Original scientific paper
Оригинални научни рад
UDC 635.52-184.7:541.123
DOI 10.7251/AGREN2402077Ž

University of Banjaluka, Faculty of Agriculture
Agro-knowledge Journal

Analyzing the impact of foliar spraying of selenium on some growth and biochemical parameters in lettuce (*Lactuca sativa* L.)

Dragan Žnidarčić

[1]*Biotechnical Centre Naklo, Naklo, Slovenia*

Abstract

Being a trace element, selenium (Se) has beneficial effects on vegetables growth and development. Nevertheless, the impact of Se on the lettuce (Lactuca sativa L.) characteristics has not been systematically examined. In our study the plants of an autochthonous local Slovenian variety of crisp lettuce 'Ljubljanska ledenka' were exposed to foliar treatment with an aqueous solution of Na_2SeO_4. The foliage of target plants underwent two rounds of spraying with a nutrient solution at a 5-day interval. In the first experiment, the concentrations used were 0 (distilled water spray as a control); 1 + 1; 2 + 2, and 5 + 5 mg Se L^{-1}. In the second experiment, the concentrations were 0; 10 + 0, 10 + 10, and 10 + 50 mg Se L^{-1}. First spraying began at 3 fully developed leaves. The experiment was carried out in a completely randomized design in a plastic greenhouse on a field near Ljubljana (46°04'46.0"N, 14°34'20.0"E). No significant differences in the shoot height, leaf number per plant, and root dry matter were observed between treatments. Leaves in control and 1+1 mg Se L^{-1} treatment had higher dry matter percentage in comparison with other treatments. The growth analysis revealed that 5+5, 10+0, and 10+10 mg Se L^{-1} treatments increased the weight of shoots. The shoots yield was reduced after foliar application of strong enlarged doses of selenate (10+10 mg Se L^{-1}). The Se concentration in shoots increased in proportion to the level of the concentration of Na_2SeO_4 and the highest values were recorded from the plants in which 10+10 mg Se L^{-1} was applied. The portion of photosynthetic pigments (chlorophylls and carotenoids) decreased from the control group to the treatment with the highest Na_2SeO_4 solution (10 + 50 mg Se $^{L-1}$).

Key words: selenate, lettuce, *Lactuca sativa*, growth components, pigments

Introduction

Selenium (Se) is a naturally occurring trace micronutrient and exists primarily in insoluble elemental and selenide forms. However, in oxidizing environments, Se is transformed to soluble selenite and selenate forms. Its chemical properties are similar to sulphur (S), leading to nonspecific substitution of S by Se in proteins and other S compounds (Dilworth & Bandurski, 1977). In the past two decades respective epidemiological studies indicated that Se is of fundamental role in animal and human biological processes (Izydorczyk et al., 2021). For example, Se is needed for the effective functioning of the immune system and appears to be a crucial nutrient in counteracting the expansion of virulence and inhibited by concomitant HIV-2 infection (Rayman, 2008). Many investigations have also shown a clear inverse correlation among the intake of Se and the risk of cardiovascular diseases, cancer (Gupta & Gupta, 2017), and coronavirus infection (Maltseva et al., 2022).

In spite of the fact that the indispensability of Se to crops has not yet been proven, the potential of some plants to acquire and convert inorganic forms of Se into bioactive organic compounds, without presenting symptoms of toxicity, has significant consequences for human food and health. Se quantity in food chain is chiefly determined by Se levels in the soil where the crops are grown (Wu et al., 2015). Availability of Se in agricultural crops is a complex function of soil and crop parameters, such as the crop development and growth stages (Montes-Bayón et al., 2002). Additionally, the accumulation rate of Se depends on the climatic conditions among locations, management practices, crop species, various physical and chemical factors of soil (pH and oxidation-reduction status, moisture, salinity, $CaCO_3$ content …), the character of competing ions, and the ability of the crop to absorb and assimilate Se (Wen, 2021).

