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Efficacy and Comparison of Different Strategies for Selenium Biofortification of Tomatoes

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Abstract: At appropriate concentrations, selenium (Se) is beneficial for humans. Tomato appears to be one of the best commodities for producing Se-biofortified fruit for dietary supplementation. To assess the efficacy of different enrichment protocols, a total of four on-plant and off-plant trials were conducted. Hydroponically grown tomato plants were sprayed with: (i) chemically synthesized Se nanoparticles (SeNPs) at 0, 1, and 1.5 mg Se L⁻¹ at blooming; (ii) sodium selenate (Na₂SeO₄) or SeNPs solution at 0, 5, and 10 mg Se L⁻¹ when the fruit entered the immature green stage. With regard to the off-plant trials, harvested mature green fruit were immersed in Na₂SeO₄ solution: (iii) at 0, 5, 10, and 20 mg Se L⁻¹ for 15 s under a vacuum; (iv) at 0, 40, and 80 mg Se L⁻¹ for 1 h. Spraying Na₂SeO₄ induced higher Se accumulation in plant tissue than SeNPs: both protocols were effective in enriching tomatoes. Postharvest Se enrichment via vacuum infiltration caused textural damage, whereas passive immersion in solution induced fruit Se accumulation without causing any damage. SeNPs appear to be quantitatively less effective than Na₂SeO₄, but might be environmentally safer. Elemental Se carried by NPs may be more easily incorporated into organic forms, which are more bioavailable for humans. Passive immersion may represent an alternative Se-enrichment strategy, allowing for the biofortification of harvested tomato fruit directly, with lower risks of environmental pollution.

Keywords: selenate; selenium nanoparticles; nutraceuticals; *Solanum lycopersicum*; foliar spraying; postharvest selenium enrichment



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1. Introduction

Nutraceuticals have become a competitive alternative to more artificial forms of food supplements, and thus the biofortification of edible plant organs is of great interest to the food industry.

Selenium (Se) plays several crucial roles in the human metabolism [1,2]. At appropriate concentrations, it is essential for DNA synthesis, fertility, reproduction, and muscle function. Se can reduce the toxic effect of mercury, contribute to muscle growth, and is essential for the synthesis of the thyroid hormone [3]. It also protects brain cells from oxidative damage and prevents mutations [4].

The margin between what are essential amounts of selenium and what is toxic is quite narrow, with a recommended dietary allowance (RDA) of 55 µg/day for adults, and a tolerable upper intake of 400 µg/day [5]. Approximately one billion people around the world are estimated to have insufficient Se intake [6]. Se uptake less than 20 µg/day may cause diseases including cancer, Parkinson's and Alzheimer's disease, thyroidal dysfunctions, and male infertility [7], as well as fatal Keshan cardiopathy and Kashin–Beck disease [8]. In healthy individuals, an appropriate intake of Se helps slow ageing, prevents

certain cancers, and reduces the incidence of viral infections, cardiovascular damage, arthritis, and alterations in the immune system [9].

People living in areas with low levels of Se in the soil have to replenish Se deficiency through medicines or nutraceuticals containing the microelement in mineral and/or organic forms. The mineral forms, including selenate and selenite, appear to be more toxic and less bioavailable for humans than the organic ones, such as the amino acids Se methionine and Se cysteine [10].

Plants can convert selenate into organic forms with less chance of damage from toxicity [11]. Therefore, Se-enriched nutraceuticals may provide a safe and effective food supplementation. Mineral biofortification of vegetables is an effective way to improve the human diet [12]. Several crops have been Se-biofortified with commercial outputs [13,14]. Se-enriched potato, carrot, garlic, and onion are already available on the European market; however, they frequently require thermal processing, which destroys the organic Se form bioavailable for humans [15]. There is no publicly available data on the amount and form of Se in these products.

There are several methods for supplementing Se in cereal or horticultural crops [16–19]. The two main techniques applied to tomatoes consist of spraying the aerial plant tissues [20–23], and facilitating Se uptake by the root system from a hydroponic solution [24–26], or the substrate [22,27]. The intensity of the uptake and distribution of Se among plant organs depends on its concentration, chemical form, application protocols, time, number of treatments, and genotype.

The methods of Se biofortification have led to partially contradictory results. Schiavon et al. [21] reported a concentration of 4.2 mg Se kg⁻¹ DW in tomato fruit as a result of single foliar spraying of 20 mg Se L⁻¹ before fruit appearance. Similar results, i.e., 5.2 mg Se kg⁻¹ DW in fruit skin, were found when a double application of a high Se amount of 1000 mg Se L⁻¹, was applied [20]. Interestingly, a ten times higher uptake, 52.24 mg Se kg⁻¹ DW, resulted from twice applying a one hundred times lower amount of Se, 10 mg Se L⁻¹ [22].

With the global focus on sustainable development, the application of selenium salts on the industrial scale needs to be safe for the environment considering that if part of the Se does not reach the desired plant organs, it remains in the environment and may cause water pollution [17] or increase soil salinity [28].

Biologically [29] or chemically [30] synthesized nanoparticles (NPs) [31] are an innovative way to provide plants with nutrients. NPs are now being considered as an alternative solution for Se enrichment of rice [32] and tomato [33]. One advantage of delivering Se in the form of NPs is that it can be used at the elemental state (Se⁰). Zero-valent Se is chemically neutral, as Se⁰ does not interfere as much with cellular processes compared to other forms, and very little is taken up and assimilated by a plant in the natural environment [34].

