

Effect of sodium selenite supplementation on some pigments and biochemical parameters of *Lactuca sativa* L. growing hydroponically

Bahaa I. Alkhafagi

*Department of biology, Babylon university, 51001 Alhilla, Iraq,
sci.evan.ibrahim@uobabylon.edu.iq*

Evan I. Merhij

Department of biology, Babylon university, 51001 Alhilla, Iraq

Abstract

Selenium (Se) is a fundamental element that is beneficial to various organisms at tiny exposure levels and toxic at large concentrations. This research aims to study the effect of selenium as NaHSeO₃ on water content, some photosynthetic pigments, carotenoids, proteins, and carbohydrate content in lettuce (*Lactuca sativa* L.). selenium used in the form NaHSeO₃ in the concentrations of 0, 1, 5, 10, and 20 mg L⁻¹ added to Hoagland solution and three growth periods (10, 20, and 30 days) was studied. The application of Selenium (Se) led to a decrease in the percentage of water in lettuce leaves, recording the least decrease on the 30th day of the treatment at a concentration of 20 mg L⁻¹, as well as the contents of total protein and carbohydrates in all concentrations compared to the control, except for the concentration 1 mg L⁻¹, which recorded a non-significant increase in total protein and a significant increase in carbohydrates on the 30th day of treatment. Chlorophyll a decreased relatively, except for a concentration of 5 mg L⁻¹, whose content increased on the 30th day of treatment, and the content of chlorophyll b, total chlorophyll and carotenoid increased in the selenium treatments compared to the control, recording the highest content of chlorophyll b and total chlorophyll in the concentration of 20 mg L⁻¹ on the 20th day of the treatment, while the concentration of 5 mg L⁻¹ recorded the highest content of carotenoid in all treatment periods. The results of our study showed that low concentrations of selenium have a positive effect on the content of total protein and carbohydrates. however, the high concentrations harm them, while the concentration of 5 mg L and 20 mg L has a positive effect on the content of chlorophyll and carotenoids.

Keywords: *carbohydrates, chlorophyll, Lactuca sativa, lettuce, selenium, sodium selenite.*

INTRODUCTION

With a little range between deficiency and excess, selenium is a fundamental element that is beneficial to various organisms at tiny exposure levels and toxic at large concentrations (El-Badri et al. 2022). Higher doses of Se have a pro-oxidant effect that lowers yields and causes metabolic disturbances (Sali et al. 2018). The Se species absorbed from the growth media determines the form and quantity of Se in plant organs in

the first place (Versini et al. 2016). Because both can be easily absorbed by plants and converted to organic Se compounds, selenite (SeO₃) and selenate (SeO₄) are the main forms of selenium that are toxic to plants. According to some studies, selenite is more toxic than selenate (Terry et al. 2000).

Lettuce (*Lactuca sativa* L.), a popular leafy vegetable with an annual intake of 11.7 kg per person, was chosen as the test plant because it is prone to nutrient deficits (Lian et al. 2021).

It is the most consumed and farmed leafy vegetable in the world, with production increasing year after year. In 2018, the overall harvested area was anticipated to be more than 1.27 million hectares, with a total yield of roughly 27.3 million tons (Medina-Lozano, Bertolín & Díaz 2021). Lettuce contains a variety of nutrients, including iron, fiber, and vitamin A. It is believed that the iron content could be still raised to satisfy daily man needs for iron (MUNAR et al., 2020); it is also one of the vegetables grown in hydroponic systems most frequently (Hawrylak-Nowak 2013).

Hydroponics agriculture relies solely on water and nutrient-rich additives to grow plants without using soil. As a result, it provides a solution to the growing problem of horticultural land shortage (Danush Ranganath, Hari Sri Rameasvar & Karthikeyan 2023). Soilless culture (culture) necessitates maintaining optimal growth and development circumstances, suitable climatic management, and the use of balanced irrigation solutions that fulfil the mineral requirements of certain crops and kinds. Plant growing in soilless cultures is one of the favored methods for the prevention of soil-borne illnesses and plant pests (Sela Saldinger et al. 2023).