Growing crops enriched with Se could be an efficient strategy of generating Se-rich foods and, thus, improving medical conditions (Lyons et al., 2005). Se biochemical activity in plants is currently well-described, and over the past 20 years a number of studies have been made to build on the Se content in agricultural crops through different methods: by adding selenite or selenate to fertilizers, spraying crops with Se solutions, treating seeds with aqueous Se before sowing and hydroponic cultivation in a nutrient solution containing Se. Banuelos & Meek (1990) note that about 70-90% of the inorganic Se applied seemed to accumulate in soil in the form inaccessible to crops. That is why choosing a suitable technique should be done with care due to possible harmful effects on the environment.

Although numerous Se biofortification programs have been developed, the effects of foliar Se application on lettuce (Lactuca sativa L.) are lacking in literature. In our research the spraying technique in a greenhouse was used. It included thorough environmental control, namely temperature, humidity, oxygen availability, and artificial lightning. Another crucial advantage is that there is no impact on the surrounding environment when enriched suspensions are used. In our case Se in the form of Na_2SeO_4 was added to the nutrient solution. Hence, the effects of different concentrations of Na_2SeO_4 in the foliar solution were investigated in relation with some growth and biochemical parameters of crisp lettuce.

Material and Methods

The experiment with crisp lettuce cv. 'Ljubljanska ledenka' was carried out at the plastic greenhouse covered with a double PE-film and passive climate control on a field near Ljubljana (46°04'46.0"N, 14°34'20.0"E), Slovenia, from 15 March to 30 April and from 5 May to 6 June 2022. Seeds were washed with distilled water and germinated in petri dishes at 20-25°C. After germination, individual plants were repotted in styrofoam seedling trays (40 cells) (cell volume: 30 cm^3) filled with a commercial Klasmann Tray substrate pH 6-6.5; N 180 mg L-1; P_2O_5 210 mg L^{-1}; K_2O 250 mg L^{-1}; MgO 85 mg L^{-1} + micronutrients). Moisture levels in growing media were maintained at 50-60%. Plants were irrigated with a tap and fertilized once a week with the Peter's® 15-30-15 commercial nutrition solution at a concentration of 100 ppm.

Daily values of sun radiation on a sunny day were between 5 and 6 kWh m-2. The climate values were recorded every hour by the automatic climate station installed in the greenhouse.

During the experiment, the temperature inside the greenhouse varied between 17 and 28 °C and at night temperature was between 10 and 14°C, while relative humidity varied between 48 and 68%.

Trials were carried out with the Se solution that was prepared by using Na_2SeO_4. The foliage of target plants were treated with two rounds of spraying with an aqueous solution with a concentration of Na_2SeO_4 (first round: 0+0-control, by using sterile distilled water without Se, 1+1; 2+2 and 5+5 mg L^{-1}; second round: 0+0; 10+0; 10+10 and 10+50 mg L^{-1}) using a hand-operated high pressure pump sprayer. The first application was done on 18 day after sowing, when the plants had 3 fully developed leaves. The interval between spraying applications was 5 days. During each treatment the same volume of solution (60 mL of Na_2SeO_4) was sprayed. The plants without Na_2SeO_4 application were cultivated next to the treated ones in the same conditions and separated to prevent contamination. Each treatment had 3 replicates.

At the end of the growth experiment, as soon as the lettuce head developed, twenty plants from each treatment were randomly taken out from trays. The plants were washed free of any contamination. After collection, samples were cleaned, and shoots and roots were separated. The height of the shoots (above ground part) was measured by the Vernier caliper. The surface areas of five developed leaves were calculated according to Ipek & Mutluay (2022). The fresh weight of the shoots was measured using an electronic balance. These shoots and roots were placed in an oven at 60°C for 3 d to measure dry weight (DW). For biochemical analysis leaves were placed in cryogenic nitrogen and stored at –20 ^{o}C until lyophilization. After lyophilization samples were ground into powder using a universal mill. Leaf photosynthetic pigment contents (chlorophyll *a*, chlorophyll *b*, and total carotenoid) were determined according to the method described by Lichtenthaler & Wellburn (1983). Hydride generation atomic fluorescence spectrophotometry was used for the Se concentration analysis of the leaves following Golob et al. (2016).

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey's HSD test. The effects of Se treatment were considered statistically significant when $P<0.05$.

