horticulturae

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Effects of Selenate Application on Growth, Nutrient Bioaccumulation, and Bioactive Compounds in Broccoli (*Brassica oleracea* var. *italica* L.)

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Abstract: The biofortification of edible crops with selenium (Se) is a common and effective strategy to address inadequate Se intake, which is suffered by millions of people worldwide. However, there is little information regarding the effects of this practice on crops belonging to the important *Brassica* family. To evaluate the efficacy of foliar Se application on broccoli, four treatments with varying Se concentrations were tested: 0%, 0.05%, 0.10%, and 0.15% (*w*/*v*), applied as sodium selenate during the early flowering stage. Although no overall effects on growth and biomass parameters were observed, the results indicate that the lowest Se dose (0.05-Se) was sufficient to notably increase Se concentration in the florets, even after boiling. Based on the increase to 14.2 mg Se kg^{-1} of dry matter in this broccoli fraction, it was estimated that consuming a 100-gram portion of boiled florets biofortified with 0.05% Se would provide approximately 140 µg of Se, which could be sufficient to potentially improve human selenium status, as previously documented. Moreover, the results obtained underscore how the application of this small dose was also adequate to reduce phytate concentration in the florets and to increase antioxidant and polyphenol concentrations, thereby improving the concentration and bioavailability of other essential nutrients, including Ca, Mg, Fe, and Zn, along with improving its quality as an antioxidant food.

Keywords: agronomic biofortification; nutrient bioavailability; selenium fertilization; sodium selenate

Citation: Poblaciones, M.J.; García-Latorre, C.; Velazquez, R.; Broadley, M.R. Effects of Selenate Application on Growth, Nutrient Bioaccumulation, and Bioactive Compounds in Broccoli (*Brassica oleracea* var. *italica* L.). *Horticulturae* **2024**, *10*, 808. https://doi.org/10.3390/horticulturae10080808

Academic Editor: Moreno Toselli

Received: 27 June 2024
Revised: 25 July 2024
Accepted: 29 July 2024
Published: 30 July 2024

1. Introduction

Selenium (Se) is an essential micronutrient that is crucial for human health, due to its involvement in various physiological processes. It is unique among trace essential elements as it is genetically encoded and constitutes the 21st amino acid, selenocysteine (SeCys), and appears in selenomethionine (SeMet), which are both incorporated into proteins [1,2]. It is widely recognized for its role in the formation of at least 25 selenoproteins [3]. Some of them are redox enzymes, such as glutathione peroxidase (GPx) or thioredoxin reductases (TrxR), which play crucial roles in cellular antioxidative defense systems and the regulation of redox states within the cell [4]. However, the functions of most of these proteins require further investigation [5].

The recommended daily intake of Se for human adults to avoid health risks is between 55 and 60 µg Se day^{-1}, with an increased intake of up to 70 µg day^{-1} during lactation [6]. Furthermore, the optimal plasma Se levels have been estimated to be 90–120 µg L^{-1} [7]. Conversely, the toxic dose has been established at 400 µg Se day^{-1} [6]. Although both a deficiency in and excess levels of Se can have detrimental effects on the body, the former is a particularly significant global health concern, affecting approximately one billion people worldwide, due to inadequate dietary intake [8]. In this context, Se intake in China and in the majority of the countries in Europe, the Middle East, and Sub-Saharan Africa [9–11]

may be considered deficient. For instance, in the case of the Spanish population, 25% of individuals fail to meet the European Food Safety Authority (EFSA) recommended intake for Se [12]. Therefore, due to the far-reaching implications for human health and well-being, it is imperative to implement interventions to address this micronutrient deficiency on a global scale [13].

Inadequate human Se intake has been attributed to the generally low Se concentrations found in soils, resulting in inadequate Se in food [14,15]. To address this deficiency, agronomic Se biofortification has been proposed as a strategy to improve dietary Se intake and prevent deficiencies and chronic diseases in humans while avoiding toxic levels of intake [16–19]. Agronomic biofortification is defined as the process of increasing the concentration and bioavailability of micronutrients in edible plant parts through the implementation of agricultural interventions [20]. Recent studies have highlighted the sustainability of biofortification strategies for increasing crop Se concentrations through the use of sodium selenate or sodium selenite when growing a diverse array of crops, including wheat (*Triticum aestivum* L.) [21], peas (*Pisum sativum* L.) [22], or broccoli (*Brassica oleracea* L. var. *italica*) [23].

The process of agronomic Se biofortification offers a number of advantages over the direct supplementation of Se. Inorganic Se that is absorbed by the plant is transformed into organic forms, which have a higher bioavailability. The implementation of Se biofortification strategies is influenced by a number of variables, including the mode of Se administration (soil fertilization, foliar spray, or hydroponics), the Se dose, the fertilizer form, the crop species and variety, and the plant growth stage [24]. In comparison to soil application, foliar application is a more straightforward, more effective, and environmentally friendly method for increasing Se concentrations in crops. This method avoids the concentration and leaching of Se in soil, facilitating rapid absorption through the leaves and concentration within the plant [25]. A series of detailed studies have demonstrated that the foliar application of Se improves the concentration of Se in cereal grains, vegetables, and fruits [2,25–27]. In recent years, foliar treatments have been increasingly explored as the best approach for the biofortification of broccoli. Studies have demonstrated the effectiveness of these treatments in improving crop yield and quality [28], including under organic conditions [29].

Brassica species, particularly broccoli, are renowned for their exceptional nutritional profile and antioxidant potential, thereby being considered functional foods due to their numerous health benefits [30]. Broccoli is a rich source of essential nutrients such as vitamins C and K, β-carotene, dietary fibers, polyphenols, fatty acids, minerals, and glucosinolates [31,32]. These components play a key role in promoting health and well-being, making brassicas a valuable addition to a healthy diet [30].

