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Effect of selenium application on mineral macro- and micronutrients and antioxidant status in strawberries

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Summary

The application of selenium (Se) as sodium selenite (Na_2SeO_3) at 0, 2, and 4 mg L⁻¹ concentrations in nutrient solution to strawberry plants was evaluated. Selenium did not modify the dry weights of the roots, stems, leaves, and fruits, or the fresh weights of the stems and fruits. The 4 mg L⁻¹ concentration caused decreases in the fresh weights of the roots and leaves and in the yield. The mineral content of different plant organs changed but was not adversely affected by Se applications, with the 2 mg L⁻¹ treatment having a lower impact on mineral concentration variation, as well as temporary positive effects on the fruits' antioxidant status. The fruit pH was not adversely affected by application of Se. Se application in nutrient solution proved to be an adequate technique to increase the Se content in all plant organs. Se concentration exhibited a differential distribution, with the highest levels in the roots, followed by the leaves and crowns; the fruits had the lowest levels, reaching an average concentration of 31.2 mg kg⁻¹ of dry weight. By contrast, fruits from the untreated plants obtained an average concentration of only 6.35 mg kg⁻¹, with no decreases in the concentrations of other mineral elements in treated plants.

Keywords: *Fragaria*, fruit quality, sodium selenite, biofortification.

Introduction

Selenium (Se) is an essential trace element for humans and other animals. Its biological importance lies in the fact that it forms a structural part of more than 30 selenoenzymes that regulate oxidative metabolism by mitigating cell damage caused by free radicals (MANGIAPANE et al., 2014). Low Se intake in humans is associated with health disorders, reduced fertility and immune function, and an increased risk of cancer (BROADLEY et al., 2006). The primary source of Se is the soil, from which it is absorbed by plants and reaches humans directly or indirectly via the food chain (STEINNES, 2009). Therefore, soils with low levels of Se are associated with human populations with a higher risk of deficiency of this element (HARTIKAINEN, 2005). Although Se is not essential for plants, it is a beneficial nutrient (PILON-SMITS et al., 2009) that enhances plant growth and antioxidant activity (BENAVIDES-MENDOZA et al., 2012). However, at high concentrations, it causes toxicity in plants (LYONS et al., 2005).

In current practice, the objective is to increase Se intake in humans through biofortification of crops, which is achieved through mineral fertilization or genetic improvement (GONZÁLEZ-MORALES et al., 2017).

Biofortification using fertilizer enriched with Se has been successfully tested in the field and greenhouse (BAÑUELOS et al., 2015). In

extensive crops such as winter wheat, Se application did not cause changes in yield or harvest index but did have a positive effect on Se concentration in grains, with increases ranging from 16 to 26 mg g⁻¹ Se of fresh weight per each gram of Se applied per hectare (BROADLEY et al., 2010). Similar results were observed in rice, in which the average content of Se was $0.025 \pm 0.011 \mu\text{g g}^{-1}$; when foliar fertilizers enriched with Se were applied, the average Se content increased until it reached 0.471 to 0.640 $\mu\text{g g}^{-1}$ (CHEN et al., 2002).

In horticultural species such as carrots, the foliar application of selenite or selenate solutions at a Se concentration of 100 $\mu\text{g ml}^{-1}$ resulted in Se levels of up to 2 $\mu\text{g g}^{-1}$ in dry matter of edible parts (KÁPOLNA et al., 2009). In potatoes, application of this mineral resulted in higher concentrations of starch in the leaves, as well as an improved yield of tubers (TURAKAINEN et al., 2004). Supplying Se through a nutrient solution, soil fertilization, or a foliar spray has been demonstrated to increase Se levels in tomato fruits (BECVORT-AZCURRA et al., 2012; CASTILLO-GODINA et al., 2016), rice grains (LIDON et al., 2018), carrots (OLIVEIRA et al., 2018), turnips (LI et al., 2018), and lettuce (HAWRYLAK-NOWAK et al., 2018). Studies have also been reported regarding Se application and its interaction with other beneficial elements such as iodine; however, the literature is very scarce concerning simultaneous biofortification with iodine and Se and possible interactions in the uptake processes (JERŠE et al., 2018).

