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Foliar Biofortification of Maize (*Zea mays* L.) with Selenium: Effects of Compound Type, Application Rate, and Growth Stage

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Abstract: Nowadays, attention is focused on the lack of selenium in the average diet, which is a highly valued element in the body's antioxidant system. The major metabolites of selenium are selenoproteins, which have an irreplaceable function in the body. This study focused on optimizing conditions for the biofortification of maize (*Zea mays* L.) with selenium (Se). Three separate pot experiments were conducted to identify the key factors influencing the efficacy of foliar selenium application. The experiments were designed to investigate the effects of different forms of selenium (selenite, selenate, and selenium nanoparticles) on maize development, the influence of the phenological stage of maize at the time of foliar Se application, and the optimal application rate of Se (100, 150, 200, or 250 µg). The results indicated that sodium selenate without a wetting agent was the most effective form for enhancing total Se content in maize, with the greatest accumulation being in leaves (3.01 mg/kg dry matter). Phenological stages (BBCH) 51 and 60 were identified as the most suitable phenological stages for Se application in terms of total Se content about 1 mg/kg in leaves and about 0.4 mg/kg in grain and the presence of organic Se compounds (mostly selenate ion and selenomethionine). We concluded from the study that a foliar application of 200 µg of sodium selenate per pot during these stages resulted in maximum Se uptake without adversely affecting plant yield. Further research is recommended to validate these findings under field conditions, paving the way for improved agricultural practices in selenium biofortification.

Keywords: sodium selenate; sodium selenite; selenium nanoparticles; phenological phase; pot experiment

Citation: Mrština, T.; Praus, L.; Száková, J.; Kaplan, L.; Tlustoš, P. Foliar Biofortification of Maize (*Zea mays* L.) with Selenium: Effects of Compound Type, Application Rate, and Growth Stage. *Agriculture* **2024**, *14*, 2105. https://doi.org/10.3390/agriculture14122105

Academic Editor: Xuebin Yin

Received: 6 October 2024
Revised: 31 October 2024
Accepted: 20 November 2024
Published: 21 November 2024

1. Introduction

Selenium (Se) is an essential trace element in various physiological functions in humans and animals. As *selenocysteine* (*SeCys*), it is incorporated into the active sites of numerous selenoproteins involved in vital metabolic processes, including thyroid hormone activity, antioxidant defense, and immune functions [1]. Beneficial bioactive organic forms of Se, such as *methyl-selenocysteine* (*MeSeCys*), are effective sources of dietary selenium. Inadequate selenium intake can lead to numerous health problems, including heart disease, hypothyroidism, decreased male fertility, compromised immune function, and heightened susceptibility to infections and cancer [2,3]. It is estimated that one billion people worldwide are affected by selenium deficiency due to the low bioavailability of this element in soil on which crops are raised [4]. In livestock, selenium deficiency can manifest as white muscle disease, characterized by skeletal and heart muscle lesions [5].

Selenium in soil can occur in common forms, such as *selenate* (Se^{VI}), *selenite* (Se^{IV}), *elemental selenium* (Se^{0}), and *selenide* (Se^{II-}) [6]. Current research has predominantly focused on elucidating the mechanism of uptake and translocation of *selenate* (SeO_4^{2-}) or *selenite* (SeO_3^{2-}), administered either via the soil–root pathway or via foliar application [7]. Nanotechnology offers promising potential for enhancing the effectiveness of Se fortification because of the unique characteristics of nanomaterials, including their small size, versatile surface chemistry, and stability [8]. There is a large body of evidence in the literature indicating that, compared to inorganic Se compounds, selenium nanoparticles (SeNPs) possess superior bioactivity, enhanced bioavailability, reduced toxicity, exceptional dispersibility, and antibacterial efficacy at low doses [9].

Compared with soil application, foliar application of Se seems to be the most suitable method of enhancing the Se content of agricultural crops in terms of safety and economic justification [10]. Another benefit of this method is the relatively low consumption of Se salts in foliar application. The increased efficacy is ascribed to the lack of need for soil-to-roots-to-shoots translocation, where the Se uptake is impeded by the large selenium reservoir on seleniferous locations (parts of USA, China, i.e.,) and the proportion of available Se in the soil [11]. The foliar application of Se^{IV} or Se^{VI} has demonstrated successful enhancement of Se concentrations in various food crops, such as potatoes, rice, soybeans, cabbage, onions, garlic, radishes, buckwheat, and carrots [12].

Maize (*Zea mays* L.) stands as the most extensively cultivated cereal globally, serving dual purposes as an animal feed and in the preparation of corn-based foods for human consumption [13]. Investigations have been undertaken in numerous countries around the world to enhance the Se content of maize through soil fertilization or foliar applications, and by utilizing different selenium forms, such as selenite, selenate, organic Se compounds, and SeNPs [14]. The capacity of maize to assimilate soil Se is influenced by its availability and soil characteristics, such as pH, redox potential, organic matter, and clay content. Climatic conditions and the variability in type of maize cultivar used also affect Se uptake regardless of the application method [15]. In this study, the main objective was to discover the optimum conditions for maximum Se uptake by plants without any reduction in plant yield and to recommend to the reader the most effective way to biofortify maize and obtain an enriched product to reduce selenium deficiency. Selenium uptake after foliar application was investigated in three separate pot experiments to determine the effect of the form of Se applied, the phenological stage of maize at the time of foliar Se application, and the application rate on the efficacy of the Se treatment.

2. Materials and Methods

The pot experiments were conducted in the outdoor weather-controlled vegetation hall of the Czech University of Life Sciences (CZU), Prague. A total of 5 kg of sieved (5 mm mesh) soil from the surroundings of the Červené Janovice (Central Bohemia, Czech Republic) was chosen for the pot experiments. The soil type was characterized as haplic luvisol (clay loam) classified by World Reference Base for Soil Resources. The physicochemical properties of the soil are shown in Table 1.

Table 1. Physicochemical properties of the experimental soil.

pH	N_{min} **	P *	K *	Ca *	Mg *	S *	Se ***
(H_2O)	(mg/kg DM)						
6.4 ± 0.1	6.23 ± 0.05	55 ± 2	260 ± 36	3212 ± 36	221 ± 4	25 ± 3	0.05 ± 0.01

* Extraction by Mehlich III; ** extraction by $CaCl_2$ (0.01 mol/L); *** extraction by $(NH_4)_2HPO_4$ (0.1 mol/L); DM, dry matter.

Nitrogen was extracted using 0.01 mol/L $CaCl_2$ and then analyzed using a flow segment analyzer (Skalar system, Breda, The Netherlands). Phosphorus, potassium, calcium, magnesium, and sulfur were extracted by Mehlich III and then measured by optical emission spectrometry with inductively coupled plasma analysis (ICP-OES; Agilent, Santa Clara, CA, USA). Selenium in soil was extracted using 0.1 mol/L $(NH_4)_2HPO_4$ and then measured by mass spectrometry with inductively coupled plasma analysi (ICP-MS; Agilent, USA).

