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Selenium Enrichment of Green and Red Lettuce and the Induction of Radical Scavenging Potential

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Abstract: Selenium (Se)-enriched vegetables are promising dietary sources of Se, which provides beneficial biological effects in humans. In this study, we investigated the effects of foliar application of Se on hydroponically grown multi-leaf green (V1) and red (V2) lettuce plants. Three selenate (SeIV) amendment levels were evaluated for their influence on plant growth, elemental composition and radical scavenging capacity. Lettuce heads biofortified with 0.598 mg Se plant^{-1} accumulated 19.6–23.6 and 14.9–17.6 µg Se g^{-1} DM in the multi-leaf green (V1) and red (V2) lettuce plants, respectively. The accumulated Se levels can contribute significantly to the recommended dietary allowance of 70 µg day^{-1} for adult men and 60 µg day^{-1} for adult women. Accordingly, both V1 and V2 lettuce cultivars grown under the Se3 foliar application condition can cover the daily requirement for adult men by approximately 100% and 85% to 100%, respectively, by consuming 75–90 g or 100 g fresh weight from V1 or V2, respectively. The ABTS radical scavenging potential of green lettuce was induced at Se2 and Se3 foliar application levels, where the IC_{50} was 1.124 ± 0.09 µg mL^{-1} at Se0 and improved to 0.795 ± 0.03 and 0.697 ± 0.01 µg mL^{-1}, respectively. There was no cytotoxicity against Vero kidney cells among all treated lettuce plants at the highest concentration tested of 1 mg/mL. Finally, a further focused investigation of the metabolic profile of lettuce plants under varied Se levels needs to be investigated in future studies.

Keywords: selenium; green lettuce; red lettuce; elemental composition; ABTS assay; radical scavenging potential; cytotoxicity

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1. Introduction

Selenium (Se) is a beneficial trace element, which is known to alleviate plant oxidative stress by stimulating the plant's antioxidant potential [1]. Subsequently, Se indirectly enhances the activity of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), in addition to promoting synthesis of non-enzymatic antioxidants such as GSH, phytochelatins, proline, ascorbate, carotenoids, flavonoids, and alkaloids, which are known antioxidants and free radical scavengers [2,3]. Se has a key role in the initiation of immunity, protection against oxidative stress, and preventing viral infections in the human body [4]. It has been reported recently that a combination of Se, vitamin C, and vitamin D might help to elevate the immune system, prevent COVID-19 spread, and inhibit the progression of the disease to severe stages [5,6]. All around the world, almost 0.5 to 1 billion people do not intake Se in their diet, while a greater number might consume less Se than recommended for better prevention against cardiovascular diseases, cancer, and devastating infectious diseases such as HIV [7]. Currently, it has been estimated that more than 15% of the world's population is affected by Se

deficiency due to inadequate intake, including people in many regions of China, Oceania, Africa, and Europe [8,9].

Se deficiency in the human body can lower immune function and has been implicated in pathogenesis of cardiovascular disease, cardiomyopathy, osteochondropathy, cognitive impairment, endothelial dysfunction, and cataracts, and Se deficiency might even lead to cancer [10,11]. Accordingly, a sufficient amount of Se is essential to maintain body Se homeostasis; however, its excess might cause toxicity and adverse health problems [12].

Se is emitted to the atmosphere from natural sources, such as volcanoes and the marine and terrestrial biosphere, but in recent years, improvements in air pollution control and reductions in coal combustion in North America and Europe have reduced emissions and deposition of Se [13]. Moreover, authors reported recently that the influence of climate change will gradually decline soil Se levels in many regions of the world, particularly in agricultural lands; these decreases are likely to enhance the prevalence of Se deficiency [14]. Previous study has highlighted the increasing prevalence of Se deficiency in agricultural soils in the United States and Europe [13]. A recent report using machine-learning algorithms suggested reductions (mean loss = 8.4%) in soil Se levels later in the 21st century, driven by a decline in soil Se retention accompanied by changes in weather and climate. This reduction might lead to Se deficiency, which is associated with complications of human health [14].

Se enrichment of crop plants, the main source of Se for consumers, has gained more attention owing to the significance of Se in human nutrition [15–18]. Se is recycled by plants within the food chain. Thus, Se biofortification of vegetables, by means of applying Se along with the basal fertilizer, is a useful method to enhance the consumption of Se by animals and humans [19,20].

Lettuce (*Lactuca sativa* L.) is an annual vegetable of the daisy family, Asteraceae. It is considered to be one of the most popular leafy vegetables, offering numerous health benefits owing to its low caloric value as well as low fat and sodium content. Additionally, it is a good source of vitamin C, fiber, iron, and folate [21].