In a recent study of strawberry plants, MIMMO et al. (2017) used sodium selenate as a Se source in concentrations of approximately 0.79 and 7.9 mg L⁻¹; supplementation with Se did not negatively affect growth, fruit yield, or mineral accumulation in the different plant organs, and fruits were obtained with Se concentrations of up to 46.04 mg kg⁻¹. On the other hand, selenate application at concentrations of up to 2.4 mg L⁻¹ favored Se accumulation in strawberry plants and increased the fresh weight of the fruits (MELO SANTIAGO et al., 2018). However, at present there are few studies of biofortification with Se – more specifically, as selenite – and its impact on the mineral composition of strawberries, which contain a wide range of nutrients and phytochemicals and are considered one of the most relevant commercial berry crops in many parts of the world (SANDHU et al., 2018).

For these reasons, this study aimed to determine the impact of fertilization with sodium selenite on growth, yield, and Se concentration, as well as the concentration of macro- and micronutrients in the roots, crowns, leaves, and fruits of strawberry plants. In addition, this study sought to verify the effect of Se on the fruits' antioxidant status.

Materials and methods

The study was conducted at Universidad Autónoma Agraria Antonio Narro, located in Saltillo, Coahuila, México. *Fragaria x ananassa* var. 'Festival' strawberry plants were used as plant material. The plants were grown under greenhouse conditions, with a temperature

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range of 12 °C to 32 °C. The plants were placed in polyethylene bags with a capacity of 5 L that contained a mixture of peat moss and perlite at a ratio of 1:3 (v:v). The bags were placed in three 0.8 × 10 m beds with black plastic mulch. For the first 15 days, the plants were irrigated with previously characterized water (Tab. 1). Crop nutrition was carried out through the application of a Steiner nutrient solution (STEINER, 1961), beginning 16 days after transplant (DAT). The nutrient solution concentration was increased according to the growth of the crop: 15% up to 30 DAT, 50% at 31-50 DAT, and 100% from 51 DAT until the end of the experiment. The pH of the solution was maintained at approximately 6.0, using sulfuric acid and a maximum electrical conductivity of approximately 2 dS m⁻¹.

The treatments consisted of a control group (without Se application) and Se addition in nutrient solution at concentrations of 2 and 4 mg L⁻¹ of Se as sodium selenite (Na₂SeO₃, Merck), initiated at 26 DAT. According to the analyses conducted before the start of the research, the basal Se concentration in the mentioned substrates was 0.175 mg kg⁻¹ (perlite) and 0.016 mg kg⁻¹ (peat moss), while in the irrigation water, it was 0.018 mg L⁻¹.

During plant development, two samplings were carried out. The first one, at 60 DAT, was conducted to determine the fresh weights (FW), dry weights (DW), Se content, and other minerals in the roots, crowns, and leaves of the plants. The second sampling, was carried out at 98 DAT, again to determine the variables above and also to analyze additional samples of mature fruits for concentrations of Se and other minerals, oxidation-reduction potential (ORP) as a measure of antioxidant status, and pH. Harvesting of the fruits began at 98 DAT and ended at 129 DAT (14 sampling dates).

Determination of biomass and yield, chemical variables, and mineral concentrations

For each sampling, five strawberry plants from each treatment condition were dissected into their various organs (roots, crowns, leaves, and fruits). The fresh weights of each fresh plant part were weighed using a Sartorius CP224S analytical balance. The samples were then placed in an Arsa drying stove at a temperature of 80 °C for 72 hours, and the dry weights were subsequently measured. The ripe strawberry fruits were collected and weighed to obtain the yield per plant. This determination began at 98 DAT and ended at 129 DAT (14 sampling dates).

The P content was obtained using a spectrophotometric method (AOAC, 1990). K, Ca, Mg, Na, Fe, Mn, Cu, and Zn levels were determined in samples submitted to acid digestion (JONES and CASE, 1990) using a Varian AA-1275 atomic absorption spectrophotometer. The Se concentration was determined in samples submitted to acid digestion (AOAC, 2000) using a Varian 725-ES inductively coupled plasma optical emission spectrometer (ICP-OES).

The pH and ORP in the fruits were determined by a Hanna HI 98121 potentiometer using the technique described by BENAVIDES-MENDOZA et al. (2002). For this determination, five fully mature fruits were collected (one replicate). The fruits were washed with

distilled water and subsequently macerated. Electrodes were placed in the macerate obtained from the five fruits, and readings were taken. Between readings, the electrodes were washed with distilled water. This procedure was performed three times (three replicates) for each treatment and for each of the 14 sampling dates (98 DAT to 129 DAT).

Experimental design

The experiment was carried out using a completely randomized design with 40 replicates (40 plants) per treatment; each Se concentration was considered a treatment and each potted plant an experimental unit.

