 4. Plants treated with nutrient treatments had a lower concentration of nitrate than untreated control garden cresses. The effect of 1 mg L^{-1} Se NPs was significantly higher than the other nutrient treatments. With the application of 1 mg L^{-1} Se NPs, the concentration of nitrate was 44.44% lower than control (0.95 μg g^{-1} DW compared with the control value of 1.71 μg g^{-1} DW) (Fig. 4).

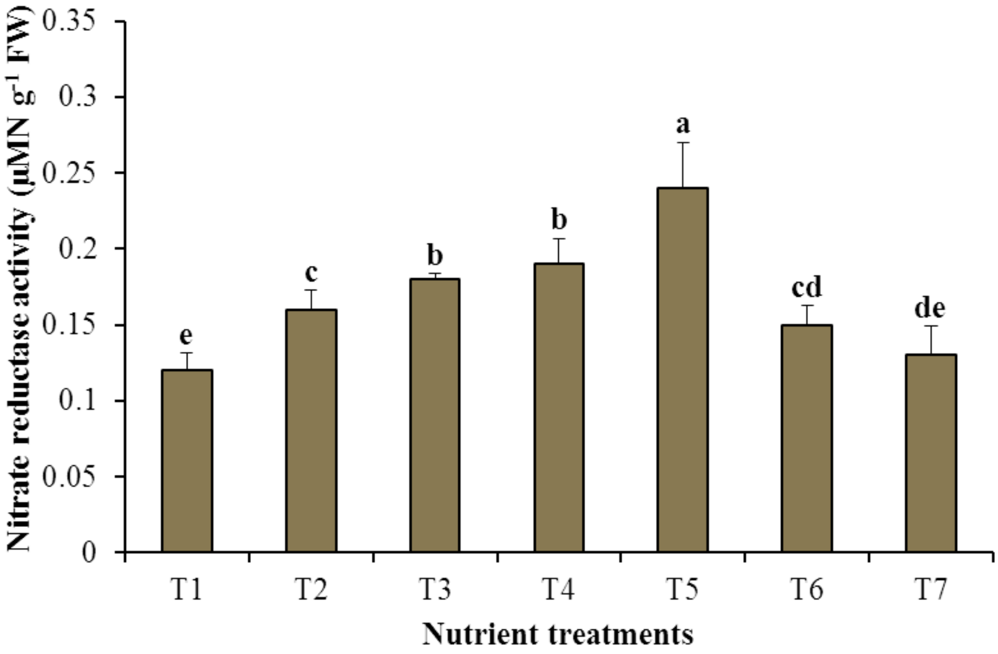

Fig. 3. Nitrate reductase activity of garden cress plants sprayed with different treatments: T1 (control), T2 (1 mg L^{-1} sodium selenite), T3 (2 mg L^{-1} sodium selenite), T4 (4 mg L^{-1} sodium selenite), T5 (1 mg L^{-1} Se NPs), T6 (2 mg L^{-1} Se NPs) and T7 (4 mg L^{-1} Se NPs). Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments. Vertical bars indicate standard deviation.