Furthermore, members of the *Brassica* genus are highly efficient in terms of Se concentration [33], with broccoli classified as a secondary accumulator of Se, which indicates its capacity to uptake and store it more readily compared to other crops [34]. It can, therefore, be concluded that the biofortification of broccoli with Se has the potential to notably increase its nutritional value without compromising its commercial quality [35]. It has been previously documented that Se biofortification can increase the total phenolic compounds in *Brassica* species, thereby prolonging shelf life and preserving food quality [15]. Moreover, previous studies have focused on the biofortification of broccoli with Se under conditions of nutrient deficiency, emphasizing the importance of Se fertilizers in enhancing the nutritional quality of broccoli [36]. Additionally, it has previously been demonstrated that the biofortification of broccoli with Se can improve its postharvest quality and nutritional value [23]. Moreover, evidence suggests that Se biofortification may enhance antioxidant activity and phenolic concentrations in broccoli [15,37].

Conversely, in the context of a biofortification program, the role of antinutrients such as phytic acid should not be overlooked. Phytic acid is renowned for its chelating properties, which allow it to bind to essential minerals, including calcium (Ca), magnesium (Mg), iron (Fe), or zinc (Zn). This process reduces the bioavailability of these nutrients, which may

result in mineral deficiencies when consuming foods that are high in phytic acid [38,39]. Although its concentration is relatively low in *Brassica* crops [40], phytic acid is an important antinutrient that may be reduced through biofortification approaches [41].

The primary goal of this study was to assess the effect of different foliar selenate dosages on yield, nutrient concentration and bioavailability, bioactive compounds, and phytate levels in broccoli. Additionally, the research aimed to assess how agronomic selenium (Se) biofortification affects Se concentration in broccoli to understand how this strategy can optimize Se uptake by consumers, considering its potential health benefits.

2. Materials and Methods

2.1. Experimental Site Conditions

The experiment was carried out in a naturally lit greenhouse at the University of Extremadura, Badajoz, Spain (38°89′ N, 6°97′ W; 186 m above sea level). The experiment was conducted from November to March, during which time, data regarding the temperature and relative humidity within the greenhouse were recorded using a datalogger (MicroLite logger LITE5032L-RH, Fourtec, Rosh HaAyin, Israel). The greenhouse maintained an average temperature of 19 ± 7 °C during the day and 12 ± 3 °C at night. The relative humidity exhibited a diurnal variation, with the lowest humidity occurring at midday (58%) and the highest humidity occurring at midnight (82%). The weekly average for the minimum and maximum temperatures, as well as the relative humidity, registered during the experiment is presented in Figure S1 in the Supplementary Materials.

2.2. Soil Characteristics and Conditioning

The Se-deficient soil used in the experiment was collected from the topsoil layer (0–20 cm) of the Tierra de Barros region in Western Spain (38°88′ N, 7°04′ W). Prior to its characterization, the soil underwent conditioning, in accordance with previous methodology [42]. Thus, the soil was air-dried and sieved to a particle size of 2 mm, then 4 subsamples were used to determine the key soil properties: sandy texture, a pH of 6.5 ± 0.2 (10 g soil:25 mL deionized water), and nutrient levels. The concentration of nitrate nitrogen was found to be 1.3 ± 0.1 mg kg^{-1}, that of ammonium nitrogen was 2.7 ± 0.2 mg kg^{-1}, available phosphorous was 15 ± 0.4 mg kg^{-1}, and that of potassium was 15 ± 0.5 mg kg^{-1}. Extractable Se was quantified at 1.23 ± 0.03 μg kg^{-1} by employing a KH_2PO_4 solution (0.016 mM, pH 4.8) at a ratio of 10 g dry soil:30 mL KH_2PO_4 [43]. Se concentrations were determined using an inductively coupled plasma spectrometer (ICP-MS) (Agilent 7500ce, Agilent Technologies, Palo Alto, CA, USA) operating in hydrogen gas mode [44]. To guarantee the accuracy and reliability of the results, a certified soil reference and blank samples were included in each batch of analyses. All results were reported on a dry-weight basis.

To ensure that Se was the only limiting nutrient for broccoli growth, the following solutions of basal nutrients (in mg kg^{-1}) were added to the soil: 150.3 $CaCl_2 \cdot 2H_2O$; 139.9 K_2SO_4; 10.0 $MnSO_4 \cdot H_2O$; 95.2 NH_4NO_3; 90.2 KH_2PO_4; 40.1 $MgSO_4 \cdot 7H_2O$; 2 $CuSO_4 \cdot 5H_2O$; 2.0 $ZnSO_4 \cdot 7H_2O$; 0.5 $CoSO_4 \cdot 7H_2O$; 0.2 $Na_2MoO_4 \cdot 2H_2O$; 0.7 H_3BO_3. Additionally, 80.9 mg of NH_4NO_3 was applied every 3 weeks to avoid nitrogen deficiency.

2.3. Experimental Design and Crop Management

The seeds utilized in the experiment were derived from a commercially available variety designated as Green Top, which is commercially distributed by Takii Seeds (Almería, Spain). This variety is characterized by its precocious growth and dense apical production and has historically been employed in Spain. The seeds of broccoli (*Brassica oleracea* var. *italica* L.) were subjected to surface sterilization by soaking in 80% v/v ethanol and in 2% v/v sodium hypochlorite for 60 s, respectively. After thorough washing with distilled water, the seeds were sown in a seedbed containing commercial substrate. Four weeks after sowing, the plants were transplanted into 30-centimeter-high and 30-centimeter-diameter free-draining pots containing 8.5 kg of the conditioned soil.

The experiment employed a completely randomized block design, with four Se treatments and four replicates. The Se treatment involved the foliar application of sodium selenate (Na_2SeO_4) at the following Se concentrations: 0% (Control) using a distilled water spray, 0.05% (0.05-Se), 0.10% (0.10-Se) and 0.15% (0.15-Se) (w/v). The spraying procedure was conducted at the early flowering stage, by manually applying 15 mL of solution per pot with a 30 mL hand sprayer, to minimize losses and ensure uniform wetting of all leaves. Tween 20 (Sigma Aldrich, St. Louis, MO, USA) was used as a surfactant for all the treatments at a concentration of 0.1% (v/v). In order to prevent contact between the foliar sprays and the soil, the pot surface was covered at the base of the plants.