However, when Se⁰ is incorporated into NPs coated with an organic shell, it becomes available in the plant tissues. This technique is more sustainable than applying salt, since it leads to a less reactive and consequently less toxic chemical state. Neysanian et al. [35] sprayed tomato plants with Se NPs and sodium selenate and reported that Se in nano form was absorbed more effectively by plant tissues. In addition, compared to Se(VI), Se⁰ appears to be more rapidly assimilated to organic forms, as observed in rice plants [32].

In this study, we compared the efficacy, in terms of Se accumulation in different tomato plant organs, among the two Se forms: sodium selenate and Se NPs. Secondly, we assessed the effectiveness of on-plant enrichment (whole-plant spraying) and designed possible alternative strategies for postharvest fruit enrichment.

We tested different Se enrichment strategies with the aim of biofortifying berries, the edible part of tomato plants: (i) pre-harvest spraying of whole tomato plants with sodium selenate or chemically synthesized Se NP solutions; (ii) postharvest immersion and vacuum infiltration of harvested mature green fruit with a sodium selenate solution.

2. Materials and Methods

2.1. On-Plant Fruit Enrichment

2.1.1. Cv. Kreos Experiment

Tomato plants (*Solanum lycopersicum* L. cv. Kreos F1) were cultivated in a temperature-controlled greenhouse at the Department of Agriculture, Food, and Environment of the University of Pisa, Italy (lat. 43°40' N) from January to June 2019. Seeds were sown into an artificial substrate and covered by vermiculite. Two hundred plantlets with developed 2 true leaves were transplanted into rock wool blocks (100 cm long × 15 cm wide × 7.5 cm tall) 4 weeks after sowing. In 3 weeks, 216 plantlets were transplanted to rock wool slabs (1.000 × 150 × 75 mm) on benches with a density of 3 plants m⁻².

The nutrient solution was provided to plants by a hydroponic drip system for 1 min three times daily. The intensity of irrigation was increased to 4 times a day 2 weeks after transplanting, and to 5 times 7 weeks after transplanting. The nutrient solution contained 14 mM L⁻¹ N-NO₃, 1 mM L⁻¹ P-H₂PO₄, 2.77 L⁻¹ mM S-SO₄, 4 mM L⁻¹ Ca, 8 mM L⁻¹ K, 1.5 mM L⁻¹ Mg, 1 μM L⁻¹ Cu, 15 μM L⁻¹ Fe, 10 μM L⁻¹ Mn, 1 μM L⁻¹ Mo, 5 μM L⁻¹ Zn. The pH and electrical conductivity (EC) values were maintained under 5.6 and 2.29 dS m⁻¹, respectively, as reported by Puccinelli et al. [24].

Se NPs were chemically synthesized by using 100 mM Na₂SeO₃ and 50 mM L-Cys as described by Li et al. [36]. They were spherical in shape, as determined by Transmission Electron Microscopy (TEM), and their hydrodynamic diameter, evaluated by dynamic light scattering (DLS) analysis, was 99.8 ± 30.2 nm.

Se NPs were diluted in distilled water in concentrations of 1 and 1.5 mg L⁻¹ and applied to the whole plant by spraying both sides of leaves, stem, and flowers 1 month after transplanting, at blooming. For each treatment regime, two benches with 36 plants on each were treated with 250 mL solution per plant. The same volume of distilled water was sprayed on the control plants. During treatments, plants were separated with 4 m high transparent plastic tent channels to prevent contamination among the treatments. The tent was removed the next day after spraying.

Apical growth was terminated by cutting plants above the first inflorescence, leaving three leaves above the flowers. Flowers exceeding 6 per plant were removed.

Eighteen days after treatment (DAT), 18 pooled leaf samples were collected for Se analysis. One sample combined the leaves collected from the middle of the 1st and 2nd branches below the inflorescence from 12 plants. Six pooled fruit samples of mature green/breaker fruit were collected from the first branch 46 DAT. The leaves and fruit were weighed and washed with distillate water.

2.1.2. Cv. Micro-Tom Experiment

Tomato plants (cv. Micro-Tom) were cultivated in a temperature-controlled greenhouse at the above-mentioned location from August 2019 to January 2020. Seeds were sown into an artificial substrate and covered by vermiculite. After developing 2 true leaves, i.e., about 4 weeks after sowing, plantlets were transplanted into 1.4 L pots filled with peat substrate. After 3 weeks, pots were moved to 6 benches, for a total of 60 plants/bench. The nutrient solution was provided by a hydroponic system. Irrigation was scheduled for 1 min twice a day for the first month, and then the irrigation mode was changed to 4 treatments per week. The composition of the nutrient solution was the same as in the above-reported trial [24].

Se NPs and sodium selenate were diluted in distilled water at concentrations of 5 and 10 mg Se L⁻¹. Spraying treatments of the whole plant were performed 2 months after transplanting, i.e., when the fruit reached the immature green stage. Thirty plants per treatment were sprayed with 100 mL solution per plant on both sides of leaves, and on the stem, flowers, and fruit. The same volume of distilled water was sprayed on control plants. During treatments, plants were separated with 4 m high transparent plastic tent channels to prevent contamination among the treatments. Tents were removed one day after the treatment. Plants from each treatment were numbered and randomized on the benches.

For Se content determination, 3 pooled samples of leaves for each treatment were collected from the middle part of plants at 3 and 53 days after the treatment (DAT), and fruit from the first truss was sampled at 3, 30, and 53 DAT. At the end of the experiment (53 DAT), the whole-plant sampling was performed to analyze Se distribution in fruit, leaves, stems, and roots. For the screening of Se content in different plant organs (53 DAT), material from individual plants was taken in quadruplicates, while for 3 and 30 DAT, pooled sampling in triplicates was performed. After fresh weight measurement, samples were labelled, washed in distillate water, and allocated in the oven at 50 °C.