Previous studies indicated that Se supplementation induces the growth, yield, and antioxidant capacity of lettuce [22]. Therefore, biofortification of crops with essential elements is one of the most powerful strategies for enhanced plant and human health. Moreover, the accumulation and distribution of Se in the tissues of vegetable plants has gained much interest. Although Se is not essential for plant growth, it can reduce detrimental effects of abiotic stresses like heavy metals, high temperature, salinity stress, and drought by up-regulating the amount and activity of antioxidant enzymes (GSH-Px, GR, SOD, and APX) and metabolites (GSH and ascorbate), resulting in higher ROS scavenging capacity of plants [23]. Therefore, there is an urgent need for updated knowledge on the state-of-the-art regarding Se biofortification and its impact on overall plant metabolism, nutrition, and health. It is also important to note that Se enrichment of food crops can help in selecting and developing better cultivars for Se biofortification with adequate Se levels for better human nutrition and health.

In our endeavor to investigate the influence of foliar application of various Se levels on Se accumulation in green and red multi-leaf lettuce plants, we hypothesized that Se foliar application may lead to accumulation of a higher concentration of Se in green lettuce in comparison to the red ones, and that it would improve its radical scavenging properties.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The seeds of the two lettuce cultivars named Hawking RZ (green multi-leaf lettuce, V1), and Barlach RZ (red multi-leaf, V2) were germinated in sandwich blots and placed in a $CaSO_4$ solution (2 mM). The germination took place in a climatic growth chamber under controlled conditions (14 h of light, 20 °C during the day, 14 °C at night, 39% humidity) for twelve days. Subsequently, the seedlings were transferred to 10 L randomly arranged individual black containers, which were kept under standard greenhouse conditions with a

day/night cycle of 18/14 °C and a 14 h photoperiod. The nutrient solution used was composed of KH_2PO_4 = 0.1 mM, $Ca(NO_3)_2 \cdot 4H_2O$ = 2 mM, $NH_4H_2PO_4$ = 0.5 mM, KCl = 0.2 mM, $MgSO_4 \cdot 7H_2O$ = 0.5 mM, and KNO_3 = 2 mM, as well as micronutrients (Fe-EDTA = 60 µM, H_3BO_3 = 10 µM, $MnSO_4 \cdot H_2O$ = 2 µM, $ZnSO_4 \cdot 7H_2O$ = 0.5 µM, $CuSO_4 \cdot 7H_2O$ = 0.3 µM and $(NH_4)_2Mo_7O_{24}$ = 0.01 µM). The experiment was designed as a completely randomized design. The nutrient solution was changed weekly. Se foliar application was performed three times, one time a day per week, early morning before sunrise, in the form of Na_2SeO_4 at three levels, namely, 0.01196 mg (Se1), 0.1196 mg (Se2), and 0.593 mg Na_2SeO_4 per plant (Se1: 0.013, Se2: 0.13, and Se3: 0.6 µM). Foliar application was performed on the upper side of the leaves with the help of a brush. To achieve better Se absorption, 0.04% Silwet was used as a wetting agent and each plant received 6 mL of the Se solution. The lettuce plants were harvested after 48 days. Fresh weight (yield), and number of leaves per head of lettuce plants were recorded. The samples were frozen in liquid nitrogen and stored in a freezing chamber at −20 °C until freeze-drying. The heads of the lettuce samples were dried at −53 °C in a freeze-dryer (Gamma1–20, Christ, Osterode am Harz, Germany) to determine the dry matter (DM). The dried lettuce heads were ground to a fine powder for further analyses.

2.2. Se Concentration and Elemental Composition

The concentrations of Se in addition to macronutrients (magnesium, Mg; phosphorus, P; potassium, K, and calcium, Ca) as well as micronutrients (iron, Fe; manganese, Mn; Copper, Cu; zinc, Zn) were performed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies 7700 Series, Böblingen, Germany) in accordance with DIN EN ISO17294-2 (Deutsches Institut für Normung, Berlin, Germany, 2005) as described by Abdalla et al. [21]. Sulfur (S) and nitrogen (N) concentrations of lettuce plants were determined by using elemental analyzer (Flash EA1112, Thermo Fisher Scientific, Milano, Italy), as described by Abdalla et al. [21].

2.3. Preparation of Plant Extract

To prepare the plant extract, the powdered dried leaves were extracted with 70% acetone using a previously described method [24]. Briefly, 10 mL of 70% acetone (Minema, Roodepoort, South Africa) was added to 1 g of each plant sample (ratio 1:10). The mixture was sonicated for 1 h and put on an orbital shaker overnight. The supernatant was then filtered through Whatman No. 1 filter paper (Merck, Kenilworth, NJ, USA) into pre-weighed labelled glass jars. The filtrate was then dried under a cold stream of air and kept in the dark prior to use for experiments.