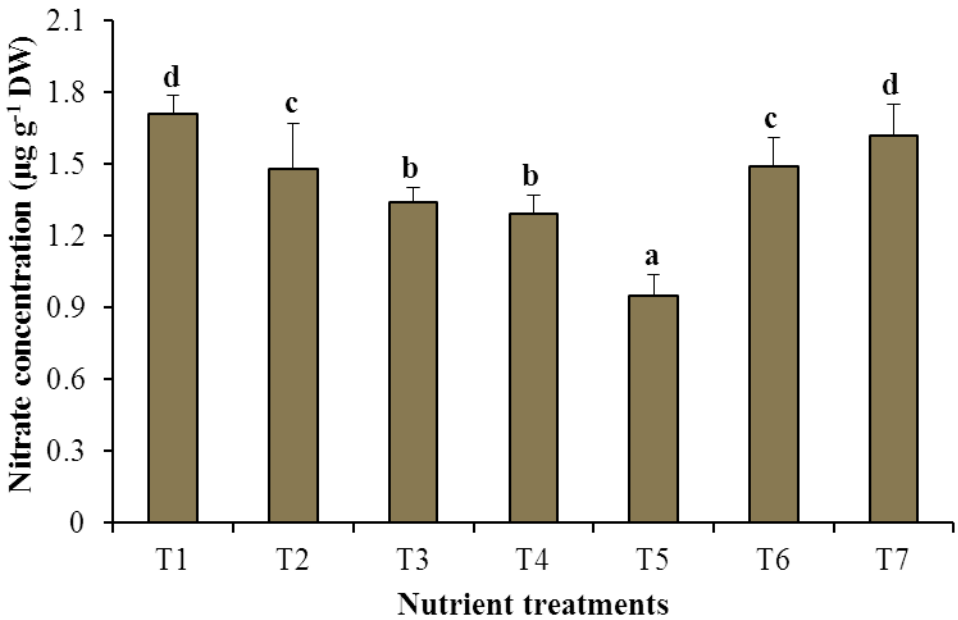

Fig. 4. Nitrate concentration of garden cress plants sprayed with different treatments: T1 (control), T2 (1 mg L^{-1} sodium selenite), T3 (2 mg L^{-1} sodium selenite), T4 (4 mg L^{-1} sodium selenite), T5 (1 mg L^{-1} Se NPs), T6 (2 mg L^{-1} Se NPs) and T7 (4 mg L^{-1} Se NPs). Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments. Vertical bars indicate standard deviation.

Leaf mineral contents

Under different treatments of sodium selenite and green synthesized Se NPs, leaf concentration of Se increased, and concentrations of Zn and P decreased (Table 3). Garden cress accumulated high amounts of Se and is an accumulator of Se (Colla et al., 2018). The highest leaf concentration of Se (5.80 ppm) was obtained with 1 mg L^{-1} Se NPs treatment, which led to about three times increase compared to Se concentration in untreated control plants (1.80 ppm) (Table 3).

The higher concentrations of sodium selenite and green synthesized Se NPs resulted in further reduction of Zn in the leaves. The lowest leaf concentration of Zn (173.00 ppm) was obtained in 4 mg L^{-1} Se NPs treatment, which showed a 30.98% decrease when compared to the control treatment (250.66 ppm) (Table 3). The leaves of plants treated with 4 mg L^{-1} Se NPs showed the lowest concentration of P (1.19%), whereas the highest value (2.40%) was observed in leaves of the control treatment (a decrease of 50.41%) (Table 3).

Table 3. Effect of foliar spray of sodium selenite and green synthesized Se NPs on leaf mineral contents of garden cress plants

Nutrient treatments (mg L^{-1})	Se (ppm)	Zn (ppm)	P (%)
		mg g^{-1} FW	
Control	1.80±0.13 f	250.68±5.85 a	2.40±0.13 a
Sodium selenite (1)	1.90±0.13 f	224.34±4.04 b	2.31±0.13 b
Sodium selenite (2)	2.50±0.10 e	217.36±2.51 b	1.52±0.12 d
Sodium selenite (4)	3.03±0.15 c	203.66±3.51 c	1.32±0.14 f
Se NPs (1)	5.80±0.15 a	185.37±5.03 d	2.10±0.11 c
Se NPs (2)	3.48±0.14 b	175.62±5.50 e	1.40±0.14 e
Se NPs (4)	2.71±0.12 d	173.00±3.00 e	1.19±0.12 g
Significance	**	**	**

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). ** is significant at $P \leq 0.01$. Values are means of seven replicates ± SD

Discussion

There are plenty of reports on the biologic synthesis of nanoparticles using plants and their application in agriculture (Abbasifar et al., 2019 and 2020). It seems plants or their extracts are the best factors for the biological synthesis of nanoparticles. They are readily available and suitable for the mass production of nanoparticles; also, their wastes are environmentally friendly (Hussain et al., 2016).

Increases in morphological parameters with exogenous use of Se have been reported in different plants, including lettuce (Liu et al., 2017; Bo et al., 2018; Zhong-hua et al., 2020), cucumber seedlings (Jozwiak et al., 2016), and pepper (Shekari et al., 2017). Selenium is not an essential element for plants, but the effects can be reflected in better growth and higher yield. The positive impact of Se on morphological parameters can be attributed to the point that this element can play a role in the synthesis of amino acids and proteins (Schiavon et al., 2013), which might result in the enhancement of plant growth. However, green synthesized Se NPs in higher concentrations negatively affect morphological parameters. The nano-fertilizers, due to their small size and high surface to volume ratio, are required in low amount, and their high concentration can be toxic to the plants (Abbasifar et al., 2019 and 2020).