The plants were watered every two days with distilled water in order to maintain soil moisture at an optimal level. Four drainage slits, each 2 cm wide and positioned 2 cm above the base of the pots, facilitated the effective drainage of excess water. Throughout the study period, no pests or diseases were detected.

2.4. Plant Material Analysis

Approximately 12 weeks after transplanting, the plants were considered to have matured and were, therefore, harvested and thoroughly washed with distilled water. Prior to separation into the broccoli head and the vegetative part of the plant (designated as Stem+Leaves), measurements of the plant's height and weight were taken. In addition, the commercial floret weight (leaving a stem of about 6 cm) was measured, as well as the major diameter (D, the widest point across the head) and the minor diameter (d, the narrowest point across the head). The floret was then divided into two subsamples: "raw floret" and "boiled floret". The latter was boiled in 400 mL of distilled water for 5 minutes. Then, to determine the total dry weight of each of the 3 plant fractions (Stem+Leaves, raw florets, and boiled florets), the samples were dried at 60 °C in an oven until they achieved a constant weight. Finally, the dried samples were finely ground in preparation for further analysis.

The total concentrations of calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), sulfur (S), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), Se, and zinc (Zn)) in each broccoli fraction (Stem+Leaves, raw florets, and boiled florets) were determined using ICP-MS, following the method previously described for measuring soil nutrient concentration. To achieve this, the plant samples were first digested in a heated mixture of concentrated nitric and perchloric acids [45]. Each digestion run included 2 operational blanks and 2 samples of certified reference material (CRM; tomato leaf SRM 1573a NIST, Gaithersburg, MD, USA). The Se-specific recovery rate by ICP-MS was 93% from the CRMs.

The bioavailability of Se, Ca, Fe, Mg, and Zn in the Stem+Leaves fraction and raw florets was estimated by quantifying the phytic acid (PA) levels in said plant fractions, using a PA-total phosphorus assay kit (Megazyme, County Wicklow, Ireland) [42]. The molar ratios between PA and these nutrients were subsequently calculated.

The results of the Se concentration analysis of boiled broccoli florets were used to determine the daily Se intake covered by a 100-gram portion of said florets. For this, 10% of dry matter was considered [35].

2.5. Antioxidant Activity

The antioxidant activity of samples of both the Stem+Leaves fraction and raw florets was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Prior to analysis, each sample was extracted by dissolving 10 g of matter in 70% v/v ethanol and incubating for 1 h at 30 °C in a shaking water bath (Unitronic Reciprocal, JP Selecta, Barcelona, Spain) under dark conditions. The solvent was then evaporated at 37 °C using a Rotavapor R-210 (Büchi Labortechnik AG, Flawil, Switzerland), then the extract was adjusted to 25 mL with ultrapure water.

The scavenging activity of DPPH free radicals was determined by mixing 50 µL of the broccoli extract with 2950 µL of DPPH reagent (Sigma, St. Louis, MO, USA). After incubating the mixture for 30 min at room temperature in the dark, the absorbance was measured at 515 nm (JP Selecta UV 3100, J.P. Selecta, Barcelona, Spain) [46].

The ABTS assay was conducted, briefly, by mixing 40 µL of the extract with 2 mL of ABTS solution (Sigma, St. Louis, MO, USA). The initial absorbance value at 730 nm was then compared with the absorbance obtained after 20 min of reaction [47].

In both cases, a calibration curve was previously prepared using Trolox (6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxylic acid; Sigma, St. Louis, MO, USA) as the standard, thereby expressing the results as mg of Trolox per kg of dried weight (mg Trolox kg^{-1} DW).

2.6. Total Phenol Concentration (TPC)

The total polyphenol concentration (TPC) was quantified using a modified version of the Folin–Ciocalteu method [48]. Each sample was extracted twice in 60 mL of 80% aqueous ethanol containing 1% of concentrated HCl, maintained for 1 h in the dark at approximately 25 °C, and then filtered. The solvent was evaporated using a Rotavapor R-210 (Büchi Labortechnik AG, Flawil, Switzerland) to determine the final weight of the extract. The total phenolic concentration was quantified spectrophotometrically (JP Selecta UV 3100) at 760 nm using the Folin–Ciocalteu reagent, and the results were expressed as mg of gallic acid equivalents per kg of dry weight (mg GAE kg^{-1} DW).

2.7. Statistical Analysis

All data were initially evaluated for normality and homogeneity of variances using the Shapiro–Wilk test and Levene's test, respectively. The growth parameters were subjected to a one-way analysis of variance (ANOVA) based on the effect of Se treatment (Control, 0.05-Se, 0.10-Se, and 0.15-Se), while the total macro-(Ca, K, Mg, Na, and S) and micronutrient (Cu, Fe, Mn, Se, and Zn) concentrations were subjected to a two-way ANOVA, with the factors being plant fraction (Stem+Leaves, raw florets, and boiled florets), Se treatment, and their interaction. In addition, the phytic acid (PA) concentration, the total phenol concentration (TPC), and antioxidant activity, as measured by ABTS and DPPH, were also subjected to a two-way ANOVA based on plant fraction (Stem+Leaves and raw floret), Se treatment, and their interaction. When significant differences were found, Fisher's protected least significant difference (LSD) test was employed at the $p < 0.05$ level of significance. All analyses were conducted using the statistical software package Statistix version 8.10 for Windows (Analytical Software, Tallahassee, FL, USA).

3. Results

3.1. Effect on Plant Growth Parameters

Selenium application significantly affected only the Stem+Leaves height. Specifically, the 0.10-Se treatment significantly increased the Stem+Leaves height to 32.0 cm, which was 13.1% higher than the Control (28.3 cm). In contrast, Se application did not consistently affect any of the other measured parameters. For instance, although 0.05-Se application increased floret weight by 11% compared to the Control treatment, said increase was not statistically significant. Regarding the rest of the determinations, plant weight and height were, on average, 293.3 g and 30.2 cm, with higher and lower diameters of 8.8 cm and 7.6 cm, respectively (Table 1).

Table 1. Broccoli growth characteristics (Stem+Leaves weight and height, floret weight, higher (D) and lower (d) diameters) under different Se treatments. Mean ± standard error; degrees of freedom (df) and F-values.