Statistical analyses

The Wilcoxon signed rank test was performed to determine the differences between treatments for the pH and ORP variables in fruits over time. The Spearman correlation coefficient was calculated to determine the degrees of correlation for the different variables evaluated in the plants and the Se concentrations in the different plant parts, using the statistical package R, version 3.1.1 (R DEVELOPMENT CORE TEAM, 2014). Data were analyzed by one-way ANOVA, also using R, version 3.1.1 (R DEVELOPMENT CORE TEAM, 2014). The LSD simultaneous test ($p \leq 0.05$) was used for means separation.

Results

Biomass and yield

There were few differences in plant biomass between the different treatments. At 60 DAT (Tab. 2), increases in fresh and dry biomass of the crowns were observed due to the effect of the Se treatments. In the second sampling, for plants treated with Se applied at a concentration of 4 mg L⁻¹, adverse effects were observed for the fresh weight of the roots and dry weight of the leaves, while all other evaluated variables demonstrated no significant effects ($p < 0.05$).

When the overall weight of plants (roots + crowns + leaves) and the yield were obtained (Fig. 1), a negative effect of treatment with Se applied at 4 mg L⁻¹ was observed in comparison with the control plants (98 DAT), which was assumed to be the result of Se accumulation.

Selenium accumulation and its effect on the content of other minerals

Selenium distribution in plants

When the Se nutrient solution was applied, increases in the concentrations of Se were found in strawberry plants at 60 and 98 DAT (Fig. 2). For both samplings of plants treated with 2 mg L⁻¹ Se and for the second sampling of plants treated with 4 mg L⁻¹ Se, the highest Se concentration was detected in the roots, followed by the leaves, crowns, and fruits. The first sampling of plants treated with 4 mg

Tab. 1: General characteristics (salinity/sodicity, cations, anions, and micronutrient determinations) of irrigation water.

Salinity / Sodicity		Cations (mg L ⁻¹)		Anions (mg L ⁻¹)		Micronutrients (mg L ⁻¹)	
pH	7.79	Ca	95.2	SO ₄	38.9	B	0.17
E.C (dS m ⁻¹)	0.85	Mg	23.4	HCO ₃	329	Fe	0.0091
SAR	0.96	Na	40.3	Cl	54.3	Mn	0.0017
SARaj	1.23	K	3.12	CO ₃	35.4	Cu	0.0003
				N-NO ₃	4.90	Zn	0.2018

E.C: electrical conductivity; ARS: absorption ratio of sodium; ARSaj: adjusted sodium adsorption ratio; ND: not detected.

Tab. 2: Fresh and dry weight averages for different organs of strawberry plants treated with different concentrations of Se in nutrient solution.

Treatment		First sampling (60 DAT)	Second sampling (98 DAT)	Treatment		First sampling (60 DAT)	Second sampling (98 DAT)
0 mg L ⁻¹ Se	RFW (g)	6.91 a ^Y	24.15 a	0 mg L ⁻¹ Se	FWL (g)	7.56 a	26.60 a
2 mg L ⁻¹ Se		4.96 a	21.74 ab	2 mg L ⁻¹ Se		7.88 a	23.67 a
4 mg L ⁻¹ Se		5.92 a	18.67 b	4 mg L ⁻¹ Se		7.22 a	16.26 a
0 mg L ⁻¹ Se	RDW (g)	1.90 a	2.89 a	0 mg L ⁻¹ Se	DWL (g)	1.81 a	6.65 a
2 mg L ⁻¹ Se		1.15 a	2.80 a	2 mg L ⁻¹ Se		1.94 a	6.13 a
4 mg L ⁻¹ Se		1.42 a	2.76 a	4 mg L ⁻¹ Se		1.86 a	4.24 b
0 mg L ⁻¹ Se	CFW (g)	0.78 b	5.79 a	0 mg L ⁻¹ Se	FFW (g)	-----	6.55 a
2 mg L ⁻¹ Se		2.07 ab	5.39 a	2 mg L ⁻¹ Se		-----	7.12 a
4 mg L ⁻¹ Se		4.70 a	5.43 a	4 mg L ⁻¹ Se		-----	7.73 a
0 mg L ⁻¹ Se	CDW (g)	0.17 b	1.34 a	0 mg L ⁻¹ Se	FDW (g)	-----	1.04 a
2 mg L ⁻¹ Se		0.61 ab	1.31 a	2 mg L ⁻¹ Se		-----	1.22 a
4 mg L ⁻¹ Se		1.54 a	1.35 a	4 mg L ⁻¹ Se		-----	1.08 a

DAT = days after transplanting. RFW = root fresh weight, RDW = root dry weight, CFW = crown fresh weight, CDW = crown dry weight, FWL = fresh weight of leaves, DWL = dry weight of leaves, FFW = fruit fresh weight, FDW = fruit dry weight. ^YAverages with different literal were statistically different according to LSD ($p \leq 0.05$).