Results and Discussion

At harvest, the plants showed an excellent overall visual quality in all treatments. All leaves were crisp and green. No visible toxic symptoms including stunting of growth, necrosis on leaf margins, and withering were observed.

The impact of foliar spraying of Na2SeO4 on the morphological parameters of lettuce are presented in Table 1. In comparison with the plants sprayed with distilled water, Na2SeO4 treatments showed no significant influences on shoot height. Observation of leaf area between the control and Se treatment groups revealed that the untreated plants were significantly different from the treatments from 1+1, 2+2, and 10+50 mg L-1. The mean leaf number per plant showed no difference among the treatment groups.

The dry matter content is the ratio between dry and fresh weight expressed as a percentage. The percentage of dry matter of leaves is an important reference parameter and is somewhat significant as well to a consumer who does not want to buy watery products. According to the ANOVA, the applied Na2SeO4 doses have a significant effect on this parameter in leaves. The dry matter content in leaves was significantly higher in control plants and in plants with limited Na2SeO4 supply (1 + 1 mg Se L-1). These results are in the agreement with those obtained by Ximénez-Embún at al. (2004), Rani at al. (2005), and Pezzarosa at al. (2007). The mean content of dry matter in roots was between 21.6 and 18.2%. Although the previous experiments demonstrated that increasing the Se concentration in nutrient solutions will also increase the dry matter production

(Khattab, 2004; Djanaguiraman et al., 2004), there was no significant difference in the share of dry matter in the roots between treatments.

Tab. 1. Mean comparison on the morphological parameters of lettuce under different Na_2SeO_4 levels

Foliar Na_2SeO_4 (mg L^{-1})	Shoot height (cm)	Leaf area (cm^2)	Leaf number ($plant^{-1}$)	Leaf dry matter (%)	Root dry matter (%)
Control (0)	22.02	352.4 ^{a}	30.6	13.8 ^{a}	20.4
1 + 1	21.84	375.8 ^{b}	29.4	14.4 ^{a}	18.2
2 + 2	22.36	381.3 ^{b}	28.9	12.1 ^{b}	19.5
5 + 5	22.18	346.5 ^{a}	31.2	12.0 ^{b}	21.6
10 + 0	21.92	342.8 ^{a}	31.9	12.2 ^{b}	20.8
10 + 10	21.45	332.4 ^{ac}	30.7	11.6 ^{b}	20.7
10 + 50	18.02	318.2 ^{c}	29.5	11.2 ^{b}	21.4

Values followed by different letters are significantly different at $P<0.05$. The absence of letters indicates that there is no statistical difference between the treatments.

The impact of the application of Na_2SeO_4 on the fresh shoot weight Se and photosynthetic pigments content are shown in Table 2. The analysis of lettuce growth revealed that a slight (5+5, 10+0 mg Se L^{-1}) and medium (10+10 mg Se L^{-1}) increase of selenate supply increased the weight of shoots. Higher solution of Na_2SeO_4 (10+50 mg Se L^{-1}) negatively affected vitality of plants. Namely, a significant decrease in shoots fresh weight was observed in response to strong enlarged doses of selenate. A similar effect was observed in the past studies. It was reported that Se at low concentrations acts as an antioxidant and can stimulate the growth of leafy vegetables, whereas at higher concentrations it acts as a pro-oxidant, reducing the yield (Xue et al., 2001; Saffaryazdi et al., 2012). Our results are in line with Gupta and Gupta (2016) who stated that high doses of Se inhibit plant growth, possibly because of toxicity. There are two mechanisms leading to Se toxicity. One is abnormal selenoproteins: SeCys/SeMet and other Se-amino acids are nonspecifically bound to the protein, replacing Cys/Met in the protein chain, resulting in insertion errors, which will damage protein function and lead to toxicity.

The Se content in shoots increased with the increase of the concentration of the Na_2SeO_4 foliar spraying solution. The results of the present study agree with those of previous studies showing that Se accumulation in plants increased proportionally with the increase of concentrations of added Se solutions (Cartes et al., 2005; Jiang et al.t, 2018).