2.4. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic Acid) (ABTS) Radical Scavenging Assay

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was done following a previously described method [25] with slight modifications. Ascorbic acid served as positive control. Methanol (Minema, Roodepoort, South Africa) and extracts without ABTS served as negative control and blanks, respectively. Briefly, ABTS (160 µL) was mixed with extracts (40 µL) at different concentrations, incubated for 7 min in the dark, and the absorbance was measured at 734 nm using a microplate reader (Epoch, BioTek, Winooski, VT, USA). Samples were tested in duplicate with two repetitions of the entire experiments (n = 4). The percentage of radical scavenging activity was calculated using the equation below:

$$\% \text{ scavenging activity} = 100 - ((\text{absorbance of sample} - \text{absorbance of sample blank}) \times 100/(\text{absorbance of control}) - (\text{absorbance of control blank})).$$

The half maximal inhibitory concentration (IC_{50}) values were obtained from the equation obtained from the plot of inhibition percentage versus the tested concentrations.

2.5. Cytotoxicity Assay against Monkey Kidney (VERO) Cells

Toxicity assays against monkey kidney (VERO) cells (ATCC® CCL-81™, Sigma-Aldrich, St. Louis, MO, USA) were conducted using the tetrazolium-based colorimetric (MTT) assay as previously described [26,27]. The Vero cells were maintained in minimal essential medium (MEM, Highveld Biological, Johannesburg, South Africa) supplemented with 5% fetal calf serum (Adcock-Ingram, Johannesburg, South Africa) and 0.1% gentamicin (Virbac, Carros, France) in a 5% CO_2 incubator. Cell suspensions were prepared from confluent monolayer cultures and plated at a density of 1×10^4 cells into each well of sterile flat-bottomed 96-well microtitre cell culture plates. Plates were incubated overnight at 37 °C in a 5% CO_2 incubator before exposure to the extracts. The crude plant extracts were dissolved in acetone, and appropriate dilutions were prepared in MEM and added to the wells. Cells were exposed to the plant extract concentrations (1–0.0075 mg/mL) for 48 h. Doxorubicin (Pfizer, New York, NY, USA) and acetone served as positive and negative controls, respectively. After incubation for 48 h, the wells were rinsed with phosphate buffered saline (PBS, Sigma, St. Louis, MO, USA) and fresh medium was added to the cells. Thirty microlitres of MTT (Sigma) dissolved in PBS (5 mg/mL) was added to each well and further incubated for 4 h. The media from the wells were discarded and 50 µL of 100% DMSO added to the wells to dissolve the formazan crystals. Absorbance was measured on a microplate reader (BioTek Synergy) at a wavelength of 570 nm. Each extract concentration was tested in quadruplicate with two repeats (n = 8). The concentration causing 50% inhibition of cell lethality (LC_{50}) was calculated.

2.6. Statistical Analysis

Data of growth, Se concentration, and elemental composition were statistically analyzed using two-way (treatment × cultivar) analysis of variance (ANOVA). The results of the radical scavenging activity assay were statistically analyzed using one-way ANOVA. Significant differences among the means were determined by Tukey's HSD test ($p \leq 0.05$).

3. Results and Discussion

Top views of the green (V1) and red (V2) multi-leaf lettuce cultivars grown in a hydroponic system and treated with 4 different Se levels are presented in Figure 1. The current results revealed some variations in terms of plant biomass production including yield, DM, and number of leaves between V1 and V2 lettuce cultivars under Se foliar application (Figure 2). Data reported in (Figure 2A–C) indicate that different Se levels (Se1: 0.019, Se2: 0.119, and Se3: 0.598 mg Se $Plant^{-1}$) did not affect the yield, the DM, and the number of leaves in both V1 and V2 significantly. However, the yield and the DM contents and the number of leaves of green lettuce (V1) grown under different Se levels were significantly greater in comparison to red lettuce (V2) plants grown under the same Se concentrations (Figure 2A–C). The lowest recorded yield, DM, and number of leaves were observed in the red lettuce (V2) grown under Se3 level in comparison to green lettuce (V1) grown under Se3 treatment (Figure 2A–C).