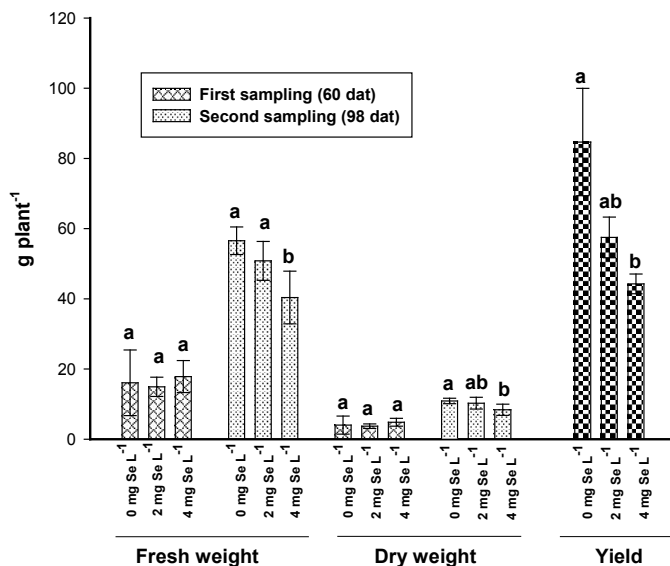


Fig. 1: Total fresh and dry weights (roots + crowns + leaves) and yields of strawberry plants treated with sodium selenite at concentrations of 0, 2, and 4 mg L⁻¹ in irrigation water. Results of the two samplings are included. The bar in each column represents the standard error. Averages with different letters were statistically different according to LSD ($p \leq 0.05$).

L⁻¹ of Se exhibited a different trend, obtaining the highest Se concentration in the roots, followed by the crowns and then the leaves. The addition of Se at 2 and 4 mg L⁻¹ increased the concentration of this element in fruits, reaching values fivefold higher than the control treatment but without differing from each other (Fig. 2).

Macro- and micronutrient concentrations in plants

The concentrations of mineral nutrients in the different organs evaluated in strawberry plants is illustrated in Tab. 3.

Roots: Changes in concentrations of K, Ca, Mg, and Zn were observed. At a concentration of 4 mg L⁻¹ Se, there were no significant effects on the first sampling date, while for the second sampling, a reduction of these elements was observed. When 2 mg L⁻¹ of Se was applied, no changes in the concentrations of these minerals were detected, except for that of Ca, which increased. Na, Cu, and Mn concentrations for the first sampling were increased by treatment with 4 mg L⁻¹ Se; a similar response was observed for the second sampling in plants treated with sodium selenite at both 2 and 4 mg L⁻¹ Se. Fe demonstrated the same behavior in the two sampling dates, with treatment at 4 mg L⁻¹ Se resulting in a higher concentration of this mineral element. P had no differences associated with treatments for either sampling date.

Crowns: For the first sampling, the treatments had no significant effect on K, Na, Cu, Mn, and Fe concentrations, while for the second sampling, treatment with 2 mg L⁻¹ Se resulted in an increase in Fe but no changes in the concentrations of K and Cu compared to the control plants. Na was increased by treatment with sodium selenite; Mn also increased, but only in plants treated with 4 mg L⁻¹ Se. The concentrations of P, Mg, and Zn were higher at 2 mg L⁻¹ Se treatment for the first sampling; for the second sampling, this effect disappeared for P and Mg, which demonstrated no significant differences, while Zn decreased at this low concentration of 2 mg L⁻¹ compared to control plants. When Se was applied at a concentration of 4 mg L⁻¹, Ca concentration in the crowns was higher for both sampling dates.