Se Treatment	Stem+Leaves Weight (g)	Stem+Leaves Height (cm)	Floret Weight (g)	D (cm)	d (cm)
Control	280 ± 23 a	28.3 ± 0.2 c	86.4 ± 3.0 a	8.4 ± 0.2 a	7.3 ± 0.1 a

Table 1. *Cont.*

Se Treatment	Stem+Leaves Weight (g)	Stem+Leaves Height (cm)	Floret Weight (g)	D (cm)	d (cm)
0.05-Se	311 ± 13 a	29.3 ± 1.0 bc	92.8 ± 3.2 a	8.8 ± 0.2 a	7.2 ± 0.2 a
0.10-Se	282 ± 21 a	32.0 ± 0.8 a	97.8 ± 5.9 a	8.7 ± 0.2 a	8.0 ± 0.0 a
0.15-Se	300 ± 11 a	31.2 ± 0.7 ab	98.8 ± 10.4 a	8.8 ± 0.3 a	7.8 ± 0.6 a
df	3	3	3	3	3
F-value	0.72	5.35 *	0.79	0.8	1.59

Means in a column with different letters were significantly different (* $p \leq 0.05$) according to Fisher's protected LSD (least significant difference) test for the Se treatment.

3.2. Effect on Nutritional Parameters

Table 2 shows that the plant fraction studied (Stem+Leaves, raw florets, or boiled florets) significantly influenced all macro- and micronutrients, with the exception of Mn and Zn. However, Se application and plant fraction × Se interaction only significantly affected total Se concentration.

Table 2. Summary of the two-way ANOVAs showing the effect of the plant fraction type (Stem+Leaves, raw florets, and boiled florets), the Se treatment, and their interaction on broccoli's nutritional characteristics, including nutrient and phytic acid (PA) levels, PA:nutrient molar ratios, antioxidant activity (ABTS and DPPH), and total polyphenol concentration (TPC).

Source	Plant Fraction (P)	Se Treatment (T)	P × T
df	2	3	6
Ca	366.28 ***	0.24	0.10
K	50.72 ***	0.44	0.58
Na	29.68 ***	1.63	1.78
Mg	91.97 ***	0.32	0.30
P	87.74 ***	4.68	0.71
S	147.31 ***	1.26	0.90
Co	269.13 ***	1.39	0.30
Cu	55.86 ***	0.41	2.66
Fe	12.46 **	0.83	0.77
Mn	4.42	0.49	0.60
Se	52.61 ***	147.04 ***	5.23 **
Zn	2.81	1.17	0.98
df	1	3	3
Phytic acid	150.99 ***	104.55 ***	132.00 ***
PA:Ca	553.14 ***	48.10 ***	44.74 ***
PA:Mg	144.03 ***	52.71 ***	44.05 ***
PA:Fe	9.04 *	6.55 *	9.21 *
PA:Se	58 **	223.19 ***	111.83 ***
PA:Zn	13.31*	15.44 **	5.99 *
ABTS	263.7 ***	14.73 ***	7.16 **
DPPH	877.82 ***	18.21 ***	0.63
TPC	72.97 ***	77.98 ***	3.38 *

The degrees of freedom (*df*), the F-values, and the levels of significance (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$) are shown for each parameter.

The analysis of nutrient concentration in different broccoli plant fractions revealed significant variations in several macro- and micronutrients. The total concentration of the Stem+Leaves fraction was significantly richer in Ca, Na, and Mg compared to the raw and boiled florets, with average increases that were 5.1-, 1.5-, and 1.3-fold higher, respectively. Additionally, the Mg concentration was significantly higher in the raw florets than in the boiled florets. In the case of K, raw florets exhibited the richest fraction, containing 1.3 times more K on average than the other two fractions. Phosphorous was significantly higher in both floret fractions (4.5 g kg^{-1} on average) compared to the Stem+Leaves fraction, with

1.4 g kg^{-1}. Sulfur concentrations exhibited a significant sequence: raw floret > boiled floret > Stem+Leaves (Table 3).

Table 3. Broccoli nutritional characteristics, phytic acid (PA) concentrations, total antioxidant activity (ABTS and DPPH), and polyphenol concentration (TPC), as affected by plant fraction (Stem+Leaves, raw florets, and boiled florets when appropriate, respectively). The results are shown as the mean ± standard error of the mean.

Parameter	Unit	Stem+Leaves	Raw Floret	Boiled Floret
Ca	g kg^{-1}	13.2 ± 0.7 a	2.6 ± 0.1 b	2.6 ± 0.1 b
K		17.1 ± 0.7 b	23.8 ± 0.5 a	18.5 ± 0.3 b
Na		618 ± 35 a	439 ± 20 b	381 ± 19 b
Mg		1.71 ± 0.06 a	1.31 ± 0.04 b	1.02 ± 0.03 c
P		3.1 ± 0.0 b	4.6 ± 0.1 a	4.4 ± 0.1 a
S		2.7 ± 0.1 c	6.8 ± 0.2 a	4.9 ± 0.1 b
Co	mg kg^{-1}	0.13 ± 0.00 c	0.26 ± 0.01 a	0.17 ± 0.01 b
Cu		1.0 ± 0.2 c	3.0 ± 0.2 a	2.3 ± 0.1 b
Fe		24.9 ± 1.9 b	38.6 ± 3.3 a	26.5 ± 1.2 b
Mn		17.4 ± 0.7 a	16.6 ± 0.6 a	14.5 ± 0.4 a
Se		11.5 ± 2.9 b	33.9 ± 8.4 a	29.0 ± 7.6 a
Zn		14.8 ± 6.8 a	27.2 ± 1.3 a	17.4 ± 0.6 a
Phytic acid	g kg^{-1}	1.6 ± 0.1 b	3.4 ± 0.7 a	-
ABTS	mg Trolox 100 g^{-1}	113 + 5 b	156 + 2 a	-
DPPH		419 ± 14 b	741 ± 14 a	-
TPC	mg GAE 100 g^{-1}	169 ± 29 b	272 ± 30 a	-

Means in a line with different letters indicate significant differences according to the LSD (least significant difference) test at $p \leq 0.05$.