Materials and Methods

Plant sampling and preparation

In the biological department at Babylon University in Iraq, lettuce (*Lactuca sativa* L.) was grown in PVC pipes with a diameter of 4 inches (deep water culture) as shown in figure 1. Five pipes had seven holes, each with a diameter of 3 inches and was filled with perlite when the seedlings were 30 days old. They remained in the pipes for 45 days after the transplant. Hoagland's solution was used for growth and refreshed every 15 days. After 15 days, sodium selenite (NaHSeO_3) in concentrations of 1, 5, 10, and 20 mg L⁻¹ was

added to the nutrient solution in addition to the control (Hoagland's solution alone). For each replicate, the samples were the middle portions of the lettuce leaves collected after 10, 20, and 30 days of treatment.

Figure 1: shows the Experiment design.



Water content of lettuce leaf

The water content was estimated by taking 1 gm from the central part of fresh lettuce leaves and then drying it in an electric oven for 48 hours, then the dry sample was weighed with a sensitive scale, and dry weight was subtracted from the fresh weight to extract the percentage of water content in the leaves.

Chlorophyll a and b, total chlorophylls and carotenoid estimation

content of chlorophylls a, b, total chlorophyll and β -carotene were estimated based on the method of (LICHTENTHALER & WELLBURN 1983) where fresh lettuce leaves were crushed with 80% acetone. The absorbance was read at wavelengths of 646, 663 and 470 nm.

Carbohydrates estimation

The method that was relied upon for the determination of carbohydrates is DuBois et al. (1956) method using ethyl alcohol 80% and phenol 5% at a wavelength of 560 nm using a spectrophotometer.

Total proteins

Bishop et al. (1985) method was dependent on protein determination. Take 200 μl of protein extract and add 800 μl of Biuret reagent to it,

then shake and leave for 30 minutes. The absorbance is read using a spectrophotometer at a wavelength of 555 nm.

Statistical analysis

The experimental design was completely randomized with a 2×3 factorial scheme: 5 Se concentrations (0, 1, 5, 10, and 20 mg L⁻¹) and three treatment periods (10, 20 and 30) days to the analysis of variance (ANOVA) and Descriptive statistics (mean, standard deviation), the averages were compared using the LSD test ($p < 0.05$). analyses were

performed by using SPSS (version 23, Chicago, Illinois, USA).

Results

Water content

From (Table 1) it can be seen that the higher the selenium concentration in the nutrient solution and the longer the treatment period, the lower the water content in lettuce (*Lactuca sativa* L.) leaves. As the lowest water content was at a concentration of 20 mg L after 30 days of treatment (87.2%), with a significant decrease compared to the control (91.7%).

Table 1: Effect of concentrations of sodium selenite on the water content of lettuce leaves. The results are average of three replicates. ($P \leq 0.05$).

NaHSeO ₃ (mg L ⁻¹)	water content (%)		
	day 10th	day 20th	day 30th
0	90.2±0.1	91.2±0.5	91.7±0.3
1	92.1±0.9	89.7±0.6	90.5±1.7
5	90.5±0.8	90.3±1.1	89.6±0.6
10	91.5±0.7	89.5±1.2	89±2.2
20	90±0.8	88.1±0.3	87.2±0.7

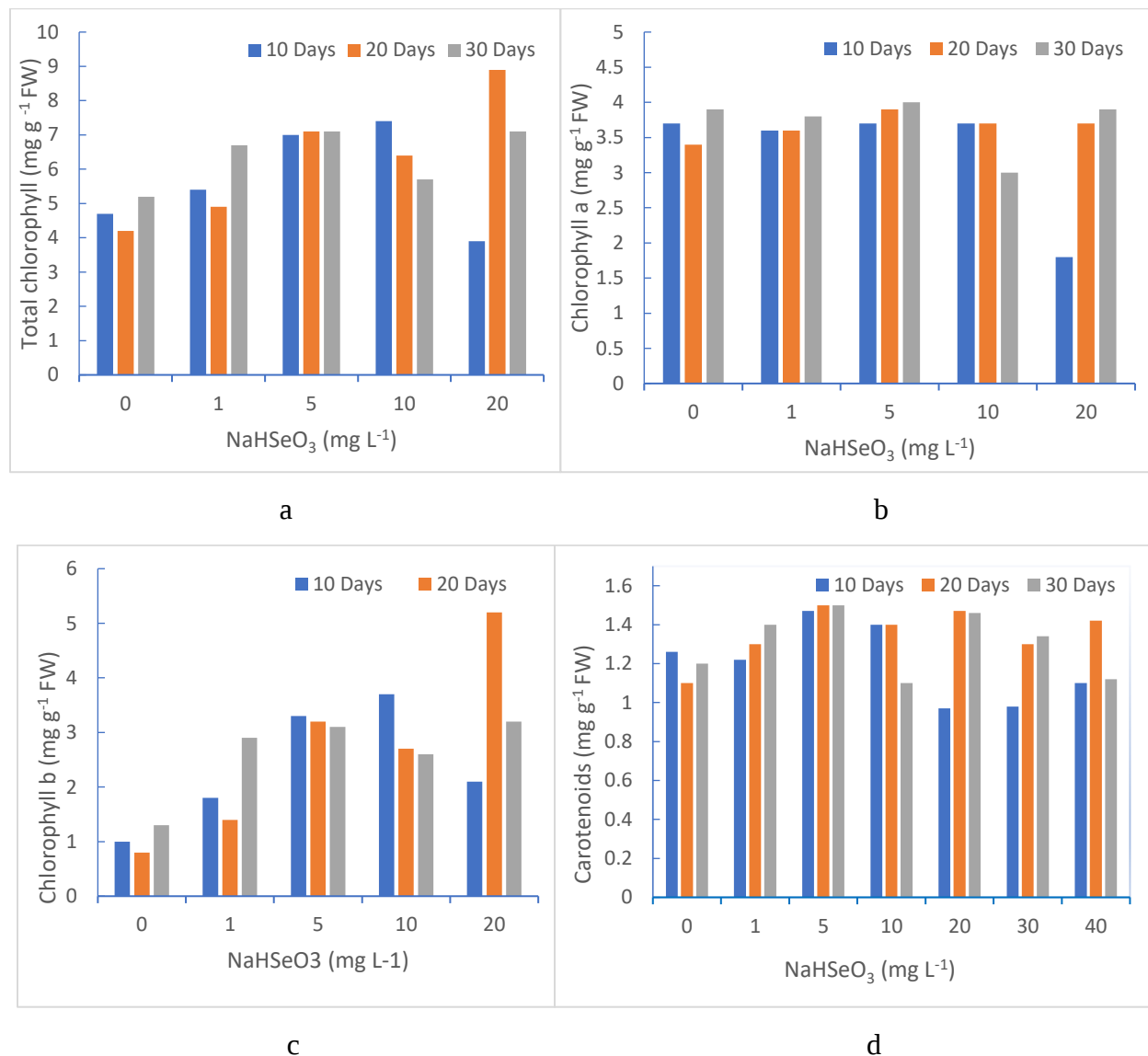
LSD (0.05) = 0.850

Chlorophyll a and b, total chlorophylls and carotenoid

Figure (2a) shows the results of the total chlorophyll of lettuce leaves treated with different concentrations of NaHSeO₃. The total chlorophyll content increased in plants treated with selenite compared to the control. The highest total chlorophyll content was recorded for the concentration of 20 mg L⁻¹ (8.9 mg g⁻¹) after 20 days of treatment and the concentration of 5 mg L⁻¹ (7.1 mg g⁻¹) after 20 and 30 days of treatment. While the concentration of 5 mg L recorded the highest content of chlorophyll A after 30 days of

treatment, compared to the control, with a non-significant difference between them figure (2b). Se led to a significant increase in the content of chlorophyll b (Fig. 2c) in all concentrations compared to the control, recording the highest content of 5.2 mg g⁻¹ after 20 days of treatment. The carotenoid content showed a significant increase at a concentration of 5 mg L⁻¹ in all treatment periods compared to the control, while the content decreased significantly at a concentration of 20 mg L⁻¹ (0.97 mg g⁻¹) and a concentration of 30 mg L⁻¹ (0.98 mg g⁻¹) on the 10th day of treatment as shown in figure (2d).

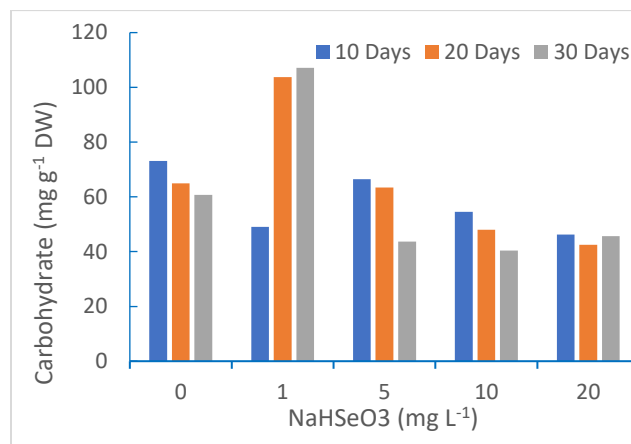
Figure 2: Effect of the different concentrations of NaHSeO₃ on (a) total chlorophyll content (LSD0.05= 1.233), (b) chlorophyll a (LSD0.05= 0.968), (c) chlorophyll b (LSD0.05= 1.240), and (d) carotenoids content (LSD0.05= 0.214), in lettuce plants at different periods of growth (10, 20 and 30) days. Results are averages of three replicates ($P \leq 0.05$).



Carbohydrates content

The content of carbohydrates significantly decreased with increasing selenite concentrations (figure 3) in all the studied periods of treatment except concentration 1 mg L⁻¹ at 20 and 30 days of treatment, which recorded a significant increase (103.8 and 107.1) mg g⁻¹.

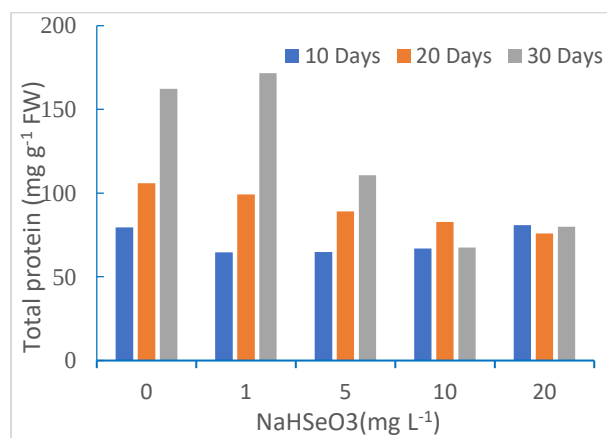
Figure 3: Effect of the different concentrations of NaHSeO₃ on carbohydrate content in lettuce plants at different periods of growth (10, 20 and 30) days. Results are averages of three replicates LSD(0.05) = 14.828.



Total protein

Figure 4 shows the effect of concentrations of selenium in the form of sodium selenite (NaHSeO₃) on the total protein content, as we notice a decrease in protein content in all treatments except for concentration 1 mg L⁻¹, where its content increased to 171.6 mg g⁻¹ after 30 days of treatment compared to control. (162.3 mg g⁻¹).

Figure 4: Effect of Se and growth stage at harvest on total protein. Data were evaluated by two-way ANOVA. A less significant difference LSD(0.05) = 14.451.



Discussion

Several studies showed that the effect of selenium on plants is different depending on the selenium concentration in the medium of growth and the plant's ability to accumulate selenium, (Boldrin et al. 2016; Yin et al. 2019; Alves et al. 2020).. Our study showed that the water content in the selenite concentrations was less than the control. Selenium leads to a decrease in plant water content compared to control (Hawrylak-Nowak 2013).

Our study agrees with the results of Skrypnik et al. (2021), where the value of chlorophyll a increased after 20-30 days of treatment than it was after 10 days of treatment, and also in the values of chlorophyll a were higher than the values of chlorophyll b, and the same study did not agree with the values of chlorophyll b, where the values were inverted, as for total chlorophyll, the results were different in some concentrations and agreed in other concentrations. Our study revealed that there is a stimulating effect of selenite on chlorophyll b values, especially at a concentration of 20 mg L⁻¹, and there was no stimulating effect of selenite on chlorophyll an except at a concentration of 5 mg L⁻¹, which was not significant, while the increase was stimulating for selenite in the total chlorophyll value at the concentrations are 5, 10 and 20 mg L⁻¹. In a study conducted by Zheng, Sun & Tang (2018) on lettuce, the highest value of chlorophyll was found at a concentration of 5 mg kg⁻¹, and this result is consistent with our results. Regarding carotenoids, selenite increased the content of carotenoids in most treatments compared to the control, especially the concentration of 5 mg L, which recorded a significant increase when compared to the control. The results of our study agree with Rao et al. (2022) when studying the comparative study between the effect of selenium nanoparticle and selenite on the soybean plant, where it was shown that an increase in selenite concentration led to an

increase in the carotenoid content, and it partially agrees with Zheng et al. (2018) that a concentration of 5 mg Se kg⁻¹ led to an increase in the carotenoid content.

From figure 3, we note that there is a decrease in carbohydrate content when using NaHSeO₃ at concentrations above 1 mg L⁻¹ compared to control, this indicates that low selenium concentrations lead to an increase in carbohydrate content. Increasing the concentration of selenium by more than 0.3 mg kg⁻¹ led to a decrease in the carbohydrate content of the potato plant *Solanum tuberosum* L. (Turakainen, Hartikainen & Seppänen 2004). In a comparative study between selenites and selenates conducted by Sharma et al. (2010) on rapeseed, it was found that selenite led to a decrease in carbohydrate content and total protein compared to the control.

Treating the plant with different concentrations of selenite resulted in a significant decrease in the total protein content in lettuce leaves, except for a concentration of 1 mg L⁻¹, where the difference was not significant. Many studies indicate a decrease in the protein content in the plant when selenium concentrations are used at concentrations higher than the normal limit that the plant can tolerate, a study by Hajiboland & Amjad (2007) on cabbage, Kohlrabi, and alfalfa plants using two selenium concentrations of 10 and 20 µmol reported lower protein content in all of these plants compared to control. In a study conducted by Gul et al. (2017) in the treatment of cornflower plant *Zea mays* exposed to salt stress with different concentrations of selenium, it was found that the total protein content increased with increasing selenium concentration, and this study does not agree with our results.

Reference

- Alves, L.R., Rossatto, D.R., Rossi, M.L., Martinelli, A.P. & Grato, P.L., 2020, 'Selenium improves photosynthesis and induces ultrastructural changes but does not alleviate cadmium-stress damages in tomato plants', *Protoplasma*, 257(2), 597–605.
- Bishop, M.C., & D.L.J.C.F.E. p. , and thirty three contributors, 1985, 'Clinical Chemistry Principles, Procedures and Correlations, 181-182.'
- Boldrin, P.F., Figueiredo, M.A. de, Yang, Y., Luo, H., Giri, S., Hart, J.J., Faquin, V., Guilherme, L.R.G., Thannhauser, T.W. & Li, L., 2016, 'Selenium promotes sulfur accumulation and plant growth in wheat (*Triticum aestivum*)', *Physiologia Plantarum*, 158(1), 80–91.
- DuBois, Michel., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, Fred., 1956, 'Colorimetric Method for Determination of Sugars and Related Substances', *Analytical Chemistry*, 28(3), 350–356.
- Gul, H., Kinza, S., Khan Shinwari, Z. & Hamayun, M., 2017, EFFECT OF SELENIUM ON THE BIOCHEMISTRY OF ZEA MAYS UNDER SALT STRESS, vol. 49.
- Hajiboland, R. & Amjad, L., 2007, 'Does antioxidant capacity of leaves play a role in growth response to selenium at different sulfur nutritional status?', *Plant, Soil and Environment*, 53(5), 207–215.
- Hawrylak-Nowak, B., 2013, 'Comparative effects of selenite and selenate on growth and selenium accumulation in lettuce plants under hydroponic conditions', *Plant Growth Regulation*, 70(2), 149–157.
- Lian, J., Liu, W., Meng, L., Wu, J., Chao, L., Zeb, A. & Sun, Y., 2021, 'Foliar-applied polystyrene nanoplastics (PSNPs) reduce