2.2. Off-Plant Fruit Enrichment

2.2.1. Vacuum Infiltration

Tomato fruit (cv. Cuore di Bue) harvested at the mature green stage (12 for each treatment) were selected based on their visual colour appearance and absence of defects and immersed in 1 L of 5, 10, and 20 mg Se L⁻¹ sodium selenate solution and subjected to vacuum pressure of -100 kPa for 15 s. After the release of the vacuum, the fruit were kept immersed in the same solution for 2 min to facilitate a rapid influx of Se solution. The same procedure, but using distilled water, was followed for the control set of fruit. The fruit were allowed to drain and dry at room temperature.

2.2.2. Passive Immersion

Tomato (cv. Camone) fruit detached at mature green-breaker stage were obtained from Lapietra farm at Monopoli (Bari, Italy). Only fruit with no sign of damage and with peduncle were preselected for trial. A sodium selenate (Chem Cruz, Dallas, TX, USA) was dissolved in tap water to reach 40 and 80 mg L⁻¹ Se. Forty fruit per treatment were kept submerged in 5 L solution for 1 h. Control fruit were immersed in the tap water. The fruit were left to drain and dry at room temperature.

2.3. Sample Preparation and Se Determination

2.3.1. Oven Drying

Plant samples were oven-dried at 50 °C and ground in a mortar to a fine powder.

2.3.2. Lyophilization (Freeze-Drying)

Approximately 20 g of chopped washed tomato fruit in 50 mL plastic falcons were placed in a Lio 5P Freeze dryer with an attached rotary vacuum pump (Kambic d.o.o.; Semic, Slovenia), and left at -90 °C under a vacuum for seven days. Dry tissue was ground in a mortar to a fine powder.

2.3.3. Selenium Determination

An amount of 0.5 g of powder from each sample type was processed by microwave-assisted acid digestion, using nitric acid and hydrogen peroxide, following EPA Method 3051a [37]. The total Se content was determined using inductively coupled plasma spectrometry (ICP OES 5900 Agilent, Santa Clara, CA, USA). Se content was expressed as mg Se kg⁻¹ DW.

2.4. Statistical Analysis

Statistical analysis was performed using RStudio (RStudio 2021.09.0 + 351 “Ghost Orchid”). Samples were compared by one-way and two-way analysis of variance (ANOVA), and *t*-test (Kruskal–Wallis and Wilcoxon test for nonparametric data, respectively) with Se treatments as the main variable. To separate means, the least significant difference (LSD) (*p* < 0.05) was used.

3. Results and Discussion

3.1. On-Plant Enrichment

Our decision to treat plants with NPs at blooming was based on preliminary trials carried out by the group of the University of Verona (Lampis et al., unpublished) and the results published by Karny et al. [38] who reported that iron and magnesium NPs sprayed on the plants penetrate the leaf and translocate in a bidirectional manner, and are distributed to other parts of the tomato plant.

In our trials, Se NPs increased the selenium concentration in the vegetative parts of plants (Figure 1A). Results from leaf analysis showed that the concentration of 1.5 mg Se L⁻¹ induced a higher selenium accumulation compared to 1 mg Se L⁻¹.

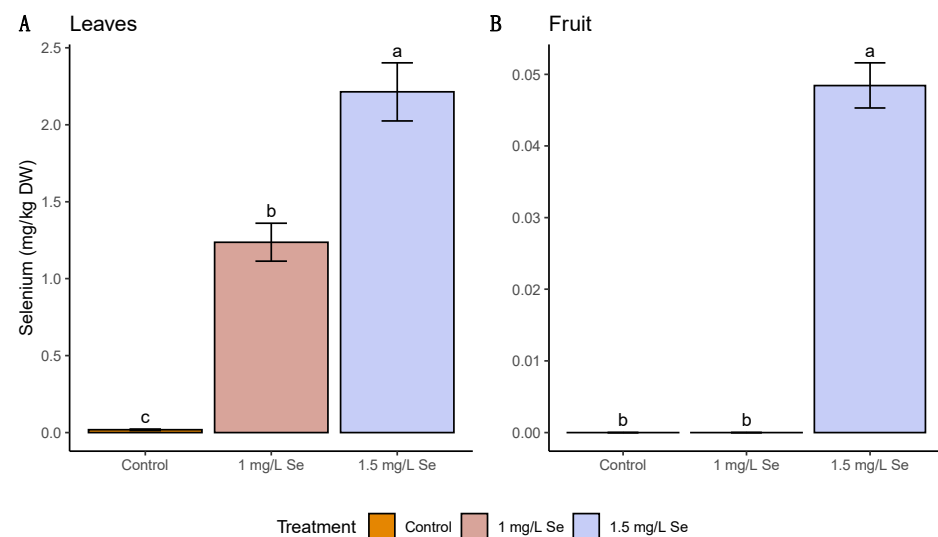


Figure 1. Total Se content in the leaves (A) and fruit (B) of tomato plants (cv. Kreos) treated 1 month after transplanting with 0, 1, and 1.5 mg Se L⁻¹ as Se NPs. Leaves and fruit were sampled 18 and 46 DAT (days after treatment), respectively. Bars indicate standard error. Letters indicate the results of the LSD test run separately for each organ. Values with the same letter are not statistically different ($p \leq 0.05$; $n = 4$).