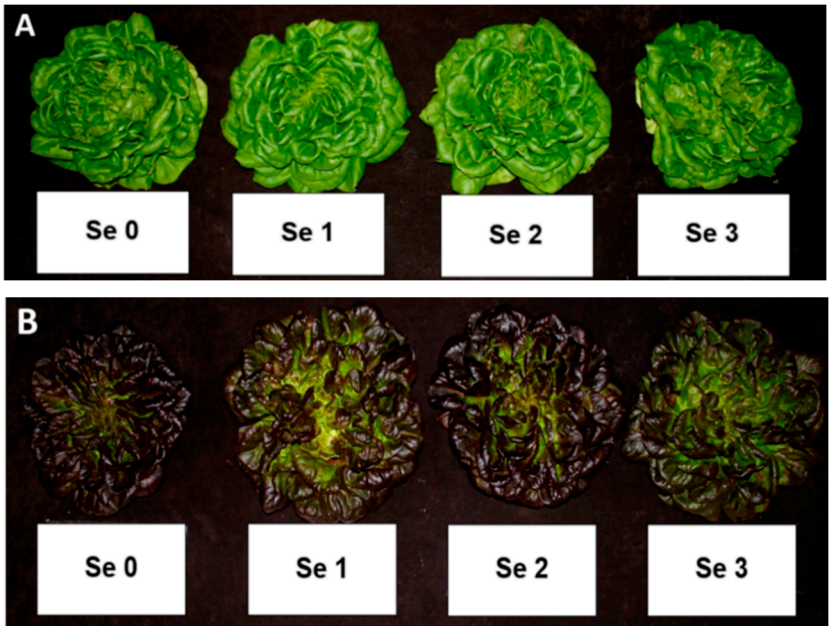

Figure 1. Lettuce heads grown in a hydroponic system and treated with 4 different Se levels (Se0: 0, Se1: 0.019, Se2: 0.119, and Se3: 0.598 mg Se Plant^{-1}). (**A**): Top view of the lettuce heads of Hawking RZ (green multi-leaf lettuce, V1). (**B**): Top view of the lettuce heads of Barlach RZ (red multi-leaf, V2).

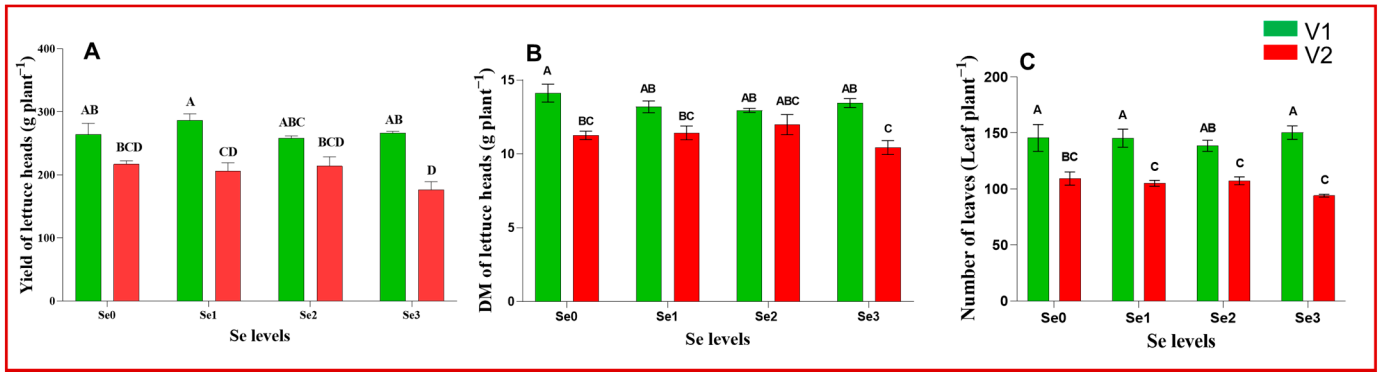

Figure 2. (**A**): Yield, (**B**): dry matter (DM) content, and (**C**): number of leaves in two lettuce cultivars; namely, Hawking RZ (green multi-leaf lettuce, V1), and Barlach RZ (red multi-leaf, V2) grown in a hydroponic system and treated with 4 different selenium levels (Se0: 0, Se1: 0.019, Se2: 0.119, and Se3: 0.598 mg Se plant^{-1}). Data presented are the means ± SDs of four replicates. Different letters show statistically significant differences among all the treatments ($p \leq 0.05$; Tukey's test).

This is consistent with our recently published findings [21], where the green multi-leaf lettuce (V1) demonstrated superior crop productivity compared to the red multi-leaf (V2).

Se is a crucial element for human nutrition and health status. Therefore, foods enriched in Se might benefit human health when incorporated into the diet. In this regard, it is very important to determine Se accumulation in all treated lettuce plants.