Leaves: For the first sampling, no significant differences were detected in the concentrations of P, Ca, Zn, and Fe between the treatments; however, for the second sampling, modifications in concentrations of these minerals were observed. Zn decreased due to the Se treatments, while Fe increased when 2 mg L⁻¹ Se was applied. Treatment with 2 mg L⁻¹ Se resulted in no changes to the concentrations of P and Ca compared to the control plants. Treatment with 4 mg L⁻¹ Se increased the concentrations of K and Cu for the first sampling, while for the second sampling, it had an opposite effect, causing the concentrations of K and Cu to decrease in comparison to the untreated plants. Neither the control plants nor those treated with 2 mg L⁻¹ Se demonstrated changes in Na concentration in the first sampling,

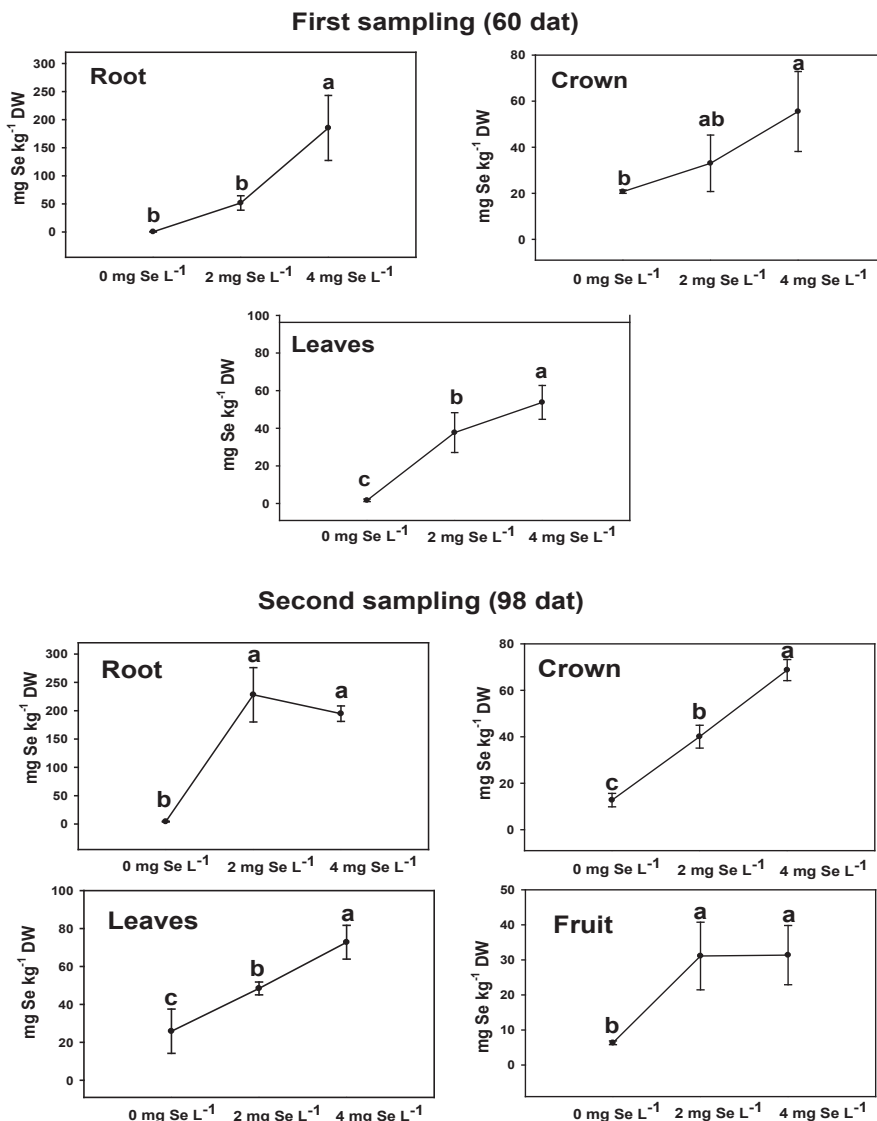


Fig. 2: Effect of Se application as sodium Se in nutrient solution on accumulation of elements in different organs of strawberry plants for samplings made at 60 and 98 DAT. The bar in each column represents the standard error. Averages with different letters were statistically different according to LSD ($\alpha \leq 0.05$).

while for the second sampling, Se treatment produced an increase in the concentration of Na. For the first sampling, Mn decreased in the presence of Se, while for the second sampling, it increased in plants treated with the higher concentration (4 mg L⁻¹) of Se. No differences in Mg concentration were detected for either sampling.

Fruits: Applying Se in nutrient solution did not have significant effects on the concentrations of Ca, Na, and Fe. When treated with 2 mg L⁻¹ Se, the concentrations of K, Zn, and Mn in the fruits did not change compared to the control plants; similar behavior was observed for Mg concentration in plants treated with 4 mg L⁻¹ Se. However, at this latter concentration, P increased, whereas Cu decreased. For Se application in nutrient solution at concentrations of both 2 and 4 mg L⁻¹, changes in the concentrations of elements in the different organs were greater for the second sampling (Fig. 3).