Tab. 2. Mean comparison on the shoot weight, Se, chlorophyll, and carotenoids content of lettuce under different Na_2SeO_4 levels

Foliar Na_2SeO_4 (mg L^{-1})	Shoot weight (g $plant^{-1}$)	Se content (mg g^{-1} DW)	Chlorophyll *a* (ng g^{-1} DW)	Chlorophyll *b* (ng g^{-1} DW)	Carotenoids (ng g^{-1} DW)
Control (0)	7.12 ^{a}	0.061 ^{a}	1652.7 ^{a}	1034.8 ^{a}	402.4 ^{a}
1 + 1	7.83 ^{a}	0.384 ^{b}	1534.2 ^{a}	956.7 ^{a}	380.6 ^{a}
2 + 2	8.04 ^{a}	1.143 ^{c}	1482.9 ^{a}	923.5 ^{a}	367.5 ^{a}
5 + 5	10.12 ^{b}	1.958 ^{d}	1456.2 ^{a}	842.0 ^{ab}	356.6 ^{a}
10 + 0	11.38 ^{b}	4.824 ^{e}	1462.7 ^{a}	712.8 ^{ab}	360.2 ^{a}
10 + 10	10.14 ^{b}	16.485 ^{f}	1358.2 ^{a}	543.8 ^{b}	320.7 ^{a}
10 + 50	5.24 ^{c}	58.254 ^{g}	985.4 ^{b}	318.2 ^{c}	125.1 ^{b}

Values followed by different letters are significantly different at $P<0.05$. The absence of letters indicates that there is no statistical difference between the treatments.

The objective of this experiment was also to investigate the effect of Na_2SeO_4 on non-enzymatic antioxidants. To our knowledge, few studies have been conducted to illustrate the impact of Se on some antioxidants. The antioxidant capability of the lettuce in our study was determined by carotenoids and chlorophyll a and b. The treatments of plants exposed to high amount of selenate (10+50 mg Se L^{-1}) led to a decrease in antioxidants, in comparison with plants obtained by other treatments. Kaya et al. (2019) argue that the pigments content of plants reflects the state of photosynthetic activity, which is sensitive to stress. Foyer and Shigeoka (2011) claimed that the decrease in the chlorophyll a and b content under stress is due to a reduction in metabolic activities of plants.

Conclusion

Although Se is not a crucial element for vegetables growth, some studies have shown that adding suitable content of Se can stimulate crop development, though disproportionate addition can cause toxicity (Kapolna et al., 2009; Li et al., 2007). Our results have indicated that the foliar application of Se could regulate plant growth which is dose-dependent, i.e. enhancing growth at low concentrations and inhibiting it at high concentrations.

However, in the future, a pot experiment is needed to illuminate the mechanisms of foliar application effects on bio-fortification in different ecotypes and varieties of leafy vegetables by means of an isotope tracer technique. Furthermore, a hydroponic experiment can be conducted to examine the uptake, transport, accumulation, and distribution of selenium with foliar application using the synchrotron radiation technique.

References

Banuelos, G.S. & Meek, D.W. (1990). Accumulation of selenium in plants grown on selenium-treated soil. *Journal of Environmental Quality*, 19: 772-777. https://doi.org/10.2134/jeq1990.00472425001900040023x

Cartes, P., Gianfreda, L., Mora, M.L. (2005). Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant and Soil*, 276: 359-367. https://doi.org/10.1007/s11104-005-5691-9

Dilworth, G.L., Bandurski, R.S. (1977). Activation of selenate by adenosine 5'-triphosphate sulphurylase from *Saccharomyces cerevisiae*. *Biochemical Journal*, 163(3): 521-529. https://doi.org/10.1042%2Fbj1630521

Djanaguiraman, M., Devi, D.D., Shanker, A.K., Sheeba, J.A., Bangarusamy, U. (2004). Selenium- an antioxidant protectant in soybean during senescense. *Plant and Soil*, 272: 77-86. https://doi.org/10.1007/s11104-004-4039-1

Foyer, C.H. & Shigeoka, S. (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiology*, 155: 93-100. doi.org/10.1104/pp.110.166181 https://doi.org/10.1104/pp.110.166181