In our experiments, only 0.05 mg Se kg⁻¹ DW was detected in the fruit of plants treated with Se NPs at 1.5 mg Se L⁻¹. On the other hand, Se was not detected in the fruit of plants exposed to 1 mg Se L⁻¹ or in the control (Figure 1B).

The findings of the first experiment on cv. Kreos tomatoes were considered for the design of the next trial. The concentrations of Se in the solution were increased from 1 and 1.5 to 5 and 10 mg Se L⁻¹, and selenium was added either as Se NPs or sodium selenate. In addition, the time of treatment was shifted from blooming to the immature green stage of fruit development. Lastly, instead of the commercial cultivar, we used the dwarf mutant Micro-Tom, a model tomato cultivar. This is because it has a small phenotype/size that enabled us to arrange the experiment in a complete randomized block and to randomize plants from different treatments throughout the trial.

The results of the total screening of the plants treated with sodium selenate and Se NPs performed 53 DAT (Figure 2) showed that leaves and stems accumulated more Se than the fruit and roots. In fact, the roots accumulated the lowest amount of the element with no significant difference among Se treatments. In leaves, 10 mg Se L⁻¹ treatments (NPs and salt) resulted in the same uptake, whereas at 5 mg Se L⁻¹ level, Se NPs were more effective than Se salt. Stems absorbed most Se at 10 mg L⁻¹ when Se was applied as sodium selenate, whereas the effect of 10 mg Se L⁻¹ as Se NPs was not statistically different from that observed in 5 mg Se L⁻¹ salt-treated plants.

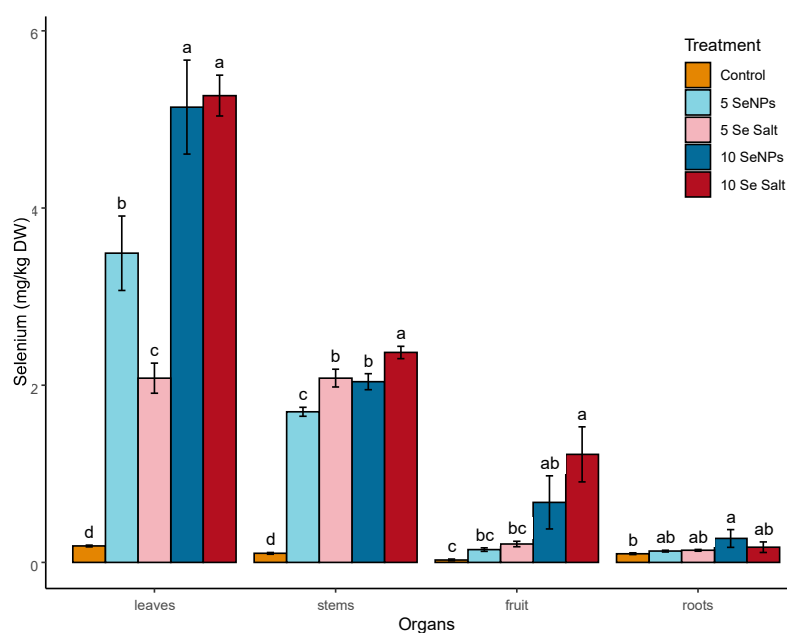


Figure 2. Total Se content in leaves, stems, fruit, and roots of tomato plants (cv. Micro-Tom) treated 2 months after transplanting with 0, 5, and 10 mg Se L⁻¹ as Se nanoparticles (SeNPs) and sodium selenate (Se Salt). Samples were collected 53 DAT. Bars indicate standard error. Letters indicate the results of the LSD test run separately for each organ. Values with the same letter are not statistically different ($p \leq 0.05$; $n = 4$).

In fruit, there was no significant difference between the two Se forms at both concentrations applied, although at 10 mg Se L⁻¹, sodium selenate samples showed an increasing trend compared with the NP-treated samples. A lower Se uptake from NPs is in line with the findings of Wang et al. [32], who reported that sodium selenate resulted in 1.7 times higher Se accumulation in rice seedlings submerged in Se solution. On the other hand, Neysanian et al. [35], who repeatedly sprayed tomato plants with 10 mg Se L⁻¹ as NPs and sodium selenate, found that NPs induced significantly higher Se accumulation in fruit compared to Se salt.

The results of the sodium selenate spraying are consistent with Schiavon et al. [21], who found an average concentration of 4.2 mg Se kg⁻¹ in the fruit of tomato plants sprayed with 20 mg Se L⁻¹ as sodium selenate before the fruit's appearance.

As highlighted by Winkel et al. [39], a positive aspect of foliar spraying is that Se accumulation in the plant is not dependent on the translocation from roots to shoots. In fact, in our trials, roots accumulated lower amounts of selenium than the leaves, stems, and fruit, and the treatment with 5 mg Se L⁻¹ as Se NPs induced the highest uptake. The effect of the other treatments was not significantly different from the control, possibly indicating a different fate in terms of translocation of absorbed Se after salt or NP treatment. The amount of Se detected in the control, i.e., 0.02 mg Se kg⁻¹, is due to the natural uptake from the environment, and does not exceed the amounts reported by White [16].

The amount of Se detected in fruit from plants biofortified with 10 mg Se L⁻¹ in our trial was up to three times higher than that reported by Zhu et al. [40]. These authors used a concentration ten times lower (1 mg Se L⁻¹) and the volume sprayed was ten times higher (1 L), compared to our experiment, yet resulting in the same total amount of applied Se. Based on this evidence, in foliar spraying experiments with selenate, the Se concentration appears to be more crucial than the volume of the solution for the effectiveness of biofortification. However, Zhu et al. [40] tested a different tomato cultivar from ours (cv. Provence), which might potentially have a different Se absorption capacity. This consequently makes interpreting the results less straightforward.