Correlation analysis

Tab. 4 indicates the correlations between Se concentration in different plant organs (roots, crowns, leaves, and fruits) and mineral content and biomass for the second sampling at 98 DAT (fruiting stage).

For purposes of presenting and discussing these results, only the Spearman correlation coefficients (ρ) that are statistically significant with absolute value equal to or greater than 0.70 were considered. A positive correlation was observed between Se concentration in the fruits and Se concentrations in the roots and crowns. In addition, Se concentration in the crowns correlated positively with Se concentration in the leaves. Regarding Se's effects on minerals, it was observed that Se in different plant organs was associated with an increase of Na in the crowns, leaves, and fruits. On the other hand, increased Se in the crowns, leaves, and fruits was associated with decreased Zn in all plant organs. It was also found that higher Se concentration in the crowns was correlated with decreased K in the roots, leaves, and fruits, while Se concentration in the leaves was negatively correlated with K in the roots and crowns. K levels decreased in the roots and leaves when there was greater Se accumulation in the fruits. For other minerals, no clear pattern due to the effect of Se was observed. In the roots, Se concentration correlated negatively with the concentration of Cu in the leaves and fruits. Se was also associated positively with the concentrations of P and Mg in the fruits. Increased Se in the crowns was associated with higher concentrations of Cu and Mn in the roots, as well as with Ca in the crowns and P in the

Tab. 3: Mineral content in roots, crowns, leaves, and fruits of strawberries plants treated with different concentrations of Se in nutrient solution.

Treatments	Minerals	Sampling 1			Sampling 2			Fruit
		Root	Crown	Leaves	Root	Crown	Leaves	
0 mg L ⁻¹ Se	P (g kg ⁻¹)	0.9 a [‡]	1.5 b	3.1 a	1.6 a	2.3 ab	4.3 a	1.7 b
2 mg L ⁻¹ Se		1.1 a	2.9 a	2.1 a	1.7 a	2.4 a	3.7 a	3.0 ab
4 mg L ⁻¹ Se		2.5 a	1.7 ab	2.7 a	1.5 a	1.6 b	2.7 b	4.5 a
0 mg L ⁻¹ Se	K (g kg ⁻¹)	5.4 a	7.6 a	6.7 b	16.2 a	12.1 a	22.1 a	18.3 a
2 mg L ⁻¹ Se		6.2 a	13.1 a	14.5 ab	12.9 a	10.9 ab	19.8 b	17.4 ab
4 mg L ⁻¹ Se		12.8 a	7.7 a	18.0 a	8.6 b	9.4 b	18.3 c	16.4 b
0 mg L ⁻¹ Se	Ca (g kg ⁻¹)	4.7 a	6.5 b	6.1 a	8.1 b	7.9 b	7.7 a	4.3 a
2 mg L ⁻¹ Se		9.1 a	6.6 b	4.9 a	10.0 a	8.4 ab	9.1 a	5.5 a
4 mg L ⁻¹ Se		12.8 a	12.7 a	4.9 a	8.2 b	10.5 a	5.7 b	4.9 a
0 mg L ⁻¹ Se	Mg (g kg ⁻¹)	2.8 a	2.3 b	3.5 a	4.2 a	2.7 a	3.4 a	2.5 a
2 mg L ⁻¹ Se		3.5 a	3.8 a	2.7 a	4.5 a	2.4 a	3.4 a	2.2 b
4 mg L ⁻¹ Se		8.4 a	2.4 ab	3.2 a	3.5 b	2.6 a	3.3 a	2.3 ab
0 mg L ⁻¹ Se	Na (g kg ⁻¹)	1.7 b	2.7 a	1.5 ab	1.9 c	2.3 b	0.7 c	1.9 a
2 mg L ⁻¹ Se		2.4 b	5.2 a	2.0 a	3.9 a	3.1 a	2.0 a	2.9 a
4 mg L ⁻¹ Se		6.9 a	2.6 a	1.4 b	3.0 b	3.2 a	1.5 b	3.0 a
0 mg L ⁻¹ Se	Zn (mg kg ⁻¹)	32.6 a	58.3 b	47.6 a	77.6 a	139.3 a	54.3 a	66.0 a
2 mg L ⁻¹ Se		59.3 a	110.0 a	41.6 a	74.0 a	112.0 b	44.3 b	74.2 a
4 mg L ⁻¹ Se		69.3 a	74.0 ab	45.0 a	62.3 b	69.0 c	37.6 c	42.3 b
0 mg L ⁻¹ Se	Cu (mg kg ⁻¹)	32.3 ab	45.3 a	8.0 b	11.3 b	27.3 ab	8.0 a	19.0 a
2 mg L ⁻¹ Se		27.6 b	36.0 a	9.2 ab	26.3 a	28.3 a	5.6 c	5.8 c
4 mg L ⁻¹ Se		67.0 a	42.0 a	11.6 a	31.3 a	23.3 b	7.0 b	9.0 b
0 mg L ⁻¹ Se	Mn (mg kg ⁻¹)	35.0 b	59.3 a	63.6 a	65.0 b	55.0 a	29.0 b	30.0 a
2 mg L ⁻¹ Se		61.3 b	73.3 a	37.0 b	93.3 a	27.6 b	18.6 c	20.8 ab
4 mg L ⁻¹ Se		313.3 a	116.6 a	41.3 b	110.3 a	50.3 a	42.3 a	10.0 b
0 mg L ⁻¹ Se	Fe (mg kg ⁻¹)	420.0 b	460.0 a	197.6 a	236.6 b	250.0 b	133.3 b	420.0 a
2 mg L ⁻¹ Se		600.0 b	646.6 a	213.3 a	253.3 b	456.6 a	213.33 a	563.3 a
4 mg L ⁻¹ Se		1646.6 a	683.3 a	253.3 a	550.0 a	270.0 b	140.00 b	350.0 a