Regarding Se concentrations in the green and red lettuce, Se foliar application at 0.019 mg plant^{-1} accumulated 0.6–0.7 and 0.4–0.6 µg Se g^{-1} DM in the green and red lettuce plants, respectively (Figure 3). The foliar application at a concentration of 0.119 mg plant^{-1} (Se2) accumulated 4.1–4.9 µg Se g^{-1} DM in the green lettuce (V1), and 3.3–4.7 µg Se g^{-1} DM in the red lettuce (V2).

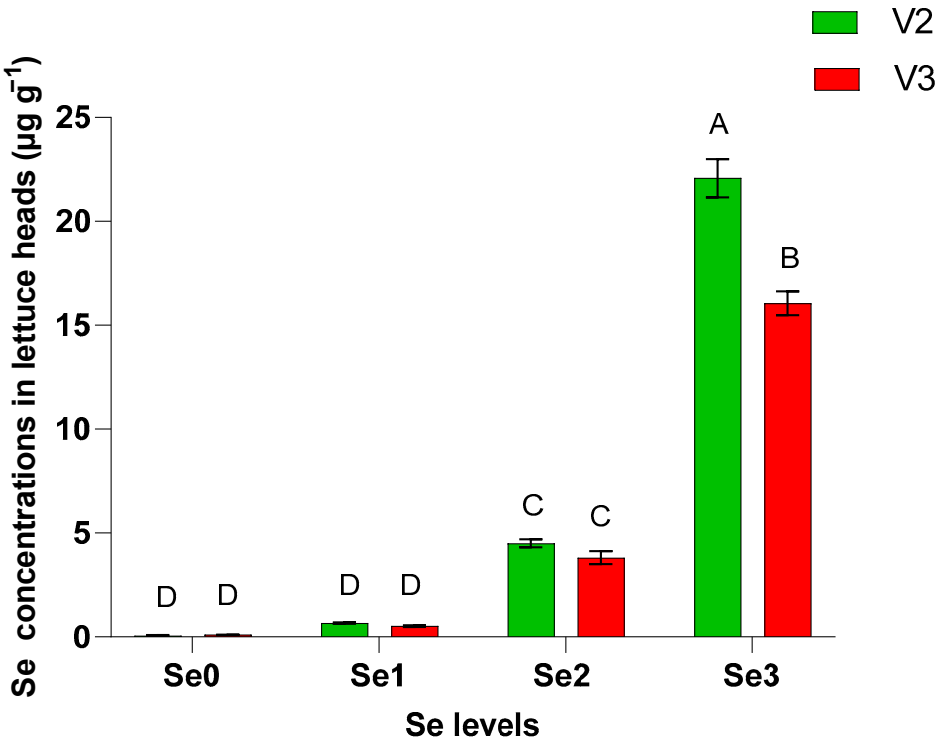

Figure 3. Se concentrations (µg g$-^{1}$ DM) in lettuce heads of Hawking RZ (V1) and Barlach RZ (V2). Data presented are the mean ± SD of four replicates. Different letters show statistically significant differences among all the treatments ($p \leq 0.05$; Tukey's test).

Additionally, significant differences were observed between green (V1) and red (V2) lettuce in the Se foliar application at Se3 level at the concentration of 0.598 mg plant^{-1}. V1 accumulated more Se (19.6–23.6 µg Se g^{-1} DM) in comparison to V2, which accumulated 14.9–17.6 µg Se g^{-1} DM at the same concentration. The current findings suggest that the accumulated Se levels in lettuce plants were adequate for providing the recommended dietary allowance of 70 µg day^{-1} for adult men and 60 µg day^{-1} for adult women [28]. Furthermore, both V1 and V2 lettuce cultivars can contribute to Se requirements with 78.4–94.4 µg Se day^{-1} and 59.6–71.6 µg Se day^{-1} respectively, by consuming 100 g fresh weight lettuce heads grown under Se3 conditions [29,30]. In this regard, we recommend that V1 and V2 can cover the dietary allowance for adult men by consuming 75–90 or 100 g fresh weight from V1 or V2 lettuce grown under Se3 treatment, respectively.

Lettuce provides significant amounts of minerals such as K, Ca, P, Mg, Fe, Cu, Mn and Zn [21]. In the current findings, different levels of Se foliar application did not significantly affect Mg, K, Ca, N, Mn, and Cu lettuce heads concentrations in both V1 and V2 lettuce cultivars (Table 1). However, Mg levels increased significantly at Se1 treatment (3.90 ± 0.1 mg/g DM) in comparison to V2 (3.33 ± 0.1 mg/g DM). Additionally, greater concentrations of P, K, and Ca were observed in V1 under Se1, Se2, and Se3 foliar applications in comparison to V2 under the same Se conditions. Moreover, Mn concentrations were higher in green lettuce (V1) than red lettuce (V3) under Se0, Se1, Se2, and Se3.