Each 6 L pot received 10 mL of fertilizer solution containing 1 g of nitrogen (N) in NH_4NO_3, 0.5 g of phosphorus (P) in $NH_4H_2PO_4$, and 0.9 g of potassium (K) in KCl. Six seeds of the maize (*Zea mays* L.) variety RGT 'Attraxxion' were sown in each pot, with the final number of plants reduced to four after emergence. The pots were randomized, and each treatment consisted of four replicates.

2.1. Experimental Design

2.1.1. Effect of Form of Selenium Applied

The first experiment aimed to determine the most suitable Se compounds for foliar maize biofortification. Sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4) (both from Sigma Aldrich, Darmstadt, Germany), and selenium nanoparticles [SeNPs (Se^0)] were tested. For each treatment, 2 × 50 µg of Se was applied per pot at the phenological phases BBCH 30 (beginning of growth elongation) (35 days after seedling emergence) and BBCH 60 (beginning of flowering of male panicles) (75 days after seedling emergence). For the control treatment, demineralized water was applied in the same amount (10 mL/pot), and four replications were performed for each treatment.

Selenium nanoparticles were synthesized via the reduction of selenium dioxide with sodium thiosulfate and subsequently stabilized with SDS (sodium dodecyl sulfate), following the modifications outlined by [16]. Additionally, each of these forms was supplemented with Silwet Star 0.1% (v/v) (polyalkyleneoxide heptamethyl trisiloxane 80%, allyloxypolyethyleneglycol 20%) (AgroBio Opava, Opava, Czech Republic) wetting agent to enhance the efficiency of biofortification by reducing the surface tension of the applied solution to ensure better penetration into the inner parts of the plants [17].

2.1.2. Choice of Optimum Phenological Stage for Se Application

The second experiment aimed to determine the optimum time for Se application. i.e., in which BBCH [18] phenological stage the Se application will achieve the maximum plant accumulation. Selenium in the form of sodium selenate (100 µg, Sigma Aldrich, Germany) was sprayed on the leaves as a solution in water for five treatments at different individual phenological stages according to the BBCH scale: BBCH 16 (6th leaf emerged), BBCH 30 (beginning of elongation), BBCH 51 (beginning of male panicle shedding), BBCH 60 (beginning of male panicle flowering), and BBCH 70 (beginning of grain formation). Additionally, split applications of 2 × 50 µg of Se per pot were applied to two treatments where different phenological stages were combined, namely BBCH 30 + 51 and BBCH 51 + 60. The control plants were sprayed with demineralized water, with four replications for each treatment.

2.1.3. Choice of Optimum Se Application Rate

In the third experiment, foliar applications of different concentrations of sodium selenate (Sigma Aldrich, Germany) were administered during the BBCH 60 phenological stage of maize (initiation of male panicle flowering). Treatments were administered at doses of 0, 100, 150, 200, and 250 µg of selenium per pot. Control plants were sprayed with demineralized water, with four replications for each treatment.

2.2. Analytical Procedures

2.2.1. Determination of Total Se Content in Maize Plants

After harvest, the plants were partitioned into leaves, grain, stover, and root segments. Each segment was weighed and subsequently subjected to drying at 35 °C. After drying to constant weight, the samples were homogenized using a grinder equipped with a sieve of 0.1 mm mesh. Samples (400 mg) were mineralized with a mixture of 8 mL of 65% nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide (H_2O_2) at 190 °C in a closed-vessel microwave system (Ethos 1, MLS GmbH, Leutkirch im Allgäu, Germany). The digested samples were analyzed using inductively coupled plasma–mass spectrometry (ICP-MS; Agilent 8900 Agilent Technologies Inc., USA) operating in hydrogen mode.

2.2.2. Selenium Speciation Analysis

Dried and ground plant samples (200 mg) were carefully weighed into 15 mL polypropylene tubes and pre-treated with 5 mL of 30 mM Tris-HCl buffer (pH 7.25) in an ultrasonic bath maintained at 38 ± 2 °C for 30 min. Afterwards, the samples were supplemented with 1 mL of 30 mM Tris-HCl buffer containing protease XIV from *Streptomyces griseus* (10 mg/mL) and protease XXIII from *Aspergillus melleus* (10 mg/mL), both sourced from Sigma Aldrich (Germany). After homogenization in a vortex mixer for 5 s, the samples were subjected to further treatment in the ultrasonic bath under the same conditions for 120 min. Following this treatment, the tubes were agitated on a rotator at 30 rpm for 30 min, centrifuged at 2690× *g* for 5 min, and filtered through a syringe filter (0.22 µm, cellulose acetate). After appropriate dilution of the filtrate, two aliquots were prepared, one for assessing the total selenium extraction efficiency and the other for speciation analysis of selenium using a chromatography–mass spectrometry technique (HPLC-ICP-MS), described by [19]. The measurement conditions and instrumental parameters adhered to those described by [20]. An isocratic elution system was used to separate five individual Se species [*selenocystine* (*SeCys$_2$*), *methylselenocysteine* (*MeSeCys*), *selenomethionine* (*SeMet*), *selenite* (*Se^{IV}*), and *selenate* (*Se^{VI}*)]. The ratio of mineral Se to the sum of Se species (Se_{min}/Se_{Σ}) was calculated as follows:

$$Se_{min}/Se_{\Sigma} = Se_{SeIV} + Se_{VI}/Se_{\Sigma} \quad (1)$$

and then converted to a decimal ratio.

2.2.3. Statistical Analysis and Calculations

Analysis of variance (ANOVA), Tukey's HSD test, and regression analysis at the significance level of $p \leq 0.05$ were performed using Statistica 12 software (Statsoft, Tulsa, OK, USA).

3. Results

3.1. Effect of Se Application on Plant Yield and Content of Total Se and Se Species

3.1.1. Effect of the Form of Selenium Applied

The effect of the applied forms of selenium on maize yield is summarized in Figure 1. The leaf weights were approximately 15.5 g/pot across all treatments. The application of SeNPs resulted in significantly higher yield in leaves, reaching 19.3 g/pot. In the grain, the lowest yield was documented in the control treatment at 16.3 g/pot, while the other treatments showed significantly higher grain yield compared to control, regardless of the Se form applied. In the case of stover, this part of the plant showed the highest yield among the analyzed plant parts, ranging from 38.7 to 50.7 g/pot. Neither the stover nor roots showed any significant differences among treatments.