Golob, A., Germ, M., Kreft, I., Zelnik, I., Kristan, U., Stibilj, V. (2016). Selenium uptake and Se compounds in Se-treated buckwheat. *Acta Botanica Croatica*, 75(1): 17-24. https://doi.org/10.1515/botcro-2016-0016

Gupta, M. & Gupta, S. (2017). An overview of selenium uptake, metabolism, and toxicity in plants. *Frontiers in Plant Science*, 7: 1-14. https://doi.org/10.3389/fpls.2016.02074

Ipek, M. & Mutluay, E. (2022). Enhancing the physiological and molecular responses of horticultural plants to drought stress through plant growth–promoting rhizobacterias. *Developments in Applied Microbiology and Biotechnology*, p. 185-199. https://doi.org/10.1016/B978-0-323-91861-9.00001-X

Izydorczyk, G., Ligas, B., Mikula, K., Krowiak, A. W., Moustakas, K., Chojnacka, K. (2021). Biofortification of edible plants with selenium and iodine - a systematic literature review. *Science of The Total Environment*, 754: 141983. https://doi.org/10.1016/j.scitotenv.2020.141983

Jiang, Y., Feng, X., Yang, Y., Qi, X., Rena, Y., Gao, Y., Liu, W., Hua, Y., Zenga, Z. (2018). Performance of common buckwheat (*Fagopyrum esculentum* M.) supplied with selenite or selenate for selenium biofortification in northeastern China. *The Crop Journal*, 6: 386-393. https://doi.org/10.1016/j.cj.2018.03.003

Kápolna, E., Hillestrom, P.R., Laursen, K.H., Husted, S., Larsen, E.H. (2009). Effect of foliar application of selenium on its uptake and speciation in carrot. *Food Chemistry*, 115: 1357-1363. https://doi.org/10.1016/j.foodchem.2009.01.054

Kaya, C., Sarioğlu, A., Akram, N. A., Ashraf, M. (2019). Thiourea-mediated nitric oxide production enhances tolerance to boron toxicity by reducing oxidative stress in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) plants. *Journal of Plant Growth Regulation*, 38(3): 1094-1109. https://doi.org/10.1007/s00344-019-09

Khattab, H. (2004). Metabolic and oxidative responses associated with exposure of *Eruca sativa* (Rocket) plants to different levels of selenium. *International Journal of Agriculture & Biology*, 6(6): 1101-1106.

Lichtenthaler, H.K., & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*. 11: 591–592. https://doi.org/10.1042/bst0110591

Li, N., Gao, Z.D., Luo, D.G., Tang, X., Chen, D., Hu, Y. (2007) Selenium level in the environment and the population of Zhoukoudian area. *Science of the Total Environment*, 381(1):105-111. https://doi.org/10.1016/j.scitotenv.2007.03.027

Lyons, G.H., Lewis, J., Lorimer, M.F., Holloway, R.E., Brace, D.M., Stangoulis, J.C.R., Graham, R.D. (2004). High selenium wheat: agronomic biofortification strategies to improve human nutrition. *Food, Agriculture and Environment*, 2(1): 171-178. https://doi.org/10.1079/NRR200255

Maltseva, V. N., Goltyaev, M. V., Turovsky, E. A., Varlamova, E. G. (2022). Immunomodulatory and anti-inflammatory properties of selenium-containing agents: Their role in the regulation of defense mechanisms against COVID-19. *International Journal of Molecular Sciences*, 23: 2360. https://doi.org/10.3390/ijms23042360

Montes-Bayon, M., Grant, D., Meija, J., Caruso, J.A. (2002). Selenium in plants by spectrometric techniques: developments in bio-analytical methods. *Journal of Analytical Atomic Spectrometry*, 17: 1015-1023. https://doi.org/10.1039/B203256M

Pezzarossa, B., Petruzzelli, G., Petacco, F., Malorgio, F., Ferri, T. (2007). Absorption of selenium by *Lactuca sativa* as affected by carboxymethylcellulose. Chemosphere, 67: 322-329. https://doi.org/10.1016/j.chemosphere.2006.09.073