In terms of micronutrients, significantly higher concentrations of Co, Cu, Fe, and Se were found in the florets. Particularly, Co and Cu were found to be significantly higher in the raw florets (0.26 mg Co kg^{-1} and 3.0 mg Cu kg^{-1}) than in the boiled florets (0.26 mg Co kg^{-1} and 3.0 mg Cu kg^{-1}), with the Stem+Leaves fraction having significantly lower levels (0.13 mg Co kg^{-1} and 1.0 mg Cu kg^{-1}). Iron concentration was also significantly higher in the raw florets (38.6 ± 3.3 mg kg^{-1}) compared to the boiled ones and the Stem+Leaves fraction. The mean Se concentration of both raw and boiled florets was 31.5 mg kg^{-1} and was 2.7-fold higher than in the Stem+Leaves fraction. However, neither Mn nor Zn concentrations differed significantly across the plant fractions (Table 3).

As shown in Table 4, only the total Se concentration was significantly influenced by Se application. The average total concentrations of macronutrients obtained were 6.1 g Ca kg^{-1}, 19.8 g K kg^{-1}, 479 g Na kg^{-1}, 1.35 g Mg kg^{-1}, 4.0 g P kg^{-1}, and 4.8 g S kg^{-1}. The average total concentrations of micronutrients were 0.19 mg Co kg^{-1}, 2.1 mg Cu kg^{-1}, 30.0 mg Fe kg^{-1}, 16.2 mg Mn kg^{-1}, and 79.1 mg Zn kg^{-1}. Notably, the total Na concentration increased by more than 50 g kg^{-1} with Se application, compared to the Control, although this difference was not statistically significant. Similarly, the application of 0.05-Se increased the Zn concentration by 32.5% compared to the Control, although this difference was not statistically significant (Table 4).

As expected, the total Se concentration increased progressively and significantly with the doses applied, going from 0.3 mg kg^{-1} in the Control to 54.0 mg kg^{-1} in 0.15-Se, multiplying by 46.7-fold from the Control to 0.05-Se (Table 4).

Studying the interaction of fraction × Se application, in all fractions, the increasing doses caused a progressive increase, with the florets, both raw and boiled, being the fractions that reached the highest concentrations. The levels were somewhat lower in the boiled florets, although without significant differences between them. Finally, the values reached by the fraction of Stem+Leaves were significantly lower at the highest doses, 0.10-Se and 0.15-Se (Figure 1). The Se concentration in the boiled floret (measured as mg Se kg^{-1}

of dry matter) and the typical 10% dry matter concentration of broccoli were considered, in order to estimate the daily Se intake provided by a 100-gram portion of boiled broccoli florets. The estimated daily Se intake, contingent on the biofortification treatment, was 3.1 µg for the Control, 142 µg for 0.05-Se, 348 µg for 0.10-Se, and 666 µg for 0.15-Se.

Table 4. Broccoli nutritional characteristics, phytic acid (PA) concentrations, total antioxidant activity (ABTS and DPPH), and polyphenol concentration (TPC), as affected by Se biofortification treatments. The results are shown as the mean ± standard error of the mean.

Parameter	Unit	Control	0.05-Se	0.10-Se	0.15-Se
Ca	g kg^{-1}	6.3 ± 0.6 a	6.2 ± 0.6 a	5.7 ± 0.6 a	6.2 ± 0.7 a
K		19.2 ± 0.4 a	19.9 ± 0.4 a	19.7 ± 0.3 a	20.4 ± 0.4 a
Na		421 ± 9 a	475 ± 12 a	504 ± 16 a	516 ± 20 a
Mg		1.34 ± 0.04 a	1.35 ± 0.03 a	1.31 ± 0.04 a	1.39 ± 0.04 a
P		3.8 ± 0.1 a	4.2 ± 0.1 a	3.9 ± 0.1 a	4.2 ± 0.1 a
S		4.7 ± 0.2 a	4.8 ± 0.2 a	4.7 ± 0.2 a	5.1 ± 0.2 a
Co	mg kg^{-1}	0.19 ± 0.01 a	0.17 ± 0.01 a	0.20 ± 0.01 a	0.20 ± 0.01 a
Cu		2.2 ± 0.2 a	2.2 ± 0.1 a	1.9 ± 0.1 a	2.2 ± 0.1 a
Fe		32.0 ± 1.4 a	27.1 ± 0.5 a	28.8 ± 1.0 a	32.2 ± 1.3 a
Mn		16.1 ± 0.4 a	16.1 ± 0.2 a	15.4 ± 0.3 a	17.0 ± 0.2 a
Se		0.3 ± 0.0 d	14.0 ± 0.6 c	31.0 ± 1.7 b	54.0 ± 2.7 a
Zn		17.8 ± 1.2 a	26.4 ± 2.7 a	16.6 ± 0.8	18.3 ± 0.9 a
Phytic acid	g kg^{-1}	4.5 ± 1.3 a	1.9 ± 0.2 b	1.6 ± 0.2 b	1.9 ± 0.1 b
ABTS	mg Trolox 100 g^{-1}	120 ± 14 c	144 ± 10 a	139 ± 10 ab	135 ± 6 b
DPPH		511 ± 73 b	600 ± 71 a	600 ± 78 a	610 ± 70 a
TPC	mg GAE 100 g^{-1}	165 ± 37 c	370 ± 28 a	219 ± 23 b	129 ± 13 c

Means in a line with different letters indicate significant differences according to the LSD (least significant difference) test at $p \leq 0.05$.