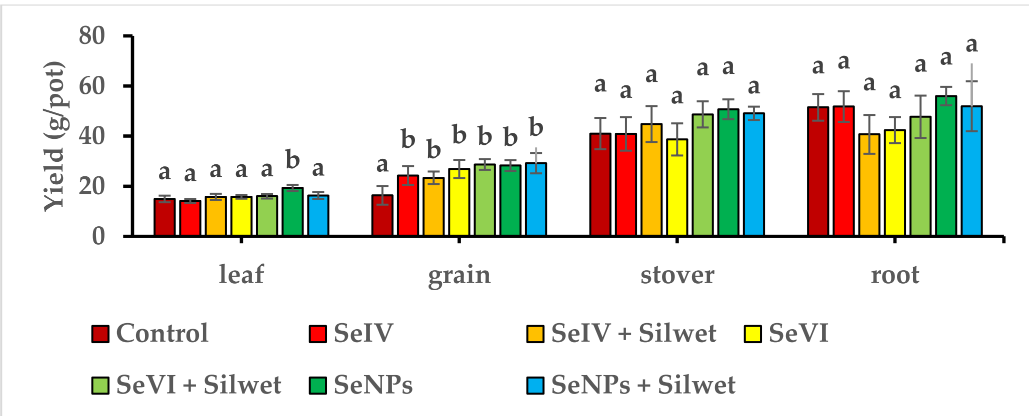

Figure 1. Yield of maize plant parts after application of different Se forms. Different lowercase letters indicate a statistically significant differences among the treatments according to a one-way analysis of variance ($p < 0.05$, $n = 4$).

Figure 2 presents concentrations of total Se in different parts of maize, with the highest Se levels recorded in the leaves, followed by the grain, stover, and roots. The most effective Se form in the leaves was selenate at 3.01 mg/kg. All selenium (Se) treatments exhibited significantly higher mean values compared to the control (without Se application) for leaves, grains, and stover. However, this trend was not observed for Se content in the roots. Only the Se^{IV} + Silwet (0.42 mg/kg) and SeNPs + Silwet (0.41 mg/kg) treatments resulted in significantly elevated root Se levels relative to the control (0.16 mg/kg). The application of SeNPs produced similar Se concentrations in leaves as the Se^{IV} + Silwet treatment.

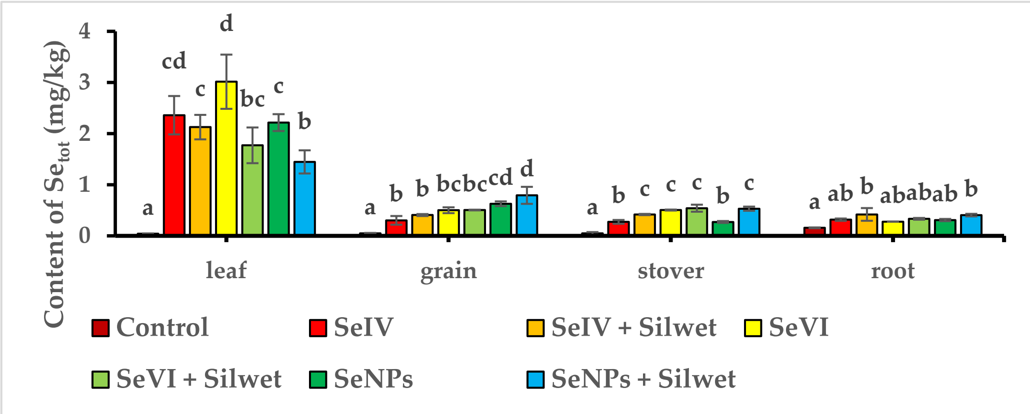

Figure 2. Total selenium content in parts of maize after application of different Se forms. Different lowercase letters indicate statistically significant differences among the treatments by a one-way analysis of variance ($p < 0.05$, $n = 4$).

The application of SeNPs did not result in a significant difference in Se content in grains compared to the Se^{VI} and Se^{VI} + Silwet treatments. However, the use of SeNPs and SeNPs + Silwet proved to be more effective when compared to the Se^{IV} and Se^{IV} + Silwet treatments (Figure 2). The highest accumulation of SeNPs was observed in maize grain, where the Se concentration reached 0.63 mg/kg (0.79 mg/kg with the use of a wetting agent). The positive effect of the wetting agent was observed for selenite as well. In the roots, there was no difference between treatments with and without Silwet. However, when examining Se levels in the stover, notable differences were detected with the use of Silwet, both between the Se^{IV} and Se^{IV} + Silwet treatments, as well as between SeNPs and SeNPs + Silwet.

3.1.2. Choice of Optimum Phenological Stage for Se Application

The yields of maize leaves ranged from 41.8 to 52.3 g/pot, and there was no significant effect of Se application time. No significant difference was observed between the means (Figure 3). The timing of Se application had no effect on grain productivity. For the maize stover, the yields tended to increase in all the treated variants compared to the control, although these observations were statistically significant only in the case of BBCH 60 (Figure 3).

The highest Se content in leaves was about 1 mg/kg with treatments at BBCH stages 51, 60, and 70, and for the combined treatment at BBCH 51 + 60 (Figure 4). These differences were statistically significant compared with treatments in other phases. An interesting observation is that the Se application at the BBCH 16 stage was the only treatment that did not lead to a significant difference in foliar Se levels compared to the control (Figure 4). Maize grain incorporated comparable quantities of Se at all BBCH stages, and the differences were significantly greater than in control. In contrast, maize stover exhibited the lowest Se accumulation. The application of Se at stages BBCH 51, 60, 70, 30 + 51, and 51 + 60 led to a significant accumulation of Se in the stover compared to the control. In roots, no unambiguous trend was observed.

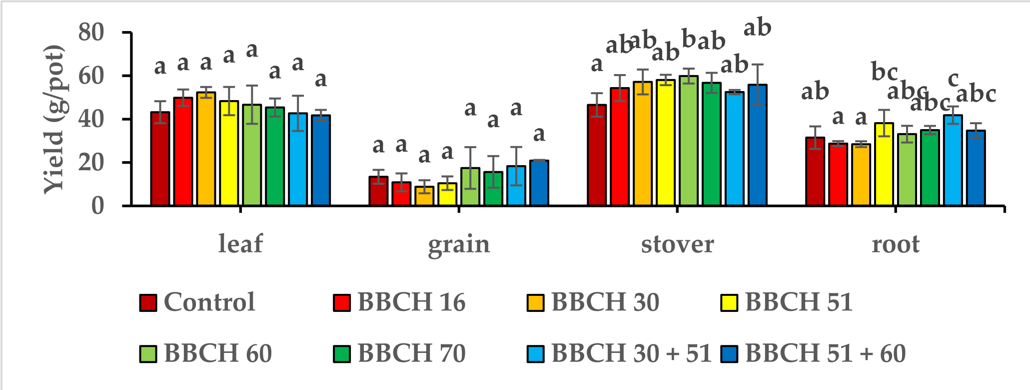

Figure 3. Yields of maize parts after the application of Se in different phenological phases. Different lowercase letters indicate statistically significant differences among the treatments by a one-way analysis of variance ($p < 0.05$, $n = 4$).