At 53 DAT, only about 2% of the total applied Se was accumulated by the various organs of the plants, and no statistical differences were detected between the chemical

form and doses of Se (Figure A1–Appendix A). A comparison of the Se distribution ratio in different plant organs for each treatment, including the control (accumulating Se only in traces), showed that at 53 DAT, most Se was accumulated in the leaves and stems (Figure A2), in accordance with Narváez-Ortiz et al. [41] who reported that foliar application caused a higher Se uptake in the shoots and a lower uptake in the roots.

Compared to Se salt application, treatments with SeNPs appeared to slightly modify the distribution pattern among plant organs, with an increased concentration in leaves. This further reinforces the hypothesis that there is a different translocation effect based on the specific form of Se applied, as previously observed for different Se salts [22,27].

Moreover, a similar general trend of higher Se accumulation in the vegetative parts of the plant was reported by Edelstein et al. [42] who supplemented tomatoes with sodium selenate added to the nutrient solution.

To investigate Se intra-plant mobility after the treatment, we analysed the Se levels in fruit at 3, 30, and 53 DAT, and in leaves at 3 and 53 DAT. In leaves, the amount of Se decreased for all the treatments from the day of treatment to fruit harvest (53 DAT) (Figure 3A).

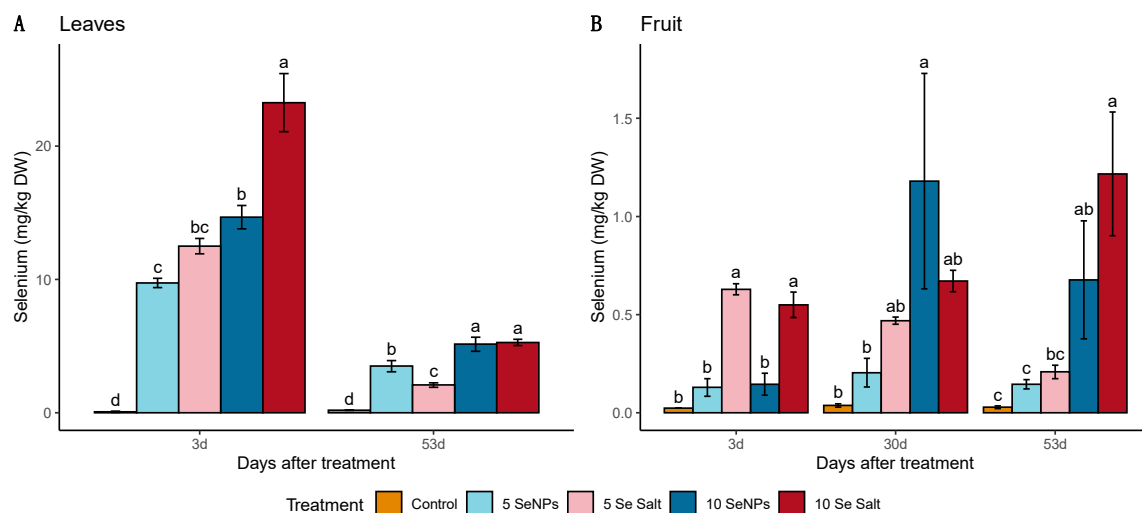


Figure 3. Total Se content in tomato leaves (A) and fruit (B) (cv. Micro-Tom) 3, 30, and 53 DAT at 0, 5, and 10 mg Se L⁻¹ as Se nanoparticles (NPs) and sodium selenate (Se Salt). Bars indicate standard error. Letters indicate the results of the LSD test run separately for each date. Values with the same letter are not statistically different ($p \leq 0.05$; $n = 4$).

The different levels of effectiveness of sodium selenate and SeNPs treatments in terms of leaf Se accumulation, which was evident at 3 DAT, decreased 50 days later. Statistically, at 53 DAT, there was no significant difference in Se accumulation between the SeNPs and salt treatments with 10 mg Se L⁻¹, while at the lower concentration, NPs resulted in a lower accumulation compared to selenate. Two-way ANOVA explains most of the difference in Se content in leaves by the effect of treatment. A comparable pattern was observed by Puccinelli et al. [43] in basil leaves, where the Se concentration, as well as the difference between different doses of sodium selenate, decreased with time from the first to the second cut.

As far as Se content in the fruit is concerned, at 3 DAT, tomatoes treated with sodium selenate accumulated more Se than fruit treated with NPs (Figure 3B).

Se salt appears to be adsorbed by fruit faster than NPs, which instead remain longer on the leaves or fruit surface. Consequently, the concentration of Se in fruit treated with 10 mg Se L⁻¹ as sodium selenate increased with time. The same concentration of Se as NPs resulted in a significantly higher Se uptake at 30 DAT, and a lower uptake at 53 DAT. Interestingly, at 53 DAT, no significant differences were observed between Se salt and NPs at the highest dose. At 5 mg L⁻¹, sodium selenate was more effective than Se NPs, and the difference between the two treatments decreased over time, as well as the amount of Se in

the fruit. Both treatment and time significantly affected the amount of Se accumulated by tomato fruit.

Se content in fruit sprayed with the salt solution was lower compared to the results reported by Pezzarossa et al. [26] and Puccinelli et al. [24]; however, the results are not directly comparable since they treated tomato plants of a different genotype (cv. Red Bunch) with a lower sodium selenate dose which was added to the nutrient solution.

Approximately 350 g of fresh tomatoes enriched with whole-plant spraying 10 mg Se L^{-1} as sodium selenate, may fulfil 50% of the recommended WHO daily Se intake for humans. Se-enriched tomatoes may be considered as an optional dietary supplementation, rather than as a solution to extreme Se deficiency cases. At the same time, consumption of the tomatoes enriched with Se following our protocol is safe: the toxic dose can only be reached in the unlikely event of someone eating 3 kg of tomatoes a day. Additionally, based on the results of Wang et al. [32], elemental Se delivered to rice seedlings in the form of NPs is transformed more efficiently into organic Se compared to Se salts.

Thus, both Se NPs and selenate were found to be efficient for plant biofortification. Our results from a time-series analysis demonstrated that Se accumulated in organs which were directly sprayed, was then redistributed in the plant following the stream of nutrients, from leaves (source organ) to fruit (sink organ). Meanwhile, the different levels and chemical forms of Se affected the amount of Se accumulated and the allocation among organs. To understand their different levels of effectiveness, further investigations of the physical processes behind the transport mechanism are needed.

3.2. Off-Plant Enrichment

Spraying the whole plant and/or aerial organs is an effective way to enrich tomatoes with Se. However, the amount of fortifying agent entering the fruit is much lower than the amount of element lost into the environment during treatment, or later while being washed from the surface of sprayed plants, or from the treated substrate of plants enriched via the root system. This leakage creates a risk of pollution, especially in the case of industrial applications.

Another issue is that the amount of accumulated Se inside fruit treated at the same concentration is not consistent. This variability may potentially cause the appearance of hyperaccumulating outliers amongst the enriched fruit. However, lowering the dosage may lead to insufficient enrichment.

Both the sustainability and precision of Se enrichment need to be optimized. One option, at least for preliminary research purposes, is postharvest enrichment that can potentially reduce production losses, due to pathogen infection, in the supply chain. One of the rare studies on the postharvest enrichment of tomatoes showed that the fruit might successfully absorb calcium chloride after vacuum infiltration [44].

In fact, in our experiment, vacuum infiltration led to a considerable accumulation of Se in the fruit. Se was mostly absorbed in the proximal parts of the fruit, while the distal parts absorbed roughly three times less Se (Figure 4).

This pattern of uneven distribution may be due to the more intensive uptake of the solution through the pedicel cavity than through the exocarp.

Vacuum infiltration was effective for tomato fruit biofortification, but led to considerable fruit injuries, as also reported by Senevirathna et al. [44]. Some fruit had tiny cracks on the surface which appeared at the moment of vacuum release or within one hour after being removed from the vacuum. Reducing the time of vacuum and increasing the time of vacuum release did not significantly decrease the damage. Some visually undamaged fruit started to show symptoms of internal browning.

Immersion was considered a possibly less harmful alternative to vacuum infiltration, and to the best of our knowledge, the literature does not report analogous experiments. The immersion of fruit in more saturated Se solution for one hour induced a slightly lower Se uptake than using a vacuum (Figure 5).

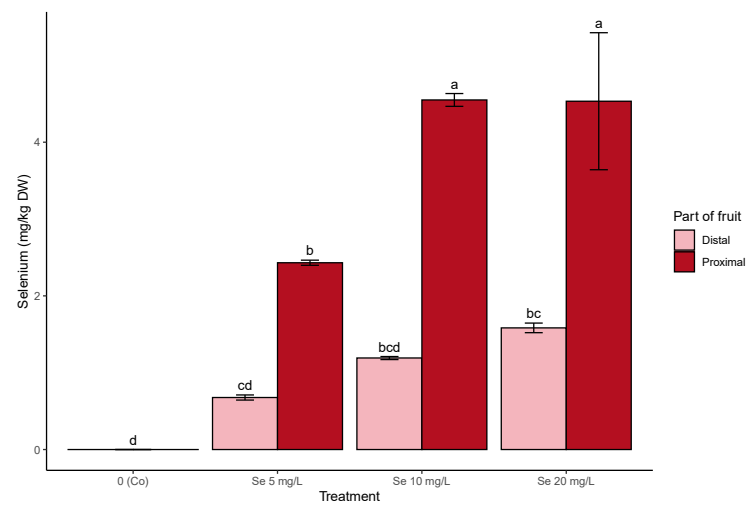


Figure 4. Content of Se in tomato fruit treated under a vacuum at 0, 5, 10, and 20 mg Se L⁻¹ in the form of sodium selenate. Values are the means \pm SE ($n = 3$). Values with the same letter are not statistically different ($p \leq 0.05$).

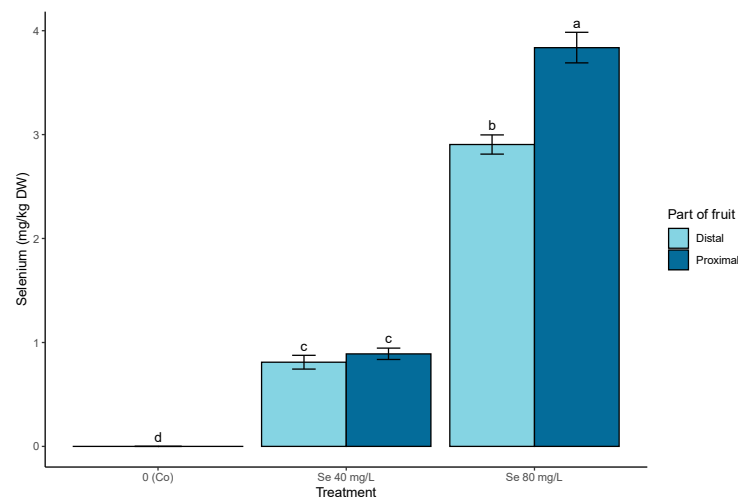


Figure 5. Content of Se was determined in tomato fruit treated under passive immersion at 0, 5, 10, and 20 mg Se L⁻¹ in the form of sodium selenate. Values are the means \pm SE ($n = 3$). Values with the same letter are not statistically different ($p \leq 0.05$).

The pressure provided by the gravity on the solution surface did not cause any damage to the immersed fruit, nor during the treatment or during on-shelf-life observation. Se was absorbed by fruit, probably more intensely through the peduncle scar, considering that the top (proximal) part of the fruit contained a higher amount of Se (Figure 5). Interestingly, a comparable amount of Se in the fruit has been reported after nutrient solution supplementation with up to 2 mg L⁻¹ sodium selenate [23], and after foliar spraying with 20 mg Se L⁻¹ [20].

Another significant advantage of our approach is the lower difference between the Se amount absorbed by proximal and distal fruit parts, compared to vacuum infiltration.

These findings suggest a new strategy for fruit biofortification, which reduces the amount of solution needed, and enables the same solution to be re-used on another set of fruit.

3.3. An Innovative Tool to Facilitate Sample Preparation for Se Analysis

A major benefit of our method is that the sample preparation time is shorter for the Se content determination. The volatility of certain chemical Se forms increases with

temperature. To limit the possible quantitative loss of Se throughout sample preparation, other researchers recommend drying plant samples in the oven at a temperature not exceeding 50 °C [43,45] or 60 °C [46]. Another suggestion for fruit samples is to cut them into relatively large pieces: halves or quarters. At the same time, only the samples with no moisture can be analysed by atomic absorption spectrometry. Such a delicate protocol is relatively time-consuming. During the first experiment reported in the present work, it took 40 days from fruit sampling to Se determination.

Consequently, we developed a shorter method for plant sample preparation. Freeze-drying rather than dehydrating samples in the oven enabled us to dry samples in seven days. Based on the results (Figure 6), the Se content determined in lyophilized samples was not statistically different from the values obtained from the same fruit traditionally dried in the oven. Moreover, the standard deviation among the freeze-dried samples was lower compared to the traditional technique. This is likely due to the more homogeneous humidity levels in the freeze-dried material.

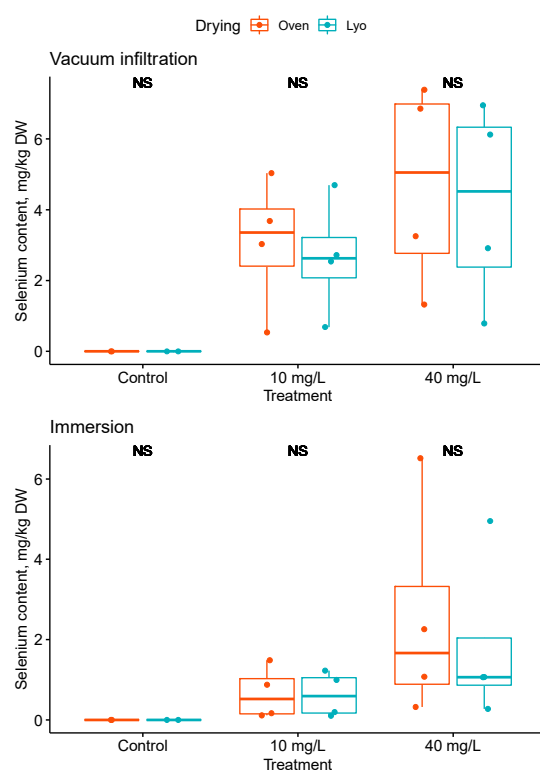


Figure 6. Content of Se in tomato fruit treated under a vacuum (top) and passive immersion (bottom) at 0, 10, and 40 mg Se L⁻¹ in the form of sodium selenate. Orange bars represent results from analysis of the samples dried in an oven, blue bars indicate results collected from lyophilized tissue (Lyo). Values are the means \pm SE ($n = 3$). Values with the same letter on the same date are not statistically different ($p \leq 0.05$).

Our results indicate that lyophilization is an alternative approach to oven drying, thus speeding up the preparation of samples for Se determination by atomic absorption spectrometry.

4. Conclusions

Our results confirmed that Se-enriched tomatoes can be produced by spraying the whole plant with Se solution. The amount of Se detected in the fruit throughout development and at harvest appeared to be mainly the result of the direct penetration of Se through the external layers of the fruit. The hypothesis of the downward redistribution of Se through a phloem-mediated translocation has been suggested in Se-hyperaccumulator *Neptunia amplexicaulis* [47] as well as in kohlrabi (*Brassica oleracea* L.) [48], maize (*Zea mays* L.) [49], and

tomato [50]. However, the pattern of Se translocation greatly depends on the form and the concentration of selenium applied. The fact that the strongest accumulation of Se in fruit was obtained after spraying at the highest levels (10 mg Se L^{-1}) and that Se concentrations detected in the roots after spraying the aerial organs, are extremely low, would indicate that, in our experimental conditions, Se accumulation is mainly (but not exclusively) the result of direct absorption.

Our data show that sodium selenate sprayed on the whole plant is more effective in enriching tomato fruit than with the same concentration of Se NPs. However, this effect disappeared after 53 DAT, with the highest dose of Se NPs inducing a similar level of Se in the fruit as the salt solution at the same dose.

After optimization, we believe that our technique could be used in industrial tomato cultivation and lead to the development of nutraceutical products.

Although the Se-enrichment of tomato fruit obtained after spraying Se NPs was less pronounced than that of Se selenate, the delivery of elemental Se (Se^0) by NPs could result, as above pointed out, in a better and more pronounced process of conversion into organic forms (Se-cysteine, Se-methionine), with fewer problems due to the reduced toxic chemical state of Se. However, this still needs to be clarified.

Postharvest application of Se might be a more sustainable enrichment technique compared to substrate supplementation or spraying, given that the same solution can be reused multiple times and there appears to be no dispersion of potentially toxic molecules. Compared to vacuum infiltration, passive immersion does not lead to fruit damage. Even though the fruit absorbs less solution without a vacuum, an increase in Se concentration compensates for this. With regard to the alternative approach of postharvest treatment, Se accumulated by tomato fruit does not appear to be effective in converting Se from inorganic into organic forms, which are valuable for human dietary uptake. Consequently, the nutraceutical impact of consuming tomato fruit enriched off-plant during post-harvest is lower than consuming fruit treated on-plant by spraying plants or supplementing the substrate.

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Appendix A

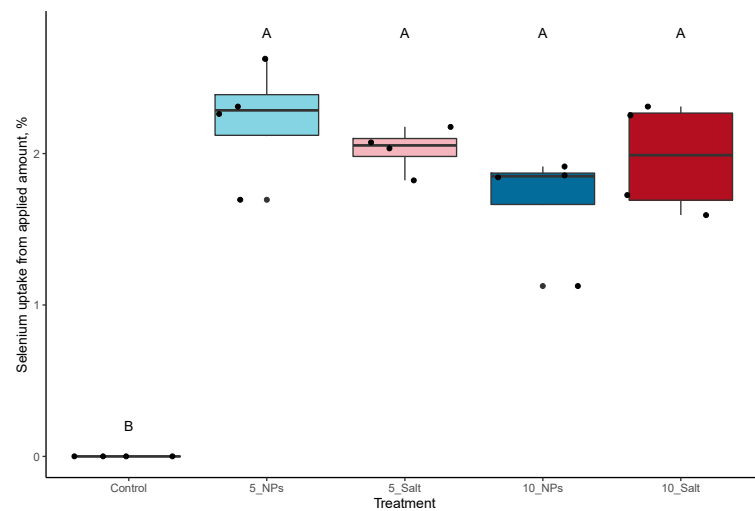


Figure A1. Percentage of total selenium cumulatively accumulated by different organs of the tomato plant (cv. Micro-Tom) 53 DAT at 0, 5, and 10 mg Se L⁻¹ as selenium nanoparticles (NPs) and sodium selenate (salt) in relation to the total amount distributed for each of the tested treatments. Bars indicate standard error. Values with the same letter are not statistically different ($p \leq 0.05$; $n = 4$).

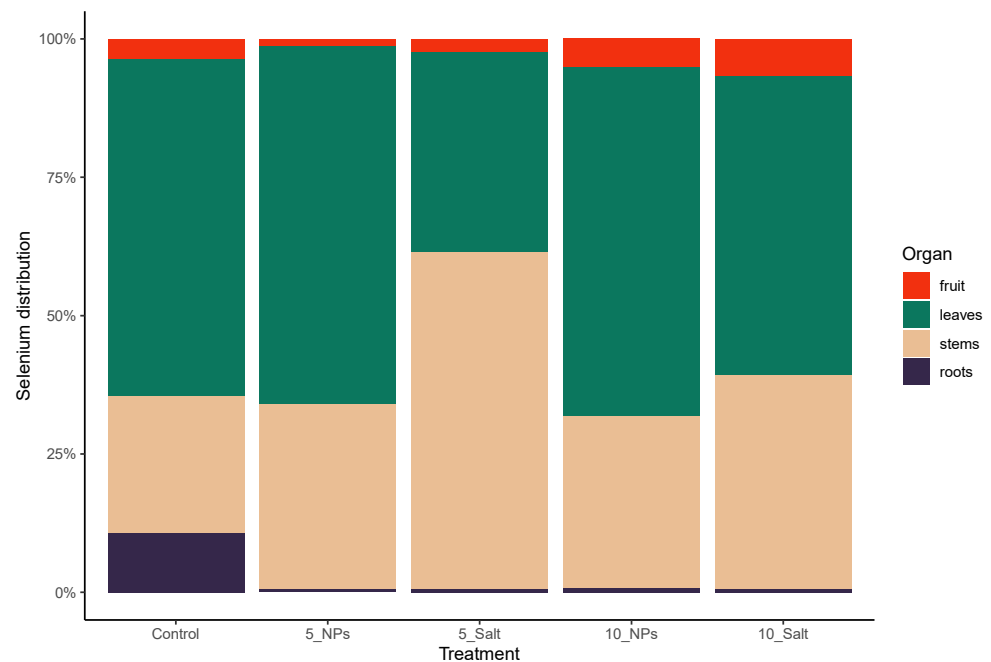


Figure A2. Percentage of distribution of accumulated Se in different tomato plant organs (cv. Micro-Tom) 53 DAT at 0, 5, and 10 mg Se L⁻¹ as Se nanoparticles (NPs) and sodium selenate (salt).

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