[‡]In each column the averages per element with different literal were statistically different according to LSD ($\alpha \leq 0.05$).

fruits. On the other hand, Se in the crowns was negatively correlated with P concentration in the leaves and Mn in the fruits. The highest level of Se in the leaves was accompanied by increases in Fe, Cu, and Mn in the roots, Ca in the crowns, and P in the fruits. A negative correlation was also found between Se concentration in the leaves and P in the leaves. Se concentration in the fruits was positively associated with P concentration and negatively associated with Mg concentration in the fruits.

The increase in Se concentration in the crowns and leaves was accompanied by a decrease in the fresh and dry weights of the leaves, as well as the fresh weight of the roots; this latter variable was also affected negatively by increased Se in the fruits.

pH and ORP dynamics in fruits

The Wilcoxon and LSD tests indicated no differences ($p > 0.05$) in the dynamic behavior of fruit pH among different treatments, as well as for the different samplings performed (Fig. 4). The above suggests that the higher Se concentration did not modify the metabolic processes that maintain the pH at between 3 and 4, which is considered

suitable for the pulp of strawberry fruits.

On the other hand, for the ORP of fruits, the Wilcoxon test indicated that differences exist ($p \leq 0.05$) between treatment with Se applied at 4 mg L⁻¹ and the other treatments: control and 2 mg L⁻¹ Se (Fig. 5). In consideration of its dynamics, the ORP demonstrated a sigmoidal behavior, observing a temporal progression from 108 to 122 DAT, with subsequent stability oscillating between approximately 20 and 60 mV. The Wilcoxon test indicated that the control condition and treatment with 2 mg L⁻¹ Se are statistically equal. Univariate analysis using the LSD test revealed significant differences in specific evaluation dates (Fig. 5) associated with the control condition and treatment with 2 mg L⁻¹ Se.

Discussion

Biomass

Similar results to those obtained for the first sampling have already been documented in other crops (LÓPEZ-GUTIÉRREZ et al., 2015; CASTILLO-GODINA et al., 2016). In *Brassica juncea* L., it has been reported that the application of 0.5 mg kg⁻¹ Se-selenite stimulated growth and yield of dry matter (SINGH et al., 1980). The same effect