Rani, N., Dhillon, K., Dhillon, S.K. (2005). Critical levels of selenium in different crops grown in an alkaline silty loam soil treated with selenite-Se. *Plant and Soil*, 277: 367-374. https://doi.org/10.1007/s11104-005-8161-5

Rayman, M.P. (2008). Food-chain selenium and human health: emphasis on intake. *British Journal of Nutrition*, 100(2): 254-68. https://doi.org/10.1017/S0007114508939830

Saffaryazdi, A., Lahouti, M., Ganjeali, A., Bayat. H. (2012). Impact of selenium supplementation on growth and selenium accumulation on spinach (*Spinacia oleracea* L.) plants. *Notulae Scientia Biologicae*, 4: 95-100. https://doi.org/10.15835/nsb448029

Wen, D. (2021). Selenium in horticultural crops. *Scientia Horticulturae*, 289: 110441. https://doi.org/10.1016/j.scienta.2021.110441

Wu, Z.L., Bañuelos, G.S., Lin, Z.Q., Liu, Y., Yuan, L.X., Yin, X.B., Li, M. (2015). Biofortification and phytoremediation of selenium in China. *Frontiers in Plant Science*, 6: 136. https://doi.org/10.3389/fpls.2015.00136

Ximenez-Embun, P., Alonso, I., Madrid-Albarran, Y., Camara, C. (2004). Establishment of selenium uptake and species distribution in lupine, Indian mustard and sunflower plants. *Journal of Agricultural and Food Chemistry*, 52: 832-838. https://doi.org/10.1021/jf034835f

Xue, T., Hartikainen, H., Piironen, V. (2001). Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant and Soil*, 237: 55-61. https://doi.org/10.1023/A:1013369804867

Анализа утицаја folijарног третмана селена на неке параметре раста и биохемијске параметре зелене салате (*Lactuca sativa* L.)

Драган Жнидарчич

Биотехнички центар Накло, Накло, Словенија

Сажетак

Елемент у траговима селен (Se) може имати корисне ефекте на раст и развој поврћа. Ипак, утицај Se на карактеристике зелене салате (*Lactuca sativa* L.) није систематски испитиван. У нашем истраживању биљке аутохтоне локалне словеначке сорте хрскаве салате 'Љубљанска леденка' фолијарно су третиране воденим раствором Na_2SeO_4. Листови испитиваних биљака подвргнуто је у два круга прскања хранљивим раствором у размаку од 5 дана. У првом експерименту коришћене су концентрације 0 (водени спреј као контрола); 1 + 1; 2 + 2 и 5 + 5 50 mg Se L^{-1}. У другом експерименту, концентрације су биле 0; 10 + 0, 10 + 10 и 10 + 50 50 mg Se L^{-1}. Прво прскање је започело на трећем правом листу који је био отворен. Експеримент је изведен у потпуно рандомизираном дизајну у пластенику на пољу близу Љубљане (46°04'46.0"N, 14°34'20.0"E). Између третмана нису уочене значајне разлике у висини изданака, броју листова по биљци и сухој твари коријена. Листови у контроли и третману 1+1 mg Se L^{-1} имали су већи постотак сухе твари у односу на друге третмане. Анализа раста показала је да третмани 5+5, 10+0 и 10+10 mg Se L^{-1} повећавају тежину изданака. Принос изданака је смањен фолијарном применом повећаних доза селената (10+10 mg Se L^{-1}). Концентрација Се у изданцима расла је пропорционално ниво концентрације Na_2SeO_4, а највеће вриједности су забиљежене код биљака у којима је примијењено 10+10 mg Se L^{-1}. Удио фотосинтетских пигмената (хлорофили и каротеноиди) се смањио од контролне групе до третмана са највећим раствором Na_2SeO_4 (10 + 50 mg Se L^{-1}).

Кључне ријечи: селенат, салата, Lactuca sativa, компоненте раста, пигменти

Corresponding author: Dragan Žnidarčič
E–mail: dragan.znidarcic@bc-naklo.si

Received: March 03, 2023
Accepted: June 04, 2024