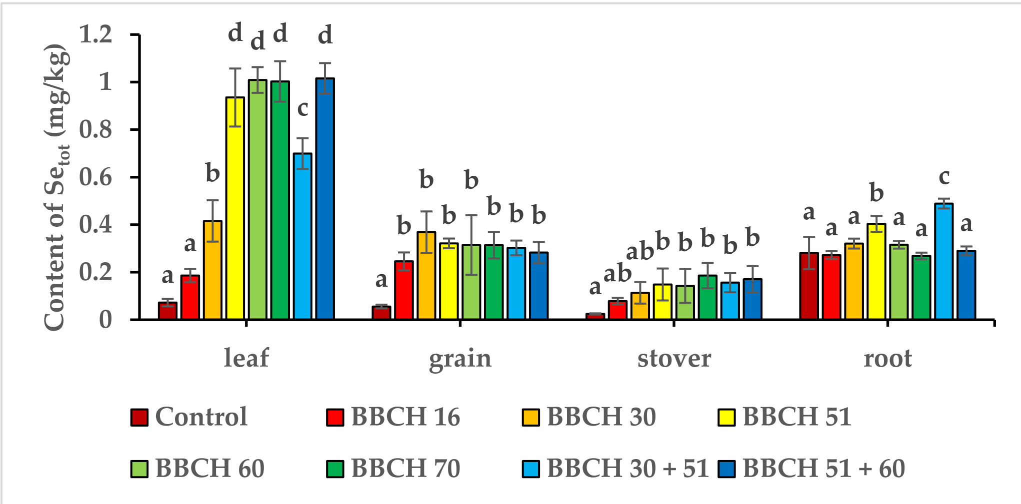

Figure 4. Contents of total selenium in maize parts (*Zea mays* L.) after application of Se in different phenological phases. Different lowercase letters indicate statistically significant differences among treatments according to a one-way analysis of variance ($p < 0.05$, $n = 4$).

3.1.3. Choice of Optimum Se Application Rate

The yield of leaves significantly differed under the 250 µg/pot treatment (37.8 g/pot), compared to the lowest determined yield under the 200 µg/pot treatment (34.3 g/pot), but the difference was relatively small, at about 10% (Figure 5). Maize grain yields were consistent across the 0, 150, and 200 µg/pot treatments, yielding 12.4, 12.0, and 11.8 g/pot, respectively. The lowest yield was observed for the 250 µg/pot treatment, which suggests that high levels of sodium selenate may have growth-inhibiting effects. For the stover, the lowest yields were observed in the control treatment, while the highest yields were recorded in the 200 µg/pot treatment. The difference between these treatments was 14.4%.

Similar to the results of the previous experiments in this study with a stepwise increase in Se treatment, maize leaves accumulated the highest Se content with increasing applications up to 200 µg/pot (Figure 6). However, the difference between the 200 and 250 µg/pot treatments was negligible at 2.71%, indicating that an accumulation plateau had likely been reached. The study reconfirmed our conclusion that the maize stover accumulated the lowest amount of Se among the parts analyzed. Selenium accumulation in the stover ranged from 0.01 to 0.35 µg for treatments with Se concentrations from 0 to 250 µg per pot, respectively. A significant effect of Se application on Se accumulation in roots was recorded between 0, 200, and 250 µg/pot, at 0.14, 0.28, and 0.44 mg/kg, respectively.

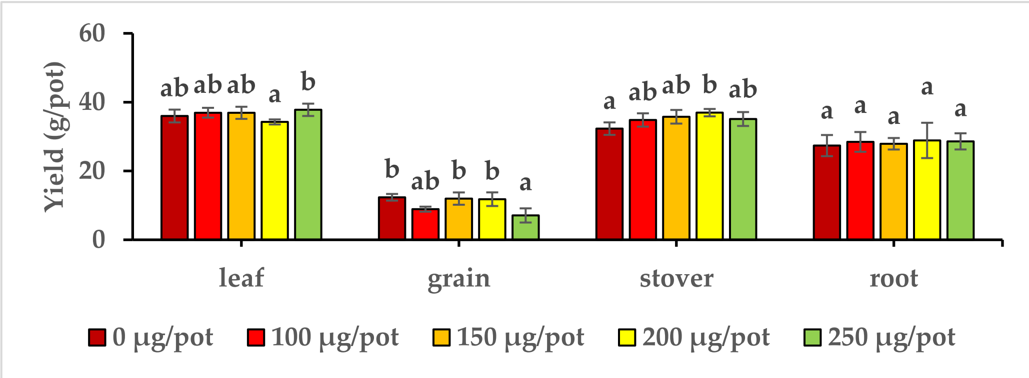

Figure 5. Yields of maize (*Zea mays* L.) parts after the application of different Se concentrations. Different lowercase letters indicate statistically significant differences among the treatments according to a one-way analysis of variance ($p < 0.05$, $n = 4$).

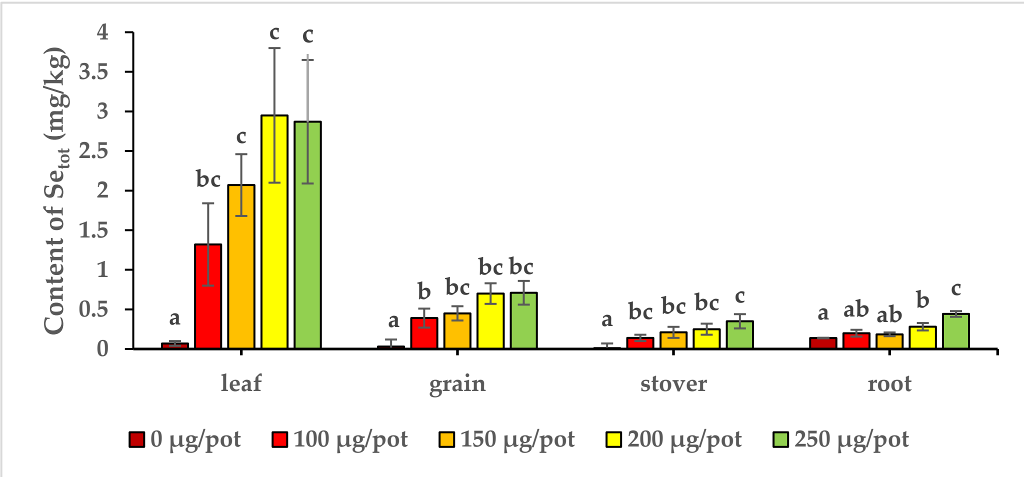

Figure 6. Content of total selenium in maize parts (*Zea mays* L.) after the application of different concentrations of Se. Different lowercase letters indicate a statistically significant differences among the treatments according to the one-way analysis of variance ($p < 0.05$, $n = 4$).