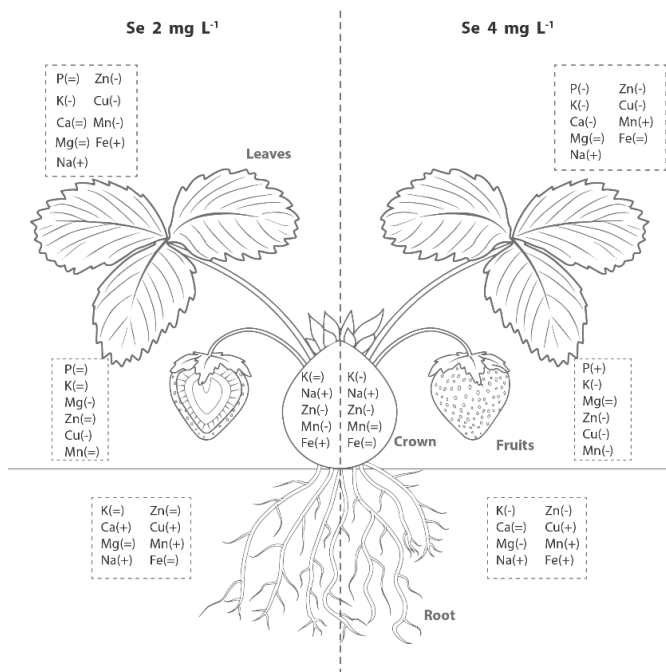


Fig. 3: Effect of Se application (2 and 4 mg L⁻¹) in nutrient solution on mineral concentration in roots, crowns, leaves, and fruits of strawberry plants at 98 DAT. To illustrate the impact of Se on mineral composition in a particular plant organ, each organ was analyzed separately for P, K, Ca, Mg, Na, Zn, Cu, Mn, and Fe. For elements not included in the figure, no significant differences were found between the treatments. Signs to the right of each element's symbol indicate increase (+), decrease (-), or equality (=) in comparison with the control.

was obtained in soybean plants with foliar application of 50 mg L⁻¹ selenate (DJANAGUIRAMAN et al., 2005). On the other hand, DA SILVA et al. (2017) found no effect associated with the application of Se-selenite in concentrations of up to 3.2 mg L⁻¹ in lettuce plants. In this study, the fresh weights obtained for roots (60 DAT) and for crowns and leaves (98 DAT) are consistent with those reported by MIMMO et al. (2017), who did not find significant differences between treatment with Se-selenate at approximately 0.79 mg L⁻¹ but observed an increase in fresh shoot weight in strawberries at a concentration 10 times higher (approximately 7.9 mg L⁻¹ Se).

The decreased biomass found for the second sampling was associated with the highest concentration of Se applied and was reflected in the weights of the roots and leaves, the organs where Se accumulation was highest; therefore, the results can be interpreted as a sign of toxicity. It is known that the impact of Se on plants depends mainly on its concentration (HAMILTON, 2004); low Se levels will promote growth, but high levels can inhibit it (BOLDRIN et al., 2016). AHMED (2010) observed a decrease in the fresh weight of tomato plants after applying Se in the forms of sodium selenite and organic Se in doses of 2 and 30 mg kg⁻¹ of soil, respectively. RAMOS et al. (2010) reported a decrease in the dry weight of lettuce plants grown in hydroponics when Se was applied in concentrations greater than 0.6 mg L⁻¹; both the selenite and sodium selenate forms were used, with selenite having a more significant effect.

It has been suggested that Se's negative effect on growth is partly due to the substitution of sulfur by Se in proteins, which could modify the functionality of those proteins and sulfur metabolism in the plant (BOLDRIN et al., 2016). Perhaps this effect is different from one plant organ to another, which could explain the positive results in some organs and either the absence of response or undesirable effects in others.

Selenium distribution in plants

The differential distribution of Se (roots > crowns > leaves) in strawberry plants was also reported for tomatoes (BECVORT-AZCURRA et al., 2012), strawberries (MIMMO et al., 2017), and beans (ARVY, 1982). For the first sampling, Se concentrations decreased as the distance of different organs from the root increased. The roots absorb the selenite through phosphate transporters dependent on the co-transport of H⁺, and once in the root tissue, it is rapidly transformed into organic forms of Se that have less mobility in the xylem than ionic forms (SÁNCHEZ-RODAS et al., 2016). The data from the first sampling seem to indicate that the distribution of Se resulted from the mobility of selenite and its organic forms. For the second sampling, however, the concentration in the leaves was higher than in the crowns, possibly indicating a type of foliar storage dependent on exposure time.

The average Se concentration in the fruits obtained in this study was 31.2 mg kg⁻¹, which is lower than that reported in strawberries treated with selenate at a concentration of 7.9 mg L⁻¹ (46 mg kg⁻¹) (MIMMO et al., 2017). Similarly, Se concentrations were higher in other types of fruits and vegetables fertilized with Se, measuring 46.7 mg kg⁻¹ for *Opuntia* (BAÑUELOS et al., 2011), 35.8 mg kg⁻¹ for tomatoes (CASTILLO-GODINA et al., 2016), and 84.32 mg kg⁻¹ for radishes (SCHIAVON et al., