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Impacts of Cobalt, Selenium and Silicon Biofortification on the Growth, Productivity and Nutritional Value of Lettuce

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RECENTLY, beneficial elements have received much attention because of their pivotal contribution in crop sustainability. The biofortification of plants with these elements is one of the brilliant strategies that provide these elements in the food chain for human consumption. Additionally, an unprecedented approach has been made to study the impact of the beneficial elements on plant growth, development, yield, and quality. Accordingly, this study was conducted to investigate the separate efficacy of the cobalt, selenium, and silicon on the biofortification of lettuce cv. Limor plants and scrutinize their potential impacts on growth and productivity during the 2020 and 2021 seasons. Ten foliar treatments, including cobalt sulphate at 100, 200 and 300 µM, sodium selenite at 10, 20 and 30 µM, and sodium silicate at 0.5, 1 and 2 mM, and distilled water as a control treatment. The experimental design was a completely randomized block with three replicates. Results clarified that the spraying of these beneficial elements significantly enhanced the vegetative growth parameters, mineral content, chlorophyll content, sugars and free amino acids as compared to the control. The treatments did not affect the content of nitrate and nitrite in the lettuce heads. The increases in head fresh weight ranged from 11.35 to 14.54% in the first season, and from 13.07 to 16.95% in the second season. In conclusion, supplying cobalt sulphate at 100 µM, sodium selenite at 20 µM or sodium silicate at 2 mM as foliar sprayings not only improves the vegetative growth and yield of lettuce plants but also enhances the nutritional quality of plants and fortifies the content of these elements in the lettuce heads.

Keywords: *Lactuca sativa*, Beneficial elements, Biofortification, Cobalt, Selenium, Silicon.

Introduction

Beneficial elements are mineral elements that are non-essential for plants but have a role in stimulating growth and have beneficial effects at very low concentrations despite not being required for plants to complete their life cycle (Piccolo et al., 2021, Nunes da Silva et al., 2022). Cobalt, selenium, and silicon are considered beneficial elements for plants (Pilon-Smits et al., 2009) and of fundamental importance to human health. However, these three elements are limiting in the diets of much of the world's population, especially in Africa, Asia, and South America. Improving the bioavailability of these elements in widely consumed food crops is an important strategy to overcome trace-element deficiencies in these crops and improve human health.

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Leafy vegetables are beneficial to human nutrition all over the world (Galić et al., 2021). Lettuce (*Lactuca sativa* L.) is a leafy vegetable greatly consumed fresh worldwide due to its high nutritional value for human health (da Cruz Ferreira et al., 2020). Therefore, fortifying lettuce with cobalt, selenium, and silicon contents is a brilliant strategy to supply these elements in the human food chain (Zhou et al., 2020, Zafeiriou et al., 2022).

Cobalt (Co) is one of the beneficial elements that exists in the forms of erythrite [$Co_3(AsO_4)_2$], cobaltite [CoAsS], and smaltite [$CoAs_2$] in the earth's crust (Nagajyoti et al., 2010). Despite not being considered a necessary element, Co is said to be good for plants (Komeda et al., 1997). The synthesis of vitamin B12, which is necessary for human and animal nutrition, requires cobalt as a crucial component (Srivastav et al., 2022). Cobalt encouraged a variety of developmental processes in higher plants, i.e., lengthening of the stem and coleoptile, opening of the hypocotyl, enlargement of the leaves, and formation of buds (Hu et al., 2021). Co is a constituent of numerous enzymes and co-enzymes, which impacts the development and metabolism of the plant since Co at low concentrations is favorable for plant growth. Still, high concentrations will have the opposite effect (Akeel and Jahan 2020). Excessive Co toxicity causes various issues, including reduced dry weight, chlorosis, premature leaf closure, leaf abscission, and decreased active transport (Mahey et al., 2020).

Selenium (Se) is one of the most researched beneficial plant elements and is frequently employed in plant biofortification projects because of its advantages for human health (Malagoli et al., 2015) in trace quantities (suggested dietary requirement level of 40 µg/day) but is toxic in increased levels (<800 µg/day) (Zhang et al., 2014, Siddiqui et al., 2021). Selenium can be found in many different forms, such as selenide, elemental selenium, selenite, selenate, dimethyl-selenide, selenoamino acids, and selenoproteins (Surai et al., 2018). Selenite and selenate are the bioavailable forms of selenium in soil. Since both ions show chemical similarities, phosphate transporters in plants are responsible for selenite uptake, while selenate is carried by sulphate transporters (Trippe and Pilon-Smits, 2021). Selenium helps plants grow their cell membranes, chloroplasts, and photosynthesis. It also functions as an antioxidant and regulates reactive oxygen species (ROS) (Pilon-Smits, 2015). Selenium can enhance plant performance and growth at lower concentrations, minimizing the potential negative impacts of abiotic stresses (Auobi Amirabad et al., 2020, Luís Oliveira Cunha and de Mello Prado, 2023).

The second most plentiful element in the crust of the earth is silicon (Si), hence, it is present in the rhizosphere in significant quantities (Vatansever et al., 2017). Si is typically recognized as a helpful element among plant micronutrients (Zhu and Gong, 2014). It promotes plant resilience to different biotic and abiotic stresses like cold, drought, salinity, temperature, and numerous bacterial and fungal infections while also enhancing canopy photosynthesis, reducing transpiration loss, and improving plant resistance to several biotic and abiotic stresses (Zhu and Gong, 2014, Salem et al., 2022, Sharifi et al., 2022). In addition, the capacity of plants to accumulate silicon is related to its positive benefits, which are more evident in high-accumulating plants and less prominent in low-Si accumulators (Ma, 2004). Monosilicic acid is the simplest form of soluble silicic acid that plants can absorb as a form of silicon (Ma and Yamaji, 2006). In addition, silicon is absorbed in and transported as silicic acid ($Si(OH)_4$), where it is transformed to hydrated amorphous silica and stocked on cell walls to create both silica cuticle and cellulose double layers on hull surfaces, leaves, and stems (Mitani et al., 2008). A favorable interaction between silicon and polyphenols and pectin promotes the strength and solidity of the cell wall (Zhang et al., 2015, Hussain et al., 2021). Also, silicon can fix nutrient imbalance in most plants (Gao et al. 2006, Romero-Aranda et al., 2006, Liang et al., 2007) and promote photosynthesis (Al-aghabary et al., 2004, Liang et al., 2007) subsequently, Si has a positive effect on the plants' growth. Also, under both favorable and unfavorable growing conditions, supplying plants with suitable quantities and forms of silicon can promote the plants' growth and development (Salem et al., 2022, Saudy et al., 2022).

Based on the previous literature, we hypothesized that the foliar application of different concentrations of cobalt, selenium, and silicon separately can fortify the heads of lettuce plants and promote the morphological and physiological development of the plants, enhancing the productivity and nutritional value of the produced heads. Therefore, the objective of this study was to investigate the impact of biofortification with foliar applications of cobalt, selenium, and silicon separately on the growth and productivity of lettuce plants and determine the appropriate concentration of each tested element.

Materials and Methods

Study site, cultivation, and experimental layout

A field experiment was carried out during the two growing seasons of 2020 and 2021 at the Experimental Vegetable Farm of the Horticulture Department (30° 06′ 46″ N, 31°14′ 37″ E), Faculty of Agriculture, Ain Shams University, Qalyubia Governorate, Egypt. The study investigates the effects of cobalt, selenium, and silicon on the growth, yield, and quality of lettuce plants. The physical and chemical properties of the experimental soil are shown in Table 1.

Lettuce (Lactuca sativa L.) cv. Limor seedlings were purchased from a commercial nursery. The transplants were cultivated on two sides of the rows at 25 cm between plants and 70 cm between rows. The plot area consisted of five rows of 5 m length. Transplants were planted on the 18th and 11th of October of the 2020 and 2021 growing seasons, respectively. The plants were irrigated using a furrow irrigation system.

Ten foliar-application treatments, including cobalt sulphate (CoSO4, MW 154.99) at concentrations of 100, 200, and 300 µM, sodium selenite (Na_2SeO_3, M.W. 172.94) at concentrations of 10, 20, and 30 µM, and sodium silicate (Na_2SiO_3, M.W. 122.063) at concentrations of 0.5, 1, and 2 mM plus distilled water, which served as a control treatment, were applied on lettuce plants. The treatments were applied as foliar applications twice, 21 and 28 days after transplanting. Early in the morning, foliar sprayings were conducted with a battery-powered backpack sprayer. To prevent interference from varying moisture levels, the control plants received an equal amount of distilled water sprayings. The volume of the spray was enough to completely cover the entire plant foliage.

The treatments were arranged in a completely randomized block design with three replicates. Each experimental plot area was 17.5 m^2.

In both growing seasons, all agricultural practices were applied as commonly recommended for commercial lettuce production in the district, according to the recommendations of the Egyptian Ministry of Agriculture.

Data recorded

Randomly, five plants were taken from the three inner rows of each experimental plot at harvest (60 days after transplanting). These plants were trimmed according to market standards to record all measured data.

Vegetative growth parameters

In the laboratory, the head diameter was measured using a caliper, and the number of leaves per head was counted. Additionally, the following equation was used by Koller (1972) to determine the average leaf area as a relationship between the area unit and the fresh leaf weight.

$$\text{Leaf area} = \frac{\text{Disk area x number of disks x fresh weight of leaves}}{\text{Fresh weight of disks}}$$

In addition, the plants were weighed to determine the fresh weight of the head before being dried at 70°C until a constant weight to determine the dry weight of the head.

Mineral content of leaves

At the Central Laboratory, Soil and Water Unit, Faculty of Agriculture, Ain Shams University, the Kjeldahl method was used to calculate total nitrogen as described by Chapman and Pratt (1982). Phosphorous, potassium, calcium, magnesium, sulfur, cobalt, selenium, and silicon were assayed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Varian Inc., Vista MPX) as described by Rodushkin et al. (1999).

TABLE 1. Soil physical and chemical properties of the 0–30 cm layer in the experimental soil before lettuce cultivation

Sand (%)	Silt (%)	Clay (%)	Texture	pH	Ca^{++} (meq/l)	Mg^{++} (meq/l)	K^+ (meq/l)	Na^+ (meq/l)	Cl^- (meq/l)	CO_3^{--} (meq/l)	HCO_3^- (meq/l)	Si^+ (mg/kg)	Se^- (mg/kg)
23.6	35.8	40.6	Clay	7.37	0.60	1.77	1.14	0.55	0.60	0	0.29	1.478	1.624

Data shown as averages for the two growing seasons

Chlorophyll content

The chlorophyll content in leaves (Chl *a* and Chl *b*) was assayed as described by Costache et al. (2012) with some modifications. A sample of 0.5 g of fresh leaves in small pieces was immersed in 10 ml of pure acetone for 24 hours at 4°C in dark bottles to prevent pigment degradation. After 15 minutes of centrifugation at 4000 rpm with the homogenate, the absorbance was recorded at 645 and 662 nm. The following equations were used:

Chl *a* (mg/g FW) = 11.75 A662 – 2.350 A645 x (V/1000xW)

Chl *b* (mg/g FW) = 18.61 A645 – 3.960 A662 x (V/1000xW)

Where A is the absorbance at 645 and 662 nm, V is the final volume of Chl extract in pure acetone, and W is the fresh weight of tissue extract. Additionally, Chl *a+b* was calculated.

Soluble sugars and free amino acids

The phenol-sulfuric acid method was used as described by Chow and Landhäusser (2004) to quantify total soluble sugars.

The ninhydrin method was used to determine the free amino acids (Sui et al., 2017). The ninhydrin reagent reacts with the amino acids in the samples to form a purple color, and the free amino acids were estimated colorimetrically at 570 nm of absorbance.

Nitrate and nitrite content

Nitrates and nitrites were determined according to Woolley et al. (1960). The method involved the reduction of nitrate to nitrite with cadmium and the subsequent diazotization of nitrite with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride. The absorbance was measured at 540 nm.

Statistical analysis

Data were subjected to a statistical analysis of variance procedure using the CoStat package's one-way ANOVA method (Microcomputer Program Analysis, Version 6.303, CoHort Software, CA, USA). The significant differences between the means of the treatments were compared using Duncan's multiple range test at a 5% level of probability (Waller and Duncan 1969). Principal component analysis (PCA) was also performed using the JMP Pro software.

Results

Vegetative growth parameters

In both growing seasons, it was clear that spraying lettuce plants with cobalt at 200 µM, selenium at 20 µM, or silicon at 2 mM showed the highest significant values of head diameter, while foliar application of silicon at 2 mM gave the highest number of leaves per head (Table 2). In addition, Table 2 shows that the impact of these beneficial elements on total leaf area was more pronounced compared with the control. Cobalt at 100 µM, selenium at 10 µM, or silicon at 1 or 2 mM revealed the highest significant values of total leaf area.

As shown in Table 3, all foliar applications with the three elements increased both fresh and

TABLE 2. Effect of cobalt, selenium and silicon foliar spraying on head diameter, number of leaves/head and leaf area of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	Head diameter (cm)		Number of leaves/head		Total leaf area (cm^2)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	10.91±0.46c	9.55±0.39d	15.67±0.48c	13.67±0.35c	1359.33±33.50d	1458.87±35.74e
Co 100 µM	11.10±0.91c	11.5±0.43ab	17.67±0.75a	14.67±1.02abc	1621.78±13.18a	1738.33±6.03a
Co 200 µM	12.32±0.54a	11.15±0.43abc	16.33±0.84abc	14.00±1.00c	1494.13±73.17b	1705.00±24.00ab
Co 300 µM	11.40±0.30c	10.58±0.52bc	16±1.02bc	13.67±0.66c	1450.67±36.50bc	1581.43±2.46cd
Se 10 µM	11.00±0.57c	10.19±0.43cd	16.67±0.66abc	15.33±1.20ab	1676.81±12.74a	1770.67±15.50a
Se 20 µM	12.02±0.77ab	11.14±0.39abc	17±1.30abc	14.67±0.21abc	1499.87±41.53b	1643.69±29.46bc
Se 30 µM	11.01±0.74bc	10.56±0.97bc	16.33±0.78abc	14.33±0.45bc	1369.45±24.01d	1474.17±15.77e
Si 0.5 mM	11.23±0.56bc	10.77±0.17bc	17.33±0.62ab	14.67±0.94abc	1408.67±84.69cd	1538.07±87.37de
Si 1 mM	11.13±0.76c	10.81±0.42bc	17.33±1.15ab	14.67±0.84abc	1663.76±22.37a	1793.22±91.93a
Si 2 mM	12.51±0.45a	11.94±0.78a	17.67±0.86a	15.67±0.67a	1686.67±15.28a	1779.67±45.00a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

TABLE 3. Effect of cobalt, selenium and silicon foliar spraying on head fresh and dry weights of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	Head fresh weight (g)		Head dry weight (g)	
	1st season	2nd season	1st season	2nd season
Control	519.67±5.51e	544.67±12.06g	26.50±0.28f	31.05±0.69f
Co 100 µM	578.67±4.04abc	637.00±23.51a	29.51±0.21c	35.61±1.29bc
Co 200 µM	545.33±12.66de	598.67±10.50cd	27.98±0.65de	33.53±0.59e
Co 300 µM	521.33±11.24e	561.00±4.58fg	27.47±0.60ef	31.81±0.26f
Se 10 µM	538.33±10.02de	612.00±11.20bc	30.04±0.56bc	33.29±0.60e
Se 20 µM	595.33±10.21ab	624.67±5.13ab	31.20±0.54ab	36.61±0.30ab
Se 30 µM	528.67±31.53e	575.67±12.50ef	30.61±1.83abc	33.39±0.73e
Si 0.5 mM	563.33±10.26cd	586.00±14.73de	29.41±0.54cd	34.40±0.86de
Si 1 mM	575.33±15.04bc	609.00±11.53bc	29.17±0.76cd	34.83±0.66cd
Si 2 mM	603.33±14.50a	632.33±13.32a	31.61±0.76a	36.99±0.78a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

dry weights of heads compared with the control plants in both growing seasons. The highest significant fresh weights of heads were obtained from spraying with cobalt at 100 µM, selenium at 20 µM, or silicon at 2 mM without significant differences between them, while selenium at 20 µM, or silicon at 2 mM gave the highest significant values of dry weights of heads in both seasons. The increases in head fresh weight ranged from 11.35 to 14.54% in the first season, and from 13.07 to 16.95% in the second season for the aforementioned superior treatments.

Mineral content of leaves

Data in Tables 4, and 5 exhibit that the cobalt, selenium, or silicon foliar applications improved the N, P, Mg, and Ca percentages in the lettuce leaves as compared with the control treatment in both seasons. Cobalt at 100 µM, selenium at 20 µM, or silicon at 2 mM gave the highest nitrogen content without significant differences, while cobalt at 100 µM, selenium at 10 µM, or silicon at 2 mM gave the highest significant values of phosphorus content. In the same manner, spraying lettuce plants with selenium at 20 µM or silicon at 2 mM resulted in significant increases in leaf magnesium content, while supplementing with silicon at 1 or 2 mM significantly improved calcium content in the leaves. In addition, it was found that selenium at any concentration significantly improved sulfur content in both seasons. Meanwhile, foliar treatments did not affect the potassium content in the leaves.

Data in Table 6 show that spraying lettuce plants with cobalt, selenium, or silicon resulted in significant accumulations of the same sprayed element in the leaves in both growing seasons. Increasing the applied element resulted in increasing the leaf content of that element.

Chlorophyll content

Data presented in Table 7 show that foliar applications of cobalt, selenium, or silicon enhanced chlorophyll *a* and total chlorophyll as compared to the control treatment in both seasons. However, there was no considerable effect of all foliar treatments on chlorophyll *b* content in the leaves. The highest significant values of the aforementioned attributes were obtained with cobalt at 100 µM, selenium at 20 µM, or silicon at 2 mM in both growing seasons. On the contrary, the control treatment showed the lowest significant values in both growing seasons.

Soluble sugars and free amino acids

As shown in Table 8, spraying lettuce plants with cobalt, selenium, or silicon improved the content of total soluble sugars and amino acids in the leaves in both seasons. Cobalt at 100 µM, selenium at 20 µM, or silicon at 2 mM significantly increased the total soluble sugars and free amino acids, as well as cobalt at 200 µM for free amino acids in both growing seasons.

Nitrate and nitrite content

There were insignificant variations between all foliar applications of these beneficial elements

TABLE 4. Effect of cobalt, selenium and silicon foliar spraying on nitrogen, phosphorus, and potassium contents of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	N (%)		P (%)		K (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.73±0.03d	2.87±0.05d	0.29±0.01d	0.34±0.06d	2.96±0.14a	2.44±0.15a
Co 100 µM	2.12±0.02a	3.15±0.09a	0.34±0.03abc	0.39±0.05ab	2.91±0.06a	2.41±0.12a
Co 200 µM	1.99±0.06bc	3.15±0.05ab	0.36±0.05ab	0.38±0.02bc	2.87±0.04a	2.42±0.11a
Co 300 µM	1.90±0.06c	2.93±0.12cd	0.32±0.02cd	0.37±0.04c	2.85±0.02a	2.45±0.09a
Se 10 µM	2.00±0.08bc	3.08±0.13abc	0.34±0.03abc	0.40±0.09a	2.96±0.08a	2.31±0.15a
Se 20 µM	2.05±0.01ab	3.18±0.07a	0.35±0.08ab	0.39±0.07bc	2.97±0.08a	2.39±0.16a
Se 30 µM	1.95±0.14bc	2.95±0.05bcd	0.32±0.01bc	0.38±0.02bc	2.85±0.10a	2.42±0.11a
Si 0.5 mM	1.95±0.12bc	2.87±0.12d	0.35±0.05abc	0.37±0.05c	2.82±0.06a	2.37±0.07a
Si 1 mM	1.90±0.05c	2.97±0.06bcd	0.36±0.10ab	0.38±0.07bc	2.98±0.10a	2.43±0.16a
Si 2 mM	2.00±0.06ab	3.20±0.10a	0.37±0.04a	0.40±0.10a	3.04±0.03a	2.39±0.14a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

TABLE 5. Effect of cobalt, selenium and silicon foliar spraying on magnesium, calcium, and sulphur contents of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	Mg (%)		Ca (%)		S (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	0.27±0.03d	0.17±0.08e	0.31±0.06d	0.16±0.06c	0.21±0.08d	0.30±0.07c
Co 100 µM	0.31±0.05abc	0.20±0.01cd	0.37±0.03a	0.23±0.03bc	0.22±0.09c	0.30±0.02c
Co 200 µM	0.30±0.08bc	0.20±0.04d	0.35±0.08ab	0.19±0.04bc	0.23±0.11c	0.31±0.06c
Co 300 µM	0.30±0.02bc	0.18±0.05e	0.32±0.02cd	0.21±0.03bc	0.20±0.04e	0.31±0.01c
Se 10 µM	0.29±0.04cd	0.22±0.09c	0.36±0.03ab	0.22±0.05bc	0.25±0.04ab	0.37±0.05a
Se 20 µM	0.32±0.01a	0.27±0.01a	0.36±0.02ab	0.26±0.05b	0.25±0.09a	0.37±0.04a
Se 30 µM	0.29±0.09cd	0.18±0.04e	0.34±0.07bc	0.16±0.04c	0.26±0.06a	0.35±0.03ab
Si 0.5 mM	0.30±0.02bc	0.20±0.07d	0.32±0.02cd	0.22±0.03bc	0.23±0.05c	0.31±0.05c
Si 1 mM	0.30±0.01bc	0.24±0.06b	0.36±0.05ab	0.34±0.11a	0.24±0.10b	0.32±0.02bc
Si 2 mM	0.32±0.07ab	0.28±0.05a	0.37±0.10a	0.38±0.05a	0.25±0.06ab	0.31±0.09bc

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

TABLE 6. Effect of cobalt, selenium and silicon foliar spraying on cobalt, selenium, and silicon contents of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	Co (ppm)*		Se (ppm)**		Si (ppm)***	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	3.246±0.027c	3.845±0.032c	0.1877±0.0190de	0.1141±0.0181cd	0.1563±0.0099c	0.132±0.0105de
Co 100 µM	3.900±0.100b	4.202±0.030b	0.1454±0.0127f	0.1331±0.0210abc	0.1763±0.0049bc	0.136±0.0040cde
Co 200 µM	3.967±0.153b	4.362±0.096a	0.1072±0.0208g	0.1277±0.0084bc	0.1687±0.0130bc	0.1373±0.0015bcde
Co 300 µM	4.273±0.241a	4.426±0.091a	0.1751±0.0141ef	0.1355±0.0045abc	0.1727±0.0080bc	0.1367±0.0006bcde
Se 10 µM	3.252±0.035c	4.069±0.083b	0.2617±0.0213b	0.1395±0.0127ab	0.1700±0.0125bc	0.1290±0.0026e
Se 20 µM	3.189±0.073c	4.085±0.068b	0.2686±0.0172b	0.1521±0.0048a	0.1727±0.0188bc	0.1313±0.0015de
Se 30 µM	3.247±0.014c	4.109±0.091b	0.3167±0.0347a	0.1586±0.0144a	0.1707±0.0107bc	0.1403±0.0023bcd
Si 0.5 mM	3.253±0.035c	4.202±0.030b	0.2384±0.0204bc	0.0966±0.0142d	0.1733±0.0058bc	0.1427±0.0057abc
Si 1 mM	3.209±0.059c	4.144±0.126b	0.2184±0.0181cd	0.0938±0.0092d	0.1783±0.0038ab	0.1453±0.0051ab
Si 2 mM	3.210±0.032c	4.069±0.081b	0.2031±0.0165de	0.1124±0.0203bcd	0.1897±0.0045a	0.1493±0.0021a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test
*Recommended dietary allowance (RDA) for cobalt: 5-8 mg/day ** Recommended dietary allowance (RDA) for selenium: ~ 55µg/day *** Recommended dietary allowance (RDA) for silicon: 10-25 mg/day

TABLE 7. Effect of cobalt, selenium and silicon foliar spraying on chlorophyll a, b and total chlorophyll in leaves of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	Chl *a* (mg g^{-1} FW)		Chl *b* (mg g^{-1} FW)		Chl *a+b* (mg g^{-1} FW)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.30±0.03c	1.39±0.01e	1.01±0.06a	1.00±0.08a	2.31±0.07d	2.39±0.09d
Co 100 µM	1.51±0.03a	1.57±0.05ab	1.03±0.07a	1.06±0.04a	2.54±0.10ab	2.63±0.04abc
Co 200 µM	1.48±0.02ab	1.54±0.08abc	1.14±0.04a	1.03±0.03a	2.62±0.02a	2.57±0.10bcd
Co 300 µM	1.45±0.02b	1.47±0.03bcde	0.99±0.03a	1.05±0.14a	2.44±0.03bc	2.52±0.15bcd
Se 10 µM	1.32±0.03c	1.39±0.07de	1.04±0.11a	1.22±0.12a	2.36±0.11cd	2.61±0.05abc
Se 20 µM	1.48±0.04ab	1.54±0.12abc	1.07±0.05a	1.11±0.17a	2.55±0.09ab	2.65±0.06abc
Se 30 µM	1.46±0.06b	1.51±0.09abcd	0.99±0.07a	0.89±0.08a	2.45±0.02bc	2.40±0.11d
Si 0.5 mM	1.46±0.02b	1.44±0.06cde	0.99±0.06a	1.03±0.11a	2.45±0.04bc	2.47±0.13cd
Si 1 mM	1.45±0.01b	1.60±0.05a	1.06±0.09a	1.10±0.06a	2.51±0.09b	2.69±0.09ab
Si 2 mM	1.47±0.05ab	1.62±0.04a	1.05±0.04a	1.16±0.11a	2.53±0.08ab	2.78±0.13a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different (P ≤ 0.05) according to Duncan's multiple range test

TABLE 8. Effect of cobalt, selenium and silicon foliar spraying on total sugars and free amino acids in leaves of lettuce cv. Limor in 2020 and 2021 seasons

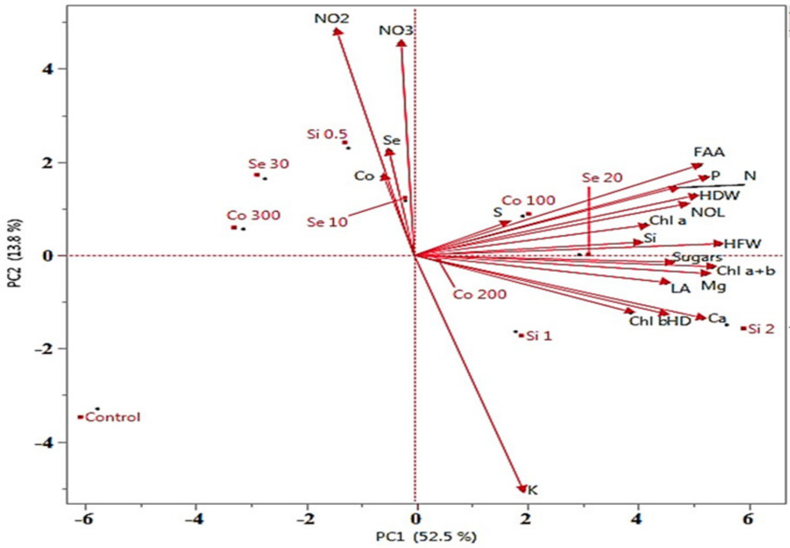
Treatments	Total sugars (mg g^{-1} FW)		Free amino acids (µg g^{-1} FW)	
	1st season	2nd season	1st season	2nd season
Control	2.75±0.05c	2.84±0.12c	191.33±15.36d	213.17±13.43d
Co 100 µM	3.01±0.14ab	3.13±0.06ab	296.96±12.13a	276.40±12.64abc
Co 200 µM	3.00±0.04ab	3.09±0.18b	275.63±9.69ab	268.67±29.75abc
Co 300 µM	2.91±0.08bc	2.87±0.15c	244.07±8.83c	264.53±7.30abc
Se 10 µM	2.91±0.24bc	3.00±0.11bc	283.70±13.6a	262.60±23.52bc
Se 20 µM	3.10±0.08ab	3.31±0.07a	277.91±25.56a	280.80±14.22ab
Se 30 µM	2.91±0.06bc	2.85±0.11c	246.37±10.34c	248.37±5.81c
Si 0.5 mM	3.04±0.08ab	3.04±0.06bc	242.80±7.44c	263.80±6.54abc
Si 1 mM	2.98±0.11ab	3.05±0.06bc	254.19±14.28bc	266.17±16.18abc
Si 2 mM	3.13±0.05a	3.12±0.13ab	295.38±9.06a	294.70±12.71a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different (P ≤ 0.05) according to Duncan's multiple range test

TABLE 9. Effect of cobalt, selenium and silicon foliar spraying on nitrate and nitrite contents in leaves of lettuce cv. Limor in 2020 and 2021 seasons

Treatments	Nitrate content (mg kg^{-1} FW)		Nitrite content (mg kg^{-1} FW)	
	1st season	2nd season	1st season	2nd season
Control	149.00±3.02a	154.67±2.31a	0.149±0.001a	0.150±0.002a
Co 100 µM	158.33±8.39a	160.33±12.66a	0.151±0.005a	0.154±0.007a
Co 200 µM	153.33±3.21a	160.00±9.85a	0.152±0.001a	0.152±0.007a
Co 300 µM	150.67±3.51a	157.33±9.07a	0.148±0.003a	0.165±0.022a
Se 10 µM	159.00±3.61a	160.67±9.02a	0.152±0.002a	0.154±0.004a
Se 20 µM	155.67±1.15a	152.33±2.31a	0.154±0.002a	0.152±0.003a
Se 30 µM	153.00±1.14a	158.67±5.13a	0.155±0.003a	0.156±0.004a
Si 0.5 mM	159.67±7.23a	166.33±9.61a	0.154±0.004a	0.157±0.004a
Si 1 mM	152.33±6.66a	156.00±2.01a	0.150±0.001a	0.152±0.005a
Si 2 mM	150.67±4.04a	153.00±5.29a	0.152±0.002a	0.150±0.005a

Values are means±SE of three replicates. Means within a column followed by the same letter are not significantly different (P ≤ 0.05) according to Duncan's multiple range test



Number	Eigenvalue	Percent
1	11.0257	52.503
2	2.8967	13.794
3	2.6598	12.666
4	1.5657	7.456
5	1.0613	5.054
6	0.9565	4.555
7	0.3628	1.728
8	0.2997	1.427
9	0.1719	0.818

Fig. 1. Principal component analysis (PCA) based on correlation matrix of effect of foliar applications of cobalt, selenium and silicon on lettuce vegetative growth characters, mineral analysis of leaves and biochemical constituents of both seasons. Abbreviations: HD head diameter, NOL number of leaves/head, LA leaf area, HFW head fresh weight, HDW head dry weight, N nitrogen content, P phosphorus content, K potassium content, Mg magnesium content, Ca calcium content, S Sulphur content, Co cobalt content, Se selenium content, Si silicon content, Chl a chlorophyll a, Chl b chlorophyll b, Chl a+b total chlorophyll, FAA free amino acids, NO3 nitrate content, NO2 nitrite content, Co 100 cobalt at 100 µM, Co 200 cobalt at 100 µM, Co 300 cobalt at 300 µM, Se 10 selenium at 10 µM, Se 20 selenium at 20 µM, Se 30 selenium at 30 µM, Si 0.5 silicon at 0.5 mM, Si 1 silicon at 1 mM, and Si 2 silicon at 2 mM.

and control treatment for nitrate and nitrite content in the leaves in both growing seasons (Table 9).

Principal component analysis (PCA)

Principal component analysis was conducted to assess the effect of cobalt, selenium, or silicon sprayings on the vegetative growth characteristics, mineral analysis of leaves, chlorophyll content, soluble sugars, free amino acids, nitrate, and nitrite content. PCA results based on the correlation matrix are presented in Figure 1. Each variable is illustrated by an arrow, and the longer its length, the greater its contribution to a given component. The angle formed by the arrows indicates the degree of correlation between variables, the smaller the angle, the greater the correlation. It was found that the first two principal components accounted for 52.5 and 13.8% of the variations for PC1 and PC2, respectively. The cumulative proportion of the variation approached 66.3% of the total variance. Most of the examined traits were discriminated by PC1, and thus explained by the larger proportion of variance (52.5%). It is clearly noted that all evaluated traits, except for potassium, cobalt, selenium, nitrate, and nitrite contents in the leaves, were positively associated with cobalt of 100 and 200 µM, selenium of 20 µM, and silicon of 1 and 2 treatments. The control treatment was positioned on the negative range which means that it gave the lowest values of all tested parameters compared with the foliar applications of the used elements.

Discussion

In sustainable agriculture, the application of beneficial elements has been increasing as an environmentally friendly technique to enhance plant growth and development in many plants under both favorable and unfavorable conditions (Vatansever et al., 2017, Piccolo et al., 2021, Khan et al., 2023). The current study generally showed that the foliar spraying of cobalt, selenium, and silicon separately on lettuce plants cv. Limor led to significant enhancements in plant growth and productivity.

Cobalt plays an important role in plant growth and development at low concentrations, but it disrupts many physiological, biochemical, and metabolic processes at higher concentrations

(Akeel and Jahan, 2020, Hu et al., 2021). In our study, lettuce plants were enriched with cobalt *via* foliar spraying since the foliar application of cobalt was more efficient than soil application (Abreu-Junior et al., 2023). The current study revealed that the foliar application of cobalt at 100 μM resulted in significant increases in leaf area, head fresh weight, leaf content of nitrogen and phosphorus, chlorophyll content, total soluble sugars, and free amino acids as compared to the control plants in both seasons. Cobalt at 100 μM increased the head yield by 11.35 and 16.95% in the first and the second seasons, respectively. Moreover, cobalt at 300 μM fortified the lettuce leaves with cobalt by an increase of 32 and 15% the first and second seasons, respectively. In this respect, cobalt treatments significantly stimulated vegetative growth in parsley (Helmy and Gad, 2002), broccoli (Gad and Abdel-Moez, 2011), and spinach (Gad and El-Bassuny, 2019). These enhancements may be due to the role of cobalt, which inhibits the action of ethylene and increases the efficiency of growth-promoting hormones such as auxins, gibberellins, and cytokines (Brengi et al., 2022). Also, cobalt is an important component of several enzymes and co-enzymes (Palit et al., 1994) and vitamin B12 (Ma et al., 2021) which are essential for healthy growth and development in plants. Zhang et al. (2021) found that an appropriate cobalt dose stimulated root growth, consequently enhanced nutrient uptake. Cobalt significantly increased leaf content of nitrogen, phosphorus, and cobalt in broccoli (Gad and Abdel-Moez, 2011), and spinach (Gad and El-Bassuny, 2019). In this regard, it is important to point out that the cobalt values in lettuce leaves obtained in this study (~ 3 ppm) are safe for human nutrition, since Young (1983) found that the daily cobalt requirements for human nutrition could reach 8 ppm without health hazards. In addition, previous studies demonstrated that the exogenous application of cobalt caused significant accumulations of total soluble sugars and total proteins in tomato fruits (Gad and Kandil, 2010) and the sugar beet roots (Gad and Ismail, 2011). Moreover, cobalt plays important roles in activating antioxidative enzymes, substituting active metals, and improving tolerance to abiotic stresses (Hu et al., 2021). In addition, it has been found that cobalt application on sweet potato plants increased L-ascorbic acid, which plays an essential role as an antioxidant in plant cells and also acts as a co-enzyme in metabolic changes and is necessary for photosynthesis and respiration processes (Franceschi and Tarlyn, 2002).

Selenium, being an essential micronutrient, enhances plant growth and development in trace amounts (Khan et al., 2023). Because of its simplicity and preferable outcomes, foliar application of selenium appears to be the most popular method (Danso et al., 2023). In this study, the effect of foliar application at different concentrations of selenium on the growth and nutritional quality of lettuce plants was investigated. The obtained results revealed that spraying lettuce plants with selenium at 20 μM significantly increased head diameter, fresh and dry weights of heads, leaf content of nitrogen and magnesium, chlorophyll content, total soluble sugars, and free amino acids compared with the control treatment in both growing seasons. It is worth noting that selenium at 20 μM increased the head yield by 14.56 and 14.69% in the first and second seasons, respectively. Similarly, Tufail et al. (2023) foliar-applied Se significantly increased fresh biomass in lettuce. Moreover, the growth-promoting response to foliar application of selenium was demonstrated in some leafy crops, including chicory (Germ et al. 2007), cilantro (Kopsell et al., 2009), pak choi (Li et al., 2015), celery (Li et al., 2020), and cabbage (Yang et al., 2022, Antoshkina et al., 2023, Yu et al., 2023). Also, the increases in nutrient contents obtained in the current study are in harmony with the previous studies for many crops (Wen et al., 2022). Furthermore, the current study demonstrated that foliar application of selenium at 30 μM significantly raised the selenium concentration in the leaves by 69 and 39% in the first and second seasons, respectively. These results are in agreement with Šindelářová et al. (2015) who stated that foliar application of selenium can increase its concentration in the edible parts of plants. Likewise, the obtained results revealed that the concentrations of chlorophyll were significantly increased by exogenous selenium application. Germ et al. (2005) suggested that selenium could stimulate respiration rates and the flow of electrons in the respiratory chain, which further accelerates chlorophyll biosynthesis. Shah et al. (2022) stated that selenium supplementation improves growth and photosynthesis by modulating the antioxidant system and gene expression of chlorophyll synthase and protochlorophyllide oxidoreductase. In this study, the increases in total soluble sugars may be attributed to the fact that selenite could modulate the activity of sucrose metabolism enzymes and affect the synthesis of soluble sugar (Ren et al., 2021). Moreover, selenium application significantly enhanced the free amino acids in lettuce leaves compared to the untreated plants. Similar results were obtained by Hu et al. (2001). Selenium can affect amino acid biosynthesis by enhancing nitrogen accumulation in plants (Puccinelli et al., 2020).