3.2. Selenium Speciation

3.2.1. Effect of Form of Selenium Applied

Selenate was the predominant selenium species in maize leaves subjected to Se^{VI} and Se^{VI} + Silwet treatments, with concentrations of 1.685 and 1.339 mg/kg, respectively (Table 2). Notably, SeNPs elevated the selenite content, resulting in a concentration of 0.588 mg/kg, which was comparable to the selenite content observed in the Se^{IV} and Se^{IV} + Silwet treatments. We conclude that inorganic compounds were more prevalent than organic species in the maize leaves, resulting in a higher ratio of Se_{min}/Se_{Σ} relative to the control.

Conversely, organic species were predominant in the maize grain, as evidenced by the Se_{min}/Se_{Σ} ratios from 0.10–0.30 ratio for the selenized treatments (Table 3). Specifically, *SeMet* was highest in the Se^{VI} + Silwet treatment (0.24 mg/kg), with similar concentrations found in the SeNPs + Silwet and Se^{IV} + Silwet treatments, at 0.23 and 0.22 mg/kg, respectively, but no statistical difference was found. Thus, for all treatments, the addition of the wetting agent enhanced the accumulation of *SeMet*, resulting in higher concentrations in the grain compared to treatments without the wetting agent. However, this claim is not statistically significant. This trend is especially interesting, as the kernels were not yet developed at the time of Se application. It suggests that the wetting agent could not have directly influenced the accumulation of each form of Se, but some shifts in the Se transformation ability due to the wetting agent can be speculated and this deserves further research.

Table 2. Content of Se species in maize leaf after application of different forms of Se.

Forms / **Treatments**	***SeCys₂***	***Se^IV***	***SeMet***	***Se^VI***	**Ratio Se_{min}/Se_{Σ}**
		(mg/kg)			
Control	0.006 ± 0.01 [a]	0.010 ± 0.01 [a]	0.014 ± 0.01 [a]	0.005 ± 0.01 [a]	0.34
Se^{IV}	0.041 ± 0.01 [bc]	0.676 ± 0.02 [b]	0.068 ± 0.01 [b]	0.213 ± 0.02 [a]	0.85
Se^{IV} + Silwet	0.043 ± 0.01 [bc]	0.548 ± 0.02 [bc]	0.080 ± 0.01 [b]	0.150 ± 0.01 [a]	0.80
Se^{VI}	0.023 ± 0.01 [ab]	0.132 ± 0.01 [ab]	0.058 ± 0.01 [b]	1.685 ± 0.25 [b]	0.93
Se^{VI} + Silwet	0.056 ± 0.01 [c]	0.084 ± 0.02 [a]	0.065 ± 0.02 [b]	1.339 ± 0.11 [b]	0.89
SeNPs	0.034 ± 0.01 [b]	0.588 ± 0.13 [b]	0.071 ± 0.01 [b]	0.092 ± 0.01 [a]	0.82
SeNPs + Silwet	0.038 ± 0.01 [b]	0.393 ± 0.01 [abc]	0.080 ± 0.01 [b]	0.142 ± 0.01 [a]	0.76

Different lowercase letters indicate statistically significant differences between the treatments in a specific parameter according to a one-way analysis of variance ($p < 0.05$, $n = 2$).

Table 3. Content of Se species in maize grain after application of different forms of Se.

Forms / **Treatments**	***SeCys₂***	***Se^IV***	***SeMet***	***Se^VI***	**Ratio Se_{min}/Se_{Σ}**
		(mg/kg)			
Control	0.002 ± 0.01 [a]	0.019 ± 0.01 [b]	0.027 ± 0.01 [a]	0.025 ± 0.01 [a]	0.53
Se^{IV}	0.025 ± 0.01 [ab]	0.011 ± 0.01 [ab]	0.086 ± 0.01 [a]	<0.05	0.27
Se^{IV} + Silwet	0.056 ± 0.04 [ab]	0.007 ± 0.01 [ab]	0.222 ± 0.11 [a]	<0.05	0.15
Se^{VI}	0.039 ± 0.01 [ab]	0.005 ± 0.01 [a]	0.142 ± 0.02 [a]	<0.05	0.19
Se^{VI} + Silwet	0.065 ± 0.02 [ab]	<0.05	0.238 ± 0.11 [a]	<0.05	0.22
SeNPs	0.056 ± 0.01 [ab]	<0.05	0.179 ± 0.02 [a]	<0.05	0.26
SeNPs + Silwet	0.078 ± 0.01 [b]	<0.05	0.232 ± 0.04 [a]	<0.05	0.22

Different lowercase letters indicate statistically significant differences between the treatments in a specific parameter according to one-way analysis of variance ($p < 0.05$, $n = 2$).

The part of the maize plant with the lowest abundance of all the Se species was the stover. The highest selenate content, not statistically significant, was observed in the Se^{VI} and Se^{VI} + Silwet treatments, with both treatments accumulating approximately 0.11 mg/kg Se in the stover. The organic *SeMet* content was particularly interesting, especially for the SeNPs + Silwet treatment, where the Se content was 0.13 mg/kg. As with the maize grain, there was a significant effect of the wetting agent on Se accumulation in all stover treatments. Again, the results indicated better conversion of Se into the organic species if the wetting agent was used (Table 4).

Table 4. Content of Se species in maize stover after application of different forms of Se.

Forms / **Treatments**	***SeCys₂***	***Se^IV***	***SeMet***	***Se^VI***	**Ratio Se_{min}/Se_{Σ}**
		(mg/kg)			
Control	0.001 ± 0.01 [a]	0.019 ± 0.01 [a]	0.020 ± 0.01 [a]	0.042 ± 0.01 [a]	0.60
Se^{IV}	0.016 ± 0.01 [ab]	0.023 ± 0.02 [a]	0.095 ± 0.01 [ab]	0.005 ± 0.01 [a]	0.15
Se^{IV} + Silwet	0.018 ± 0.01 [ab]	0.026 ± 0.01 [a]	0.110 ± 0.01 [b]	0.005 ± 0.01 [a]	0.15
Se^{VI}	0.014 ± 0.01 [a]	0.004 ± 0.01 [a]	0.054 ± 0.02 [bc]	0.118 ± 0.01 [a]	0.51
Se^{VI} + Silwet	0.022 ± 0.01 [ab]	0.009 ± 0.01 [a]	0.097 ± 0.01 [ab]	0.106 ± 0.01 [a]	0.40
SeNPs	0.014 ± 0.01 [a]	0.018 ± 0.01 [a]	0.067 ± 0.01 [bc]	0.001 ± 0.01 [a]	0.34
SeNPs + Silwet	0.026 ± 0.01 [b]	0.035 ± 0.01 [a]	0.127 ± 0.01 [c]	0.010 ± 0.01 [a]	0.18

Different lowercase letters indicate statistically significant differences between the treatments in a specific parameter according to a one-way analysis of variance ($p < 0.05$, $n = 2$).