Table 1. Macro- and micronutrients (in mg/g DM and mg/kg DM, respectively) in lettuce heads of Hawking RZ (V1) and Barlach RZ (V2).

	Multi-Leaf Green Lettuce (Hawking) V1				Multi-Leaf Red Lettuce (Barlach) V2			
	Different Selenium Levels							
	Se0	**Se1**	**Se2**	**Se3**	**Se0**	**Se1**	**Se2**	**Se3**
Macro in mg/g								
Mg	3.87 ± 0.2 AB	3.90 ± 0.1 A	3.61 ± 0.1 ABC	3.47 ± 0.1 ABCD	3.37 ± 0.1 BCD	3.33 ± 0.1 CD	3.2 ± 0.04 CD	3.06 ± 0.04 D
P	8.63 ± 0.1 BCD	9.24 ± 0.2 ABC	9.56 ± 0.1 AB	9.95 ± 0.3 A	7.78 ± 0.4 D	7.64 ± 0.3 D	8.2 ± 0.3 CD	8.48 ± 0.3 BCD
K	75.9 ± 1.1 A	78.6 ± 2.4 A	75.6 ± 0.6 A	74.1 ± 1.2 AB	66.0 ± 4.0 ABC	62.1 ± 3.8 BC	61.9 ± 2.3 BC	58.1 ± 2.9 C
Ca	7.09 ± 0.3 A	7.02 ± 0.2 A	6.57 ± 0.1 AB	6.75 ± 0.3 AB	5.95 ± 0.3 ABC	5.76 ± 0.3 BC	5.68 ± 0.2 BC	5.08 ± 0.2 C
N	43.4 ± 0.9 A	43.1 ± 0.9 AB	44.5 ± 0.5 A	41.3 ± 0.5 ABC	38.1 ± 1.3 CD	37.5 ± 1.3 CD	38.5 ± 0.8 BCD	36.1 ± 1.2 D
S	1.8 ± 0.1 A	1.08 ± 0.04 BC	1.0 ± 0.1 C	1.88 ± 0.1 A	1.93 ± 0.1 A	1.34 ± 0.1 B	2.05 ± 0.1 A	2.07 ± 0.1 A
N/S	24.4 ± 1.5 BC	39.9 ± 1.3 A	45.3 ± 3.1 A	22.1 ± 0.9 CD	19.9 ± 0.8 CD	28.2 ± 1.7 B	18.9 ± 1.3 CD	17.5 ± 0.7 D
Micro mg/kg								
Mn	32.9 ± 0.92 AB	34.8 ± 0.91 A	33.95 ± 1.03 A	33.3 ± 0.89 A	23.1 ± 1.30 C	22.1 ± 2.02 C	25.3 ± 2.26 BC	23.4 ± 2.16 C
Fe	100.9 ± 2.39 B	120.6 ± 7.35 A	93.57 ± 2.86 B	99.6 ± 3.53 B	98.9 ± 2.31 B	104.2 ± 4.12 AB	95.7 ± 3.93 B	95.3 ± 2.16 B
Cu	5.42 ± 0.30 A	5.31 ± 0.65 A	4.98 ± 0.62 A	4.18 ± 0.34 A	3.51 ± 0.16 A	4.59 ± 0.49 A	3.6 ± 0.40 A	3.33 ± 0.44 A
Zn	110.9 ±5.56 AB	128.1 ± 5.50 A	80.9 ± 4.26 C	79.9 ± 3.57 C	103.2 ± 2.62 B	103.9 ± 2.16 B	68.3 ± 1.89 C	71.9 ± 4.88 C

Data presented are the mean ± SD of four replicates. Different letters show statistically significant differences among all the treatments ($p \leq 0.05$; Tukey's test).

P concentration was enhanced significantly in V1 (9.95 ± 0.3 mg/g DM) in response to Se3 treatment in comparison to the control (Se0). S levels were affected dramatically in both green and red lettuce plants, where the concentration decreased significantly from 1.80 ± 0.1 and 1.93 ± 0.1 mg/g in both V1 and V2 plants under Se0 treatment to 1.08 ± 0.04 and 1.0 ± 0.1 mg/g DM in V1 under Se1 and Se2 foliar application, and to 1.34 ± 0.1 mg/g DM in V2 under Se1 treatment. Subsequently, S level increased again significantly (1.88 ± 0.1 mg/g DM), in response to higher concentrations of Se foliar application (Se3). Additionally, S was enhanced significantly in V2 to 2.05 ± 0.1 and 2.07 ± 0.1 mg/g DM with Se2 and Se3 foliar application. In a previous report which studied the interaction between Se and S in *Arabidopsis thaliana* (L.) Heynh. plants grown on agar plates and supplied with different concentrations of S and Se, the authors found that a higher Se level in agar enhanced both Se and S levels in shoots but decreased the shoot fresh weight [31,32].