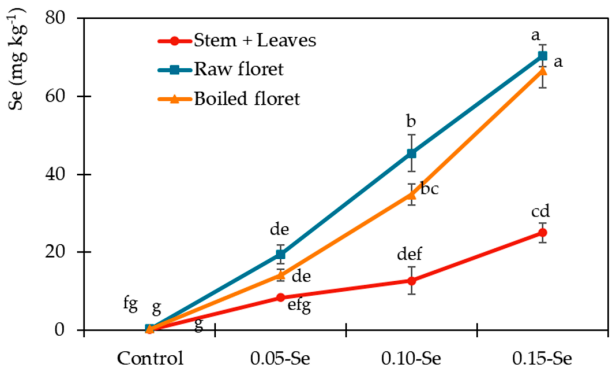

Figure 1. Effect of the interaction between the plant fraction (Stem+Leaves, raw florets, and boiled florets) and the biofortification treatment (Control, 0.05-Se, 0.10-Se, and 0.15-Se) on the total Se concentrations of the broccoli samples. The results are shown as the mean ± standard error (error bars). Different letters indicate significant differences according to Fisher's protected LSD (least significant difference) test at $p \leq 0.05$.

3.3. Effect on Phytic Acid, Antioxidant Activity and Polyphenol Concentration

The phytate (PA) antinutrient and the PA:nutrient ratios, along with total polyphenol concentration and antioxidant activity, were measured by ABTS and DPPH methods and were found to be influenced by both plant fraction and Se application, with the plant fraction × Se interaction being significant for all these parameters except via DPPH (Table 2).

The phytic acid concentration was found to be significantly higher in the raw florets, with a 2.1-fold increase. The antioxidant activities (ABTS and DPPH) were also observed to be higher in the floret than in the Stem+Leaves fraction, with a 1.4- and 1.8-fold increase, re-

spectively. Furthermore, the total phenol concentration (TPC) was found to be significantly higher in the raw florets than in the Stem+Leaves fraction, with a 1.6-fold increase (Table 3).

The application of Se at any of the studied doses resulted in a significant reduction in phytic acid concentration, with a decrease from 4.5 g kg^{-1} to less than 2 g kg^{-1} (Table 4). As illustrated in Figure 2a, there was a clear decrease in phytic acid concentration with the application of Se in the Stem+Leaves fraction. However, this reduction was not observed in the florets. In this context, the application of sodium selenate at a concentration of 0.05% resulted in a significant decrease in the phytate molar ratios studied in terms of Se (Figure 2a) and Zn (Figure 2f), in comparison to the Control. The effect on the PA:Se ratio showed no differences between Se doses, while the PA:Zn ratio had a minimum peak with the 0.05-Se treatment, which was significantly lower than the Control. However, this ratio increased again with the higher concentrations of sodium selenate (0.10-Se and 0.15-Se). Conversely, in the florets, the application of a minimum concentration of Se (0.05-Se) significantly reduced the phytate molar ratios for Se (Figure 2b), Ca (Figure 2c), Fe (Figure 2d), Mg (Figure 2e), and Zn (Figure 2f), showing no significant differences compared to the other biofortification treatments. It is crucial to highlight that all the biofortification treatments reduced the phytate:Se ratio to nearly zero for both plant fractions.

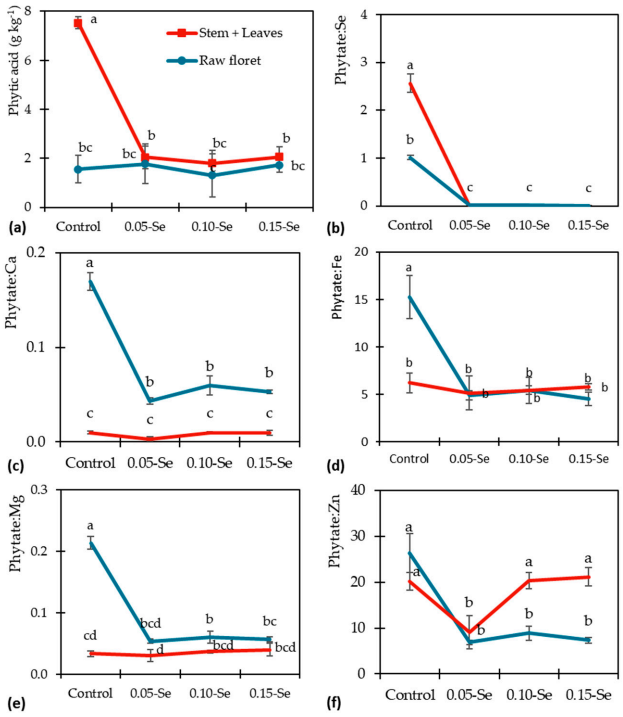

Figure 2. Effect of the interaction between plant fraction (Stem+Leaves and raw florets) and biofortification treatment (Control, 0.05-Se, 0.10-Se and 0.15-Se) on (**a**) total phytic acid concentration, along with the phytate molar ratios of (**b**) Se (PA:Se), (**c**) Ca (PA:Ca), (**d**) Fe (PA:Fe), (**e**) Mg (PA:Mg), and (**f**) Zn (PA:Zn) in the broccoli samples. The results are shown as the mean ± standard error of the mean (error bars). Different letters indicate significant differences according to Fisher's protected LSD (least significant difference) test at $p \leq 0.05$.

The antioxidant capacity increased significantly following the application of Se. In the ABTS assay, the lowest doses (0.05-Se and 0.10-Se) yielded the highest values. In contrast, in the DPPH assay, all 3 doses studied showed a significant increase of more than 100 mg Trolox 100 g^{-1} compared to the Control. However, there were no significant differences between the 3 doses. Figure 3a illustrates the effect of the interaction between the different doses of Se and plant fraction on the antioxidant capacity. While the 0.15-Se dose in the raw florets exhibited a significant decrease in ABTS concentration compared to the 0.05-Se dose, no such decrease was observed in the Stem+Leaves fraction.

With regard to total phenols (TPC), the maximum value for each plant fraction (although significantly higher in the raw floret) was obtained with the application of 0.05-Se, with 418.7 mg GAE 100 g^{-1} and 321.5 mg GAE 100 g^{-1} in the florets and Stem+Leaves fraction, respectively. This value decreased significantly and progressively with the increasing application of Se, reaching levels that were significantly lower than even the Control in the case of the raw floret (Table 4 and Figure 3b).