3.2.2. Choice of Optimum Phenological Stage for Se Application

Table 5 shows that the predominant selenium (Se) species identified in the leaves was the *selenate ion* (Se^{VI}) with smaller concentrations of *selenocystine* ($SeCys_2$) and *selenomethionine* (*SeMet*).

Table 5. Content of Se species in maize leaf after the application of Se in different phenological phases.

Treatments Timing	$SeCys_2$	Se^{IV}	SeMet (mg/kg)	Se^{VI}	Ratio Se_{min}/Se_{Σ}
Control	0.002 ± 0.01 [a]	0.005 ± 0.01 [a]	0.008 ± 0.01 [a]	0.006 ± 0.02 [a]	0.11
BBCH 51	0.044 ± 0.01 [a]	0.014 ± 0.02 [a]	0.092 ± 0.04 [b]	0.338 ± 0.01 [b]	0.65
BBCH 60	0.097 ± 0.06 [a]	0.023 ± 0.01 [a]	0.077 ± 0.01 [ab]	0.546 ± 0.07 [c]	0.72
BBCH 70	0.039 ± 0.03 [a]	0.010 ± 0.01 [a]	0.135 ± 0.01 [c]	0.349 ± 0.01 [b]	0.64
BBCH 51 + 60	0.080 ± 0.08 [a]	0.034 ± 0.02 [a]	0.144 ± 0.01 [c]	0.540 ± 0.03 [c]	0.68

Different lowercase letters indicate statistically significant differences between the treatments in a specific parameter according to a one-way analysis of variance (p < 0.05, n = 2).

The highest concentrations of Se^{VI} were observed at the BBCH 60 growth stage, and significant levels of both inorganic and organic selenium forms were measured, with selenate reaching 0.546 mg/kg, *SeMet* at 0.077 mg/kg, and $SeCys_2$ at 0.097 mg/kg. However, inorganic selenium was most abundant in the leaves, as indicated by the range of values of the ratio Se_{min}/Se_{Σ} from 0.60 to 0.70 outside the control treatment (Table 6).

Table 6. Content of Se species in maize grain after the application of Se in different phenological phases.

Treatments Timing	$SeCys_2$	Se^{IV}	SeMet (mg/kg)	Se^{VI}	Ratio Se_{min}/Se_{Σ}
Control	0.002 ± 0.01 [a]	<0.01	0.011 ± 0.02 [a]	0.002 ± 0.01 [a]	0.34
BBCH 51	0.034 ± 0.01 [a]	<0.05	0.106 ± 0.02 [b]	0.043 ± 0.01 [ab]	0.33
BBCH 60	0.031 ± 0.02 [a]	<0.05	0.108 ± 0.03 [b]	0.026 ± 0.01 [ab]	0.29
BBCH 70	0.057 ± 0.02 [a]	<0.05	0.057 ± 0.01 [ab]	0.067 ± 0.02 [b]	0.42
BBCH 51 + 60	0.022 ± 0.01 [a]	<0.05	0.074 ± 0.01 [ab]	0.029 ± 0.01 [ab]	0.35

Different lowercase letters indicate statistically significant differences between the treatments in a specific parameter according to a one-way analysis of variance (p < 0.05, n = 2).

Organic Se compounds were predominantly abundant in maize grain, with the highest statistically significant differences of *SeMet* observed at the BBCH 51 and BBCH 60 growth stages, measuring 0.106 mg/kg and 0.108 mg/kg, respectively. The ratio was more inclined towards organic selenium in the grain (0.30 to 0.40). Mineral forms of selenium, specifically *selenate* Se^{VI}, were more prevalent at later growth stages, such as BBCH 70, where concentrations reached 0.067 mg/kg (Table 7).

Table 7. Content of Se species in the maize stover after application of Se in different phenological phases.

Treatments Timing	$SeCys_2$	Se^{IV}	SeMet (mg/kg)	Se^{VI}	Ratio Se_{min}/Se_{Σ}
Control	0.001 ± 0.01 [a]	<0.01	0.008 ± 0.01 [a]	0.002 ± 0.01 [a]	0.39
BBCH 51	0.021 ± 0.01 [a]	<0.05	0.027 ± 0.01 [a]	0.005 ± 0.01 [a]	0.51
BBCH 60	0.007 ± 0.01 [a]	<0.05	0.023 ± 0.01 [a]	0.078 ± 0.02 [b]	0.62
BBCH 70	0.033 ± 0.02 [a]	<0.05	0.007 ± 0.01 [a]	0.064 ± 0.01 [b]	0.56
BBCH 51 + 60	0.016 ± 0.01 [a]	<0.05	0.013 ± 0.01 [a]	0.074 ± 0.02 [b]	0.61

Different lowercase letters indicate a statistically significant difference between the treatments in one parameter according to a one-way analysis of variance (p < 0.05, n = 2).

In the maize stover, selenate ions predominated during the later application stages of BBCH 60 and 70 and the combined application stages, BBCH 51 + 60, as indicated by higher Se_{min}/Se_{Σ} ratios of 0.50 to 0.60, relative to the control treatment.

3.2.3. Choice of Optimum Se Application Rate

When stepwise rates of sodium selenate were administered, a linear trend of increasing Se content, where a similar response for all the Se species was expected (Figure 7).

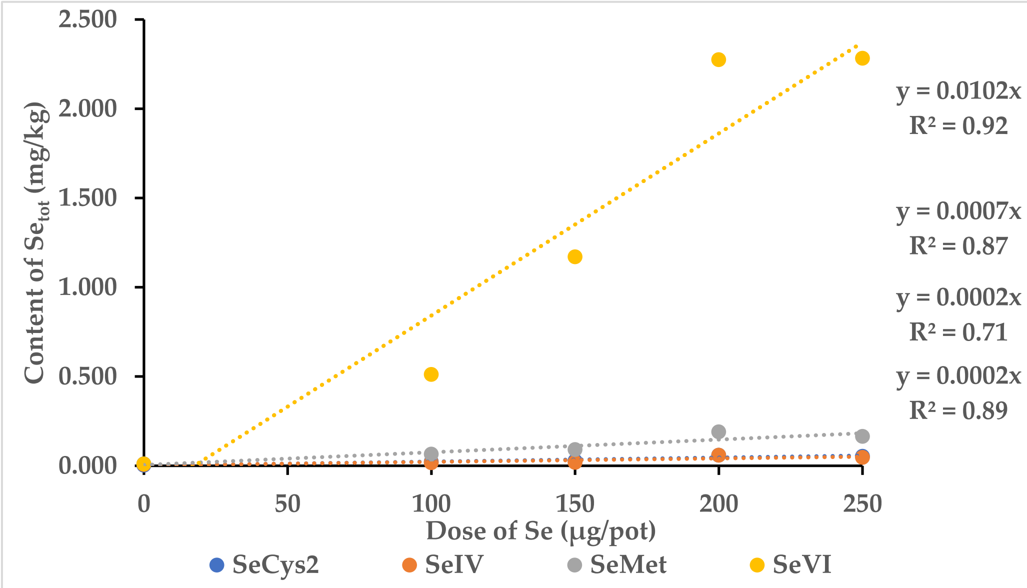

Figure 7. Regression curve slopes modelling the relationship between the applied Se dose and Se content in maize leaf.