S is an essential macronutrient required for plant growth and development, in addition to the synthesis of important S-containing compounds like the amino acids methionine and cysteine, GSH, specialized peptides and hormones, proteins, co-enzymes, prosthetic groups, vitamins, and other S-containing secondary metabolites [33–35].

The N/S ratio was enhanced in V1 plants to 39.9 ± 1.3 and 45.3 ± 3.1 mg/g DM with Se1 and Se2 application and to 28.2 ± 1.7 mg/g DM in V2 plants with Se1 application (Table 1). Although the N/S ratio indicates that the plants may have been S deficient, this ratio should be interpreted with caution because it might be affected by high level of N without S deficiency. Moreover, in all lettuce plants under different Se foliar applications no symptoms of S deficiency have been observed. Fe concentration increased significantly with Se1 (120.6 ± 7.4 mg/g DM) in V1 plants and remained unchanged in V2 plants at all Se levels (Table 1). Although Zn levels were not affected by Se1 application, the concentrations decreased dramatically with Se2 and Se3 treatments in both V1 and V2 plants.

Se is an essential element with antioxidant properties mediated through glutathione peroxidases and other selenoenzymes [36]. Regarding the antioxidant activity quantified by ABTS, green and red lettuce heads biofortified with varied Se concentrations were positively associated with higher radical scavenging activity (Figure 4).

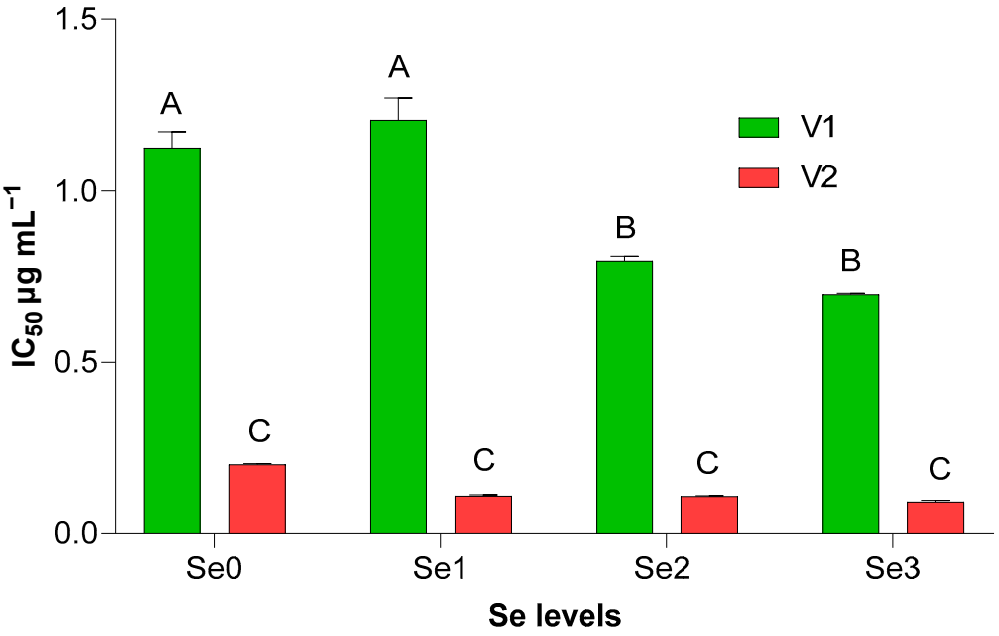

Figure 4. Antioxidant activity (ABTS scavenging potential) of V1 and V2 multi-leaf lettuce plants. Data presented are the mean ± SD of four replicates. Different letters show statistically significant differences among all the treatments ($p \leq 0.05$; Tukey's test).

The effect of Se supplementation of lettuce on in vitro scavenging effects of the ABTS free radical is presented in Figure 4. The ability of V1 multi-leaf green lettuce extracts to scavenge ABTS radicals was increased significantly ($p < 0.05$) in response to Se2 and Se3 foliar applications. Moreover, the IC_{50} values of examined extracts indicated that the ABTS radical scavenging ability of V1 was greatest, as it had the lowest IC_{50} (0.795 ± 0.02 and 0.697 ± 0.01 µg mL^{-1}, respectively).