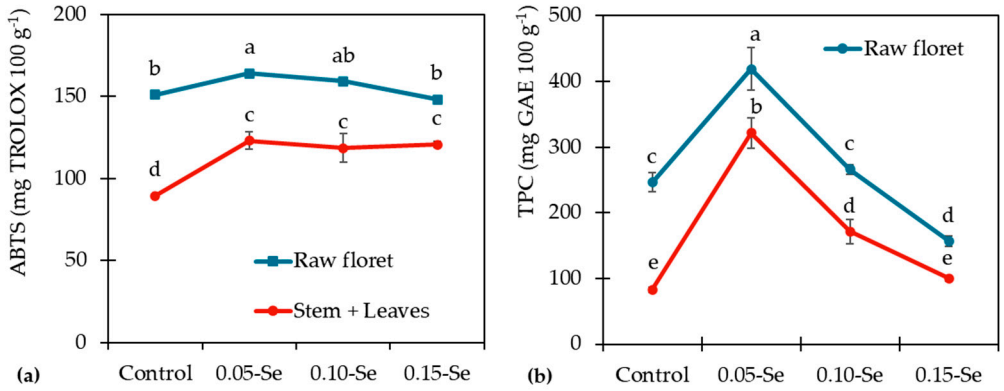

Figure 3. Effect of the interaction between the plant fraction (Stem+Leaves and raw florets) and the biofortification treatment (Control, 0.05-Se, 0.10-Se, and 0.15-Se) on (**a**) ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity and (**b**) TPC (total polyphenol concentration) of the broccoli samples. The results are shown as the mean ± standard error of the mean (error bars). Different letters indicate significant differences according to Fisher's protected LSD (least significant difference) test at $p \leq 0.05$.

4. Discussion

This study aimed to expand the knowledge base regarding Se biofortification and its effect on broccoli growth, nutrient concentration and bioavailability, and antioxidant potential. We used one variety (Green Top) to evaluate the effects of the different foliar treatments of sodium selenate on the parameters analyzed and to determine the appropriate Se dosages. To this end, the efficacy of different doses of sodium selenate (0%, 0.05%, 0.10%, and 0.15% w/v) was compared. Further research should be oriented to evaluate the different responses to these treatments, especially the effect of 0.05-Se, on a wider range of broccoli varieties and cultivars, to delve even further into the relationship between the biofortification process and the broccoli genotypes.

The foliar application of different doses of sodium selenate on broccoli growth had a significant impact on Stem+Leaves height. Specifically, the treatments with concentrations of 0.10% and 0.15% led to significant increases in the height of this plant fraction of 13.1% and 9.3%, respectively, compared to the Control group, indicating a positive effect on this particular parameter. With regard to the remaining parameters, while the 0.05-Se treatment resulted in an increase in floret weight of 11% in comparison to the Control, this improvement was not statistically significant. These results are in accordance with previous studies [49,50], which observed that the dry matter of the florets was not affected by the Se treatment or the application mode. The lack of consistent effects on broccoli growth parameters could be explained by the fact that, although it has been proven to be beneficial, this element is not considered an essential nutrient for plants [51,52]. Therefore, even when Se application can sometimes improve crop yield [53,54], especially under stress conditions [55], due to its potential to induce the concentration of antioxidant compounds in the plant to reduce the concentration of reactive oxygen species (ROS) [56], the relationship between Se application and broccoli development must be complex, with certain parameters being more responsive to Se treatments than others [57]. Additionally, considering the non-significant tendencies to higher mean values in the Se-treated plants, the specific Se concentration could have influenced this outcome [58]. In our tests, the doses were

sufficient enough to significantly increase Se concentration in the florets without them being toxic for the plant, which would have reduced the head weight, as observed in previous studies with higher doses of this element [59].

With regard to the concentration of nutrients, our study showed that the plant fraction under consideration was more decisive for the nutrient distribution than the effect of foliar Se application. Specifically, the levels of most macro- and micronutrient levels, with the exception of Mn and Zn, significantly differed depending on the plant fraction. In contrast, the application of Se and the interaction between plant fraction and Se only significantly affected the total Se concentration in broccoli. This outcome is of significant importance, as it confirms that the biofortification approach used in our study can effectively increase Se levels in the edible parts of broccoli without negatively affecting the concentrations of other nutrients. These findings are consistent with previous studies [60,61], which observed that Se application had a limited influence on nutrient bioaccessibility in plants. This outcome was confirmed by other experiments [58], which determined that the mineral statuses of cauliflower and broccoli were the least affected by sodium selenate foliar application among the *Brassica* crops.

The variations in macro- and micronutrient concentrations in different fractions of broccoli plants after the foliar application of sodium selenate exhibited a similar pattern to that observed in similar research [62], where the biofortification of broccoli was achieved by both soil and foliar applications of Zn. In said study, the nutritional relationship between the Stem+Leaves fraction and the florets was consistent with the present study, except for Mn and Zn, which did not show significant differences in our case. In both studies, the Stem+Leaves fraction showed significantly higher levels of Ca, Na, and Mg compared to the florets. These results may be explained by the fact that the delivery of Ca to developing florets is limited, while the reduced Na concentration in the floret may be part of a mechanism to contribute to the healthy mineral profile of broccoli [63,64]. Meanwhile, the florets presented higher levels of K, P, and S, as well as Co, Cu, Fe, and Se compared to the Stem+Leaves fraction. As previously outlined, the concentration of micronutrients like Cu and Fe may be possibly related to their higher mobility within the plant [65].

Raw florets showed higher levels of K, Mg, S, Co, Cu, and Fe compared to boiled florets, suggesting that cooking may affect the retention of these nutrients. Particularly, Fe concentration was notably higher in raw florets, as found previously [62], emphasizing the impact of processing on nutrient retention. However, the processing of florets did not significantly affect Ca, Na, P, and Se levels.

The results for the Se concentration were of particular importance, since the level was significantly higher in both raw and boiled florets compared to the Stem+Leaves fraction, highlighting the effectiveness of sodium selenate foliar application in enhancing Se levels in broccoli. In our study, it was observed that increasing doses led to a progressive increase in Se concentration in the different plant fractions. Specifically, the florets, both raw and boiled, exhibited the highest Se concentrations, which is in accordance with the results of other research, which found that broccoli accumulated Se mainly in the head of the plant, with less in the leaves [50].