This is particularly apparent for the *selenate ion* (Se^{VI}), where the difference between doses of 200 µg/pot and 250 µg/pot was merely 0.35%, with almost identical Se concentrations of 2.28 mg/kg. Similar patterns were observed for *SeMet*, $SeCys_2$, and Se^{IV} forms (Figure 8).

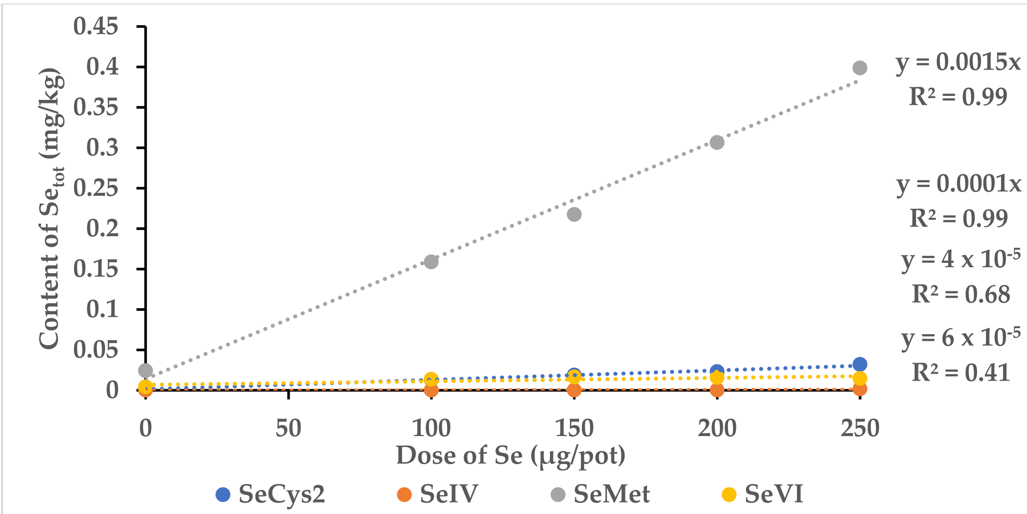

Figure 8. Regression curve slopes modelling the relationship between the applied Se dose and Se content in maize grain.

The anticipated linear trend was not observed for Se^{VI} in maize grain. Instead, the predominant Se species was the organic form, primarily *SeMet*, with a concentration of 0.398 mg/kg for the 250 µg/pot treatment. For *SeMet*, there was a clear dose-dependent increase in content. For $SeCys_2$ at the 250 µg/pot rate the abundance of this compound increased significantly compared to the 200 µg/pot rate (Figure 9).

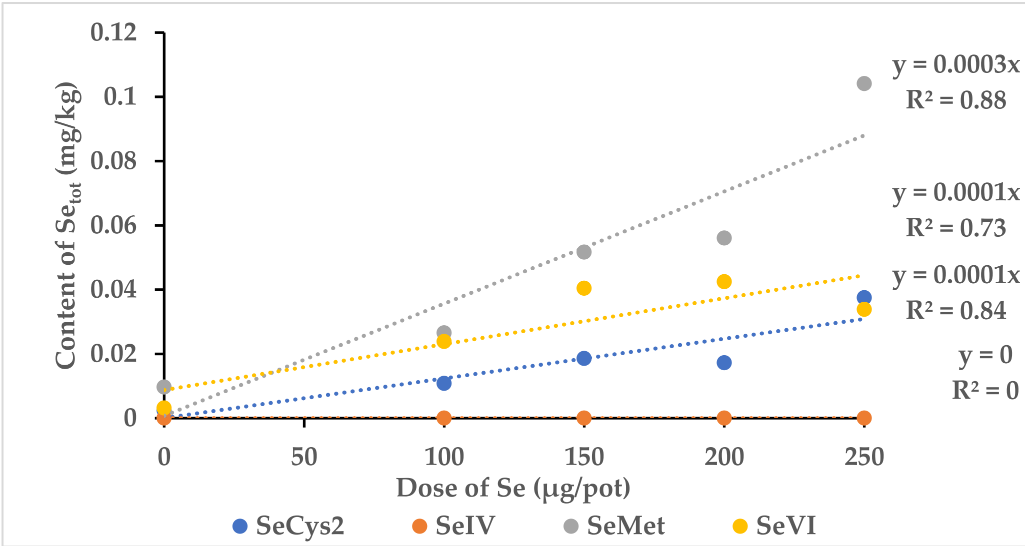

Figure 9. Regression curve slopes modelling the relationship between the applied Se dose and Se content in maize stover.

Se^{IV} concentrations were below detection limits. The maize stover primarily contained the organic selenium compounds *SeCys*$_2$ and *SeMet*. However, Se^{VI} was also detected, with the highest concentrations at 150 and 200 µg/pot, representing values of 0.040 and 0.042 mg/kg, respectively.

4. Discussion

Our results indicate that the use of different selenium forms did not significantly affect maize yield. This is consistent with the findings of [15], who reported no significant differences in yield across various plant parts, including grain and stover, following the application of an aqueous sodium selenate solution to maize in comparison with control treatments. It was found that the application of sodium selenite did not influence grain yield across various maize cultivars [21]. Similarly, some authors observed no effect on seed and straw yield in soybean, a protein crop, after sodium selenate application compared with the control treatment [19].

A comparative study on various forms of selenium across different crops was conducted to assess the effect of selenization on yield, as documented by other researchers [6]. Their findings indicated that selenate was the most effective form of Se for foliar application on maize. Across various crops, selenate generally proved to be more effective than selenite. In crops with a higher protein content, such as soybean, it was observed that these crops also accumulated significantly higher contents of Se following selenate application in comparison with other forms [19]. In this study, the application of sodium selenate was confirmed as the most effective method for elevating the total selenium concentrations in the leaves and other parts of maize. However, the use of a wetting agent to improve the efficacy of selenate application was not supported by the findings and, in fact, had a negative impact on selenate uptake in leaves. We observed that selenate treatment alone on leaves resulted in a 41% higher selenium uptake compared to treatments that included a wetting agent. This contradicts the findings of [22], who reported enhanced selenium uptake by plants with the use of a wetting agent. It was also reported that a wetting agent helped tomato plants maintain their ionic balance [23]. In addition, [24] showed that wetting agents increased the wettability of the plant surface, and [25] claimed that some wetting agents may penetrate the plant cuticle, enhance water conductance, and increase foliar uptake of applied solutions.