The multi-leaf red lettuce extracts (V2) demonstrated higher ABTS radical scavenging capacity with IC_{50} values of 0.109 ± 0.01, 0.108 ± 0.003, and 0.092 ± 0.01 µg mL^{-1} with Se1, Se2, and Se3 treatments, respectively, in comparison to multi-leaf green lettuce extracts (V1). However, ABTS radical scavenging potential of V2 remained unaffected in response to all Se applications. In our recent published results, the red lettuce (V2) showed the best radical scavenging activity against the DPPH radical [21]. The significantly greater antioxidant potential of V2 in comparison with V1 might be attributed to the presence of anthocyanins, such as cyanidin 3-*O*-galactoside, which was only detected in V2 lettuce plants [21]. The IC_{50} of ascorbic acid (positive control) was 0.0013 mg/mL.

The enhanced scavenging effects of both V1 and V2 lettuce might be attributed to interaction of the phenolic components in Se-enriched lettuce and their synergistic effects on inducing antioxidant potential. A change in the polyphenolic compounds in response to Se biofortification has been recorded, where D'Amato et al. [37] reported enhanced levels of oleacein, ligustroside aglycone, and oleocanthal in olive oil, in comparison to the untreated plants. Additionally, a previous study of Se biofortification of radish plants indicated that the hydroxycinnamic acids, including kaempferol derivatives, were enhanced in radish leaves, while other detected phenolic metabolites remained unchanged or decreased [38]. Moreover, Se application of 1 mg Se/L in tomato plants resulted in a significant increase in quercetin level, where both β-carotene and lycopene concentrations were decreased and rutin level did not change [39]. In our previous report, we identified phenolic compounds such as quercetin 3-*O*-malonylglucoside, quercetin, isoquercetin, quercetin 3,4′-diglucoside, quercetin-3-*O*-glucose-6″-acetate, quercetin 3′,4′-di-*O*-β-D-glucopyranoside, caffeic acid hexose, luteolin-7-glucuronide, 5-*O*-caffeoylquinic acid, dicaffeoyltartaric acid, dicaffeoylquinic acid in V1 and V2 lettuce plants V2 red multi-leaf lettuce contains cyanidin-3-*O*-galactoside which was responsible for its high antioxidant activity [21]. Following several recent studies reporting

that Se supplementation of plants can elicit the production of primary and secondary metabolites [40–42], our study recommends further investigation of metabolites in all lettuce plants treated with varying Se concentrations. As the antioxidant potential of lettuce plants increased under Se treatment, this may be attributed to the induction of specific metabolites with antioxidant properties.

The cytotoxicity assay (LC_{50}) (Table 2) revealed that extracts of all green and red lettuce plants biofortified with different Se levels were not cytotoxic at the highest tested concentration (1 mg/mL). These findings suggested relatively low or no cytotoxicity against Vero kidney cells. Doxorubicin was included as a positive control and the LC_{50} was 0.013 mg/mL.

Table 2. Toxicity (LC_{50}) against Vero kidney cells of all treated lettuce plants.

Treatments	LC_{50} (mg/mL)
V1/Se0	>1
V1/Se1	>1
V1/Se2	>1
V1/Se3	>1
V2/Se0	>1
V2/Se1	>1
V2/Se2	>1
V2/Se3	>1
Doxorubicin	0.013

4. Conclusions

The level of Se supplementation is important in meeting nutritional recommendations and requirements. Both green (V1) and red (V2) multi-leaf lettuce plants behaved differently when subjected to varied concentrations of Se treatments, where V1 accumulated more Se in comparison to V2 at the higher Se application (Se3). However, both V1 and V2 lettuce cultivars can cover the nutritional requirements of 70 μg day^{-1} for adult men and 60 μg day^{-1} for adult women with relatively low or no cytotoxicity against Vero kidney cells. In this regard, the enrichment of lettuce plants with Se may be an effective approach to enhance Se nutritional status in Se-deficient populations. The biofortification of Se by Se foliar application had a positive effect on the antioxidant (ABTS) capacity of the leaf extracts, especially with regard to the green lettuce. The novelty of the current study lies in the investigation of different lettuce cultivars for Se biofortification with adequate Se levels coupled with the effect on free radical scavenging activity of the leaf extracts. The green lettuce was highlighted as the better cultivar for Se content and antioxidant activity. Further work needs to be done to elucidate the nature of the secondary metabolites which might be responsible for the entire free radical scavenging properties under Se biofortification.

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