Regarding the daily Se intake, considering an average of 10% of dry matter [35] and that the Se concentration in the boiled floret for the 0.05-Se treatment was approximately 14.2 mg kg^{-1} of dry matter, it can be estimated that a 100-gram portion of boiled broccoli florets would provide more than sufficiently the recommended amount of Se per day (142 µg vs. 55 µg) without exceeding toxic levels, established at 400 µg Se day^{-1}. Therefore, this Se application type may be the best option for increasing Se uptake in humans, compared to the other tested treatments.

Apart from nutrients, broccoli contains some antinutritional components, such as phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphoric acid), which interfere with the metabolism of nutrients like Ca, Fe, Mg, or Zn [66] and limit their absorption [67]. In our study, Se biofortification proved to be an important factor in reducing this compound in the edible part of broccoli, by reducing it at least 3.63 times in the florets when compared to the

Control (2.07 g kg^{-1} vs. 7.53 g kg^{-1}, for 0.15-Se and the Control, respectively). By reducing phytic acid concentration in broccoli, the bioavailability of these essential minerals can be enhanced, making them more accessible for absorption and utilization by the human body [68]. In this context, the PA:mineral molar ratios were found to be below the respective thresholds in the Se-biofortified plants. The PA:Ca ratio was below 0.24 [69] for all the treatments, including the Control. However, the application of Se significantly reduced it to one-third in the case of the raw florets. The PA:Fe, PA:Mg, and PA:Zn ratios exceeded the threshold values of 10 [70], 0.2 [71], and 15 [72], respectively, in the florets of the Control treatment. Nevertheless, the application of Se resulted in the reduction of these values below the aforementioned threshold for the florets in the treated plants. Furthermore, it is noteworthy that the Se application resulted in a near-complete reduction of the PA:Se ratio, indicating that the biofortification of broccoli, even with a minimal dose of 0.05%, not only has the potential to significantly enhance Se concentration but also to maximize the bioavailability of that Se concentration. This is crucial for improving the overall nutritional quality of broccoli and ensuring that individuals consuming this vegetable can benefit from its mineral concentrations.

The antioxidant activity, as measured by ABTS, and the total polyphenol concentration of broccoli were higher in the florets than in the Stem+Leaves fraction. These results are in accordance with diverse studies [73,74], since the broccoli's head normally accumulates more phenols than the broccoli stems, thereby presenting an improvement in antioxidant potential. Additionally, the antioxidant activity of broccoli, as measured by the ABTS assay, was significantly affected by the interaction between the Se applications and the plant fractions studied. In this sense, although the antioxidant activity was lower in the Stem+Leaves fraction, the application of the lowest dose of Se (0.05-Se) significantly increased the scavenging of ABTS radicals for both plant fractions, the Stem+Leaves and raw florets (123 mg Trolox 100 g^{-1} and 164 mg Trolox 100 g^{-1}), when compared to their respective Controls (89.6 mg Trolox 100 g^{-1} and 151.1 mg Trolox 100 g^{-1}). The potential of Se biofortification to improve the antioxidant activity of the treated plants has been outlined previously in other important horticultural crops such as red and green lettuce [75]. This outcome may be explained by the results obtained for the interaction between both factors in terms of the total polyphenol concentration, as these are the main compounds responsible for the antioxidant activity of this plant [37,75,76]. In this sense, the application of the 0.05-Se treatment supposed an increase of 70.1% in the polyphenol concentration of the raw florets and a 2.9-fold increase in the case of the Stem+Leaves fraction. However, it is noteworthy that the scavenging of ABTS radicals and the phenolic concentration significantly decreased with increasing Se doses beyond optimal levels, underscoring the importance of maintaining a balance in Se supplementation to maximize phenolic concentration and antioxidant properties.

5. Conclusions

Although the results indicate that higher doses of Se were more effective in significantly increasing the Se concentration in broccoli florets, the lowest Se dose (0.05-Se) was sufficient to notably increase Se concentration. Additionally, since there were no significant differences in Se concentration between the various doses in both raw and boiled florets, this means that the 0.05-Se treatment could be enough to potentially improve human nutritional status. This lower dose was also adequate to reduce phytate concentration in the florets without causing differences among the biofortification treatments. Moreover, this dose resulted in significantly higher polyphenol concentrations in both the florets and the plant, compared to other treatments. Regarding antioxidant activity, the 0.05-Se treatment increased DPPH radical scavenging compared to the Control, although no differences were observed with other treatments. Additionally, this dose significantly increased ABTS radical scavenging in the florets, matched only by the 0.10-Se treatment. These findings highlight how the application of small quantities of sodium selenate to an important horticultural crop, such as broccoli, can significantly increase both the concentration and bioavailability

of Se, as well as the total phenolic concentration in the edible parts of the plant, thereby increasing its antioxidant activity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10080808/s1, Figure S1: Greenhouse minimum and maximum temperatures (°C), together with the relative humidity during the experiment. Weekly average values are shown for each date, starting from the specified day.

Author Contributions: Conceptualization, M.J.P. and M.R.B.; methodology, M.J.P., M.R.B. and R.V.; software, C.G.-L.; validation, M.J.P., M.R.B. and R.V.; formal analysis, M.J.P. and C.G.-L.; investigation, M.J.P. and R.V.; resources, M.J.P. and M.R.B.; data curation, M.J.P.; writing—original draft preparation, C.G.-L. and M.J.P.; writing—review and editing, C.G.-L., M.J.P., M.R.B. and R.V.; visualization, M.J.P., C.G.-L. and R.V.; supervision, M.J.P. and M.R.B.; project administration, M.J.P.; funding acquisition, M.J.P. and M.R.B. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support was provided by the Ministry of Science, Education and Universities of Spain (Program: Salvador de Madariaga, PRX22/00335) for Poblaciones's stay in the Sustainable Soils and Crops Strategic Area at Rothamsted Research (UK). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors would like to thank Teodoro Garcia for his invaluable help in the laboratory work.

Conflicts of Interest: The authors declare no conflicts of interest.

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