Nanoencapsulation is a popular technique for delivering a variety of compounds into cells with enhanced stability and penetrability. Thus, we synthesized nanoparticles, modified according to [16]. The mean diameter was 20 nm ± 6 nm, which was within the nanoparticle size recommended by [26] for optimal plant uptake. In our investigation, the SeNPs exhibited good uptake by leaves, reaching 2.21 mg/kg, and significant uptake in maize grain at 0.79 mg/kg when used with a wetting agent. According to the study, wheat accumulated more selenium in the leaves following selenite application compared to selenate application [7]. They also observed a significant variation in Se accumulation in the leaves when the application was made during the later growth stage, with wheat accumulating 15.05 mg/kg after selenite application during grain filling, compared with 13.47 mg/kg when applied at the flowering stage. Here, we observed the opposite trend, where selenate application resulted in higher Se content in the leaves compared to selenite application. Furthermore, selenate application during the later growth stages (BBCH 51, 60, and 70) did not increase Se accumulation.

Our results revealed that the Se content in leaves was highest at growth stages 51, 60, and 70. The Se concentration in maize grain remained relatively stable if Se was applied at BBCH 30 or later. Conversely, other authors observed a different trend, reporting an increase in grain Se accumulation in wheat from the panicle stage to the grain filling stage [27]. Similar conclusions were reached by [28], who found that, on average, wheat had a 14.7% greater ability to accumulate Se at the later growth stage (visible flag leaves) compared to the tillering stage. Potatoes also demonstrated an increased ability to accumulate Se in leaves, stems, and roots when selenate was applied at later stages of tuber growth and development, compared to earlier stages. However, the highest Se levels occurred during the tuber filling stage [29]. Further studies need to be carried out to determine the optimal conditions for Se application and accumulation with regard to the transformation ability of different crops.

A linear increase was observed for selenate doses up to 200 μg/pot, with a plateau occurring at 250 μg/pot. It was demonstrated that higher doses resulted in increased Se concentrations, particularly in leaves [30]. They applied Se to wheat leaves at rates of 20 g/ha and 100 g/ha to observe a consistent elevation in Se content within the plants. This finding was corroborated by [10], who reported similar results when Se was applied to carrots. A linear increase in Se content in whole wheat plants following the application of increasing rates (5–20 g/ha) was also confirmed by [31]. Utilizing maize as an experimental plant, authors observed a linear trend of Se accumulation in shoots without evidence of a plateau [32].

The content of specific bioactive Se compounds in maize and their distribution among plant parts represent crucial factors in the evaluation of the nutritional value of the Se-enriched maize. It was demonstrated that *SeMet*, the predominant organic form of Se in maize grain, represented over 90% of the Se found in the grain [33]. In this work, *SeMet* was also identified as the most abundant Se species in maize grain, with a peak of 89.5% observed at the Se rate of 250 μg per pot. It can be concluded that following the application of sodium selenite, maize leaves exhibit an increased concentration of mineral Se forms, primarily selenite ions. This is attributed to the fact that leaves serve as contact organs through which Se enters in its primary form and can subsequently be stored in the plant's vacuoles [34].

Conversely, there was a higher content of organic Se in the grain, primarily *SeMet*, and to a lesser extent, $SeCys_2$, at the expense of mineral forms. This observation was corroborated by [35], who found similar patterns in wheat, noting that *sodium selenate* application resulted in elevated levels of inorganic Se in the leaves and increased levels of organic Se in the grain. Additionally, it was confirmed that the application of Se at rates of 15, 40, and 100 g/ha in a field experiment resulted in a higher percentage of organic forms of Se in soybean seeds, from 2.5 to 16.1 mg/kg, relative to inorganic Se [18].

We identified two pathways to meet the minimum Se intake for humans: supplementation through grain and through animal products (specifically dairy products). The total Se uptake for both pathways was calculated and compared to a control treatment.

4.1. Grain Pathway

The average total Se uptake by grains was 7.48 µg/pot in the best treatment, compared to 0.65 µg/pot in the control. The average grain yield was 18.72 g/pot under optimal conditions and 14.03 g/pot for the control. Based on these values, a human would need to consume 138 g of optimally supplemented grain to meet the minimum daily Se requirement of 55 µg/day. In contrast, to achieve the same intake using the control grain, a person would need to consume 1188 g of grain.

4.2. Animal Pathway

The average total Se uptake by biomass for the optimal application was 66 µg/pot, whereas the control resulted in a total uptake of only 3.88 µg/pot. The average yield of biomass was 96.11 g/pot for the optimal application and 85.35 g/pot for the control. Conversely, 1 kg of biomass from the control supplied only 15.73 µg Se, falling significantly short of the requirement.

Se_{min} was the predominant form of Se in maize leaves across all experiments, exhibiting an average Se_{min}/Se_{Σ} ratio of 0.86, whereas organic Se was the most prevalent species in maize grain. The uptake of *SeNPs* needs to be considered as a special case, because their concentrations significantly differed from the control in maize leaves, grain, and stover, with values of 2.22, 0.63, and 0.27, respectively.

The major recommendation for further research is to test this biofortification methodology in field experiments and to determine the optimal foliar Se application rate and phenological phase for field conditions. These insights will contribute to a deeper understanding of selenium's role in maize cultivation, paving the way for improved agricultural practices.

5. Conclusions

Our findings highlight the significant impact of selenium form and application timing on maize Se content, with information about specific dosages for optimizing these effects.

Sodium selenate without a wetting agent was identified as the most suitable form for maize biofortification, as it provided high total Se levels (about 3 mg/kg Se in leaves, 0.4 mg/kg Se in grain, 0.4 mg/kg Se in stover and 0.2 mg/kg Se in roots) and a good representation of individual Se species in various maize parts. The most suitable phenological stages for selenate application, in terms of total Se content and species representation, were BBCH 51, 60, and the combination of 51 and 60 (about 1 mg/kg of Se in leaves). The optimal application Se dose in a pot is 200 µg/pot. However, it should be noted that the applied doses were per pot, and a correction for field application is necessary. Thus, the optimal application should involve sodium selenate applied between BBCH stages 51 and 60 at a rate of 200 µg per pot.

Author Contributions: T.M.: methodology, writing, data curation, visualization—original draft, investigation; L.P.: methodology, analysis, writing—review and editing; J.S.: supervision, writing—review and editing; L.K.: investigation; P.T.: supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the NAZV project QK22010037: "The implementation of selenization measures into the agricultural systems for production of milk and dairy products as functional food".

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available in FigShare at [https://doi.org/10.6084/m9.figshare.27205065.v1].

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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