Using silicon as a foliar application to improve plant growth and increase crop productivity has been increasing. It is well documented that silicon-deprived plants are weaker than silicon-enriched plants, demonstrating reduced growth, development, and these plants are also more susceptible to biotic and abiotic stresses (Laane, 2018) since silicon plays an essential role in plant growth by regulating physiological and biochemical processes (Souri et al., 2021). In our study, lettuce plants were foliarly applied with silicon since the foliar application of silicon was more efficient than soil application (Savvas and Ntatsi, 2015). The effect of different concentrations of silicon on the growth, and nutritional quality of lettuce plants was evaluated. The current study demonstrated that foliar spraying of 2 mM significantly increased all evaluated vegetative growth parameters in lettuce plants. In addition, the same concentrations resulted in significant increments in leaf mineral content of nitrogen, phosphorus, magnesium, calcium, and silicon, chlorophyll content, total soluble sugars, and free amino acids compared with the control in both tested seasons. Noteworthy, selenium at 2 mM increased the head yield by 16.10 and 16.09% in the first and the second seasons, respectively. Moreover, foliar application of silicon at 2 mM significantly raised the selenium concentration in the leaves by 21 and 13% in the first and second seasons, respectively. The beneficial effects of foliar silicon application on plant growth have been reported for several leafy vegetables, including chard and kale (De Souza et al., 2019), pakchoi (Wang et al., 2020), oak leaf lettuce (Othman et al., 2021), land cress and chicory (Garcia Neto et al., 2022), and flowering Chinese cabbage (Liu et al., 2022). The increases in the vegetative growth, chlorophyll content, and mineral content parameters can be attributed to the role of silicon in improving the transpiration and stomatal conductance, photosynthetic efficiency, net CO_2 assimilation rate, antioxidant capacity, and plant cell water (Pavlovic et al., 2021). Also, silicon improved the uptake of various nutrients (Liang et al., 2007, Yassen et al., 2017), resulting in strong plants that, in turn, improved the effectiveness of photosynthesis and helped in maintaining the mineral balance and water conservation of plants (Zhu and Gong, 2014). Furthermore, silicon contributes in forming a mechanical barrier (cuticle-silica double layer) that protects the plant from pests (Teixeira et al., 2017). Also, silicon makes the plant leaves rougher in texture, hence, it increases the leaf rigidity (Ouzounidou et al., 2016) and delays leaf senescence by elevating the chlorophyll content and RuBisCo activity (Ma and Yamaji, 2006). Silicon may increase plant growth by enhancing leaf erectness, thereby increasing light interception and concurrently canopy photosynthesis process (Gong et al., 2003). Moreover, our results agree with D'Imperio et al. (2016) who found that silicon applying resulted in silicon accumulations in purslane, chicory, and Swiss chard, while silicon did not influence the nitrate content in these vegetables.

Conclusion

It can be concluded that using beneficial elements such as cobalt, selenium, and silicon as foliar applications on lettuce plants cv. Limor can improve the vegetative growth, yield, quality, and nutritional value of lettuce heads. Providing cobalt at 100 µM, selenium at 20 µM or silicon at 2 mM as foliar sprayings not only improves the vegetative growth and yield of lettuce plants but also raises the nutritional quality of the heads by fortifying the content of these elements. Furthermore, farmers can increase the productivity of lettuce cv. Limor by 14.15, 14.62, and 16.09 % with cobalt at 100 µM, selenium at 20 µM and silicon at 2 mM, respectively, as compared with the control treatment. Further work needs to be done to investigate the combined effects of these elements on the growth and productivity of lettuce.

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Conflict of interest

The authors declare that they have no competing interests.

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تأثير التعزيز الحيوي بالكوبلت والسيلينيوم والسيليكون على النمو والإنتاجية والقيمة الغذائية للخس

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في الآونة الأخيرة، نجد أن العناصر المفيدة حاذت على الكثير من الإهتمام وذلك لدروها المحوري والهام في مساهمتها في استدامة المحاصيل. ان التعزيز الحيوي للنباتات بهذه العناصر هو احد الاستراتيجيات البارعة لامداد هذه العناصر خلال سلسلة الغذاء للاستهلاك الأدمي. قد تم استخدام نهج غير مسبوق لدراسة تأثير هذه العناصر على نمو، تطور، المحصول وجودته لهذه النباتات. وفقاً لذلك، أجريت هذه الدراسة لدراسة التأثير والفعالية المنفردة لكل من عناصر الكوبلت، السيلينيوم والسيليكون على التعزيز الحيوي بهذه العناصر على نباتات الخس صنف ليمور ودراسة تأثيراتها المحتملة على النمو والانتاجية خلال موسمي ٢٠٢٠ و٢٠٢١. تم استخدام ١٠ معاملات رش ورقي، والتي تشمل استخدام سلفات الكوبلت بتركيز ١٠٠، ٢٠٠ و ٣٠٠ ميكرومولار و سيلينيت الصوديوم بتركيز ١٠، ٢٠ و ٣٠ ميكرومولار وسليكات الصوديوم بتركيز ٠,٥، ١ و ٢ ملليمولار والماء المقطر كمعاملة مقارنة (كنترول). التصميم التجريبي للتجربة كان قطاعات كاملة العشوائية في ثلاث مكررات. أظهرت النتائج أن الرش بهذه العناصر قد عزز قراءات المجموع الخضري، محتوى العناصر، محتوى الكلوروفيل والسكريات والأحماض الأمينية الحرة مقارنة بمعاملة المقارنة. بالإضافة انه لم تؤثر المعاملات على محتوى النترات والنيتريت في رؤوس الخس. وقد كانت الزيادة في الوزن الطازج للرؤوس تتراوح من ١١,٣٥ إلى ١٤,٥٤ ٪ في الموسم الأول، ومن ١٣,٠٧ إلى ١٦,٩٥ ٪ في الموسم الثاني. يستنتنتج ان معاملة الرش الورقي بسلفات الكوبلت بتركيز ١٠٠ ميكرومولار أو سيلينيت الصوديوم بتركيز ٢٠ ميكرومولار لم تزيد فقط المجموع الخضري والمحصول لرؤوس الخس ولكن ايضاً حسنت من القيمة الغذائية للنباتات وتعزز من محتوى هذه العناصر في رؤوس الخس.

الكلمات الدالة:

Lactuca sativa L . العناصر المفيدة. التعزيز الحيوي. الكوبلت. السيلينيوم . السيليكون.