- the growth and nutritional quality of lettuce (*Lactuca sativa* L.)', *Environmental Pollution*, 280, 116978.
- 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(5), 591–592.
- Medina-Lozano, I., Bertolín, J.R. & Díaz, A., 2021, 'Nutritional value of commercial and traditional lettuce (*Lactuca sativa* L.) and wild relatives: Vitamin C and anthocyanin content', *Food Chemistry*, 359, 129864.
- MUNAR, A., ... A.A.-... A. and N. & 2020, undefined, no date, 'Utilization of Various Fish Dung on the Growth and Production of Lettuce (*Lactuca sativa* L.) in the Aquaponic System', *jurnal.umsu.ac.id*.
- Rao, S., Xiao, X., Wang, Y., Xiong, Y., Cheng, H., Li, L. & Cheng, S., 2022, 'Comparative study of the effects of selenium nanoparticles and selenite on selenium content and nutrient quality in soybean sprouts', *Folia Horticulturae*, 34(2), 223–234.
- Sali, A., Zeka, D., Fetahu, S., Rusinovci, I. & Kaul, H.-P., 2018, 'Selenium supply affects chlorophyll concentration and biomass production of maize (*Zea mays* L.)', *Die Bodenkultur: Journal of Land Management, Food and Environment*, 69(4), 249–255.
- Sela Saldinger, S., Rodov, V., Kenigsbuch, D. & Bar-Tal, A., 2023, 'Hydroponic Agriculture and Microbial Safety of Vegetables: Promises, Challenges, and Solutions', *Horticulturae*, 9(1), 51.
- Sharma, S., Bansal, A., Dhillon, S.K. & Dhillon, K.S., 2010, 'Comparative effects of selenate and selenite on growth and biochemical composition of rapeseed (*Brassica napus* L.)', *Plant and Soil*, 329(1–2), 339–348.
- Skrypnik, L., Styran, T., Savina, T. & Golubkina, N., 2021, 'Effect of Selenium Application and Growth Stage at Harvest on Hydrophilic and Lipophilic Antioxidants in Lamb's Lettuce (*Valerianella locusta* L. Laterr.)', *Plants*, 10(12), 2733.
- Terry, N., Zayed, A.M., Souza, M.P. de & Tarun, A.S., 2000, 'Selenium Application and Growth Stage at Harvest on Hydrophilic and Lipophilic Antioxidants in Lamb's Lettuce (*Valerianella locusta* L. Laterr.)', *Plants*, 10(12), 2733.
- Terry, N., Zayed, A.M., Souza, M.P. de & Tarun, A.S., 2000, 'Selenium Application and Growth Stage at Harvest on Hydrophilic and Lipophilic Antioxidants in Lamb's Lettuce (*Valerianella locusta* L. Laterr.)', *Plants*, 10(12), 2733.
- Turakainen, M., Hartikainen, H. & Seppänen, M.M., 2004, 'Effects of Selenium Treatments on Potato (*Solanum tuberosum* L.) Growth and Concentrations of Soluble Sugars and Starch', *Journal of Agricultural and Food Chemistry*, 52(17), 5378–5382.
- Yin, H., Qi, Z., Li, M., Ahammed, G.J., Chu, X. & Zhou, J., 2019, 'Selenium forms and methods of application differentially modulate plant growth, photosynthesis, stress tolerance, selenium content and speciation in *Oryza sativa* L.', *Ecotoxicology and Environmental Safety*, 169, 911–917.
- Zheng, S., Sun, G. & Tang, Y., 2018, 'Effects of Different Selenium Concentrations on Eco-Physiology of *Lactuca sativa*', *IOP Conference Series: Earth and Environmental Science*, 199, 032013.