Among plants, the most considerable beneficial effects of Se on growth (up to 2.8-fold higher biomass with Se) have been detected in the Se hyperaccumulator plants such as garden cress, and Se has been suggested to be essential for these species (Shrift, 1969).

There are various reports on the effect of Se on chlorophyll content by the researchers. Some researchers described that chlorophyll content was not affected by Se application (Duma et al., 2011), while some others showed that chlorophyll content was increased by Se application (Hawrylak et al., 2007; Saffaryazdi et al., 2012; Bo et al., 2018), which is in agreement with the results achieved in the current study (Table 2). This increase in chlorophyll content can be attributed to the Se effect in reduction of chlorophyll degradation, protection of chloroplast enzymes, and thus increasing the biosynthesis of photosynthetic pigments (Pennanen et al., 2002, Feng et al., 2015).

The nitrate reductase is a rate-limiting enzyme for nitrate assimilation, catalyzing nitrate to nitrite (Sivasankar and Oaks, 1996). It is generally recognized that Se enhances the activity of nitrate reductase, leading to a decrease in the level of nitrates in plants (Pilon-Smits, 2015). Increases in nitrate reductase activity with Se fertilization have been reported in different plants, including potatoes (Munshi and Mindy, 1992), sunflowers (Ruiz et al., 2007), wheat (Hajiboland and Sadeghzade, 2014), and lettuce (Zhong-hua et al., 2020) which is in line with the results obtained in the current study. Our findings are in agreement with previous reports that showed the exogenous application of Se decreased the accumulation of nitrate in plants (Rios et al., 2010; Golubkina et al., 2017; Bo et al., 2018; Zhong-hua et al., 2020). The decreases in the accumulation of nitrate in plants with exogenous use of Se possibly ascribed to the reduced uptake or transport of nitrate toward the aerial part of the plant, in addition to enhancing nitrate reductase synthesis and activity (Bo et al., 2018).

Garden cress accumulated high amounts of Se and is an accumulator of Se (Colla et al., 2018). The highest leaf concentration of Se was obtained with 1 mg L^{-1} Se NPs treatment, which led to about three times increase compared to Se concentration in untreated control plants (Table 3). Our results are in consistent with those of previous works that the exogenous application of Se increased leaf concentration of Se in potato (Turakainen et al., 2004), tea (Hu et al., 2003), garden cress (Yáñez Barrientos et al., 2012), and lettuce (Zhong-hua et al., 2020).

There are various reports on the effect of the exogenous application of Se on the leaf concentration of Zn by the researchers. Our findings are in line with those obtained by Silva et al. (2018) and Silva and Cadore (2019), reporting that the exogenous application of Se decreased leaf concentration of Zn. In contrast, Rios et al. (2013) indicated that the exogenous application of Se did not influence leaf concentration of Zn.

Selenium modify the transport or absorption of other mineral nutrients (Pilon-Smits and LeDuc, 2009). The leaves of plants treated with 4 mg L^{-1} Se NPs showed the lowest concentration of P. These results are inconsistent with the findings of Silva et al. (2018) and Wu and Huang (1992). They are also similar to the findings of Rios et al. (2013), who suggested that the exogenous use of Se reduces leaf concentration of P. It is known that ions are absorbed through the transcellular and paracellular pathways. Therefore, when ions bind with Se, absorption of these molecules would be negatively influenced in the two transcellular and paracellular, thus decreasing the element bioavailability to the plant, and as a consequence, its accumulation by the plant (Silva et al., 2018), as determined for Zn and P in this study. The antagonistic effect of Se on P detected in the leaves can probably be as the result of the competition between these ions (Hopper and Parker, 1999) because both of them are absorbed through phosphate transporters (Zhao et al., 2010).

Conclusion

The findings of present investigation showed that the foliar application of Se, especially 1 mg L^{-1} Se NPs, improved the morphological parameters, the chlorophyll content, and the concentration of Se, as well as the activity of nitrate reductase, whereas reduced the accumulation of nitrate in the garden cress plants. Hence, an effective method of biofortifying food crops with selenium could be the foliar application. This is the time to incorporate nano-fertilizers in the agriculture system with further studies and practical applications in the fields s needed. Nevertheless, extensive studies are required to understand the mechanism for nanoparticle toxicity to avoid their adverse impacts on the natural environment and to avoid the hazards experienced with this technology.

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Conflict of interests

Authors have declared that no competing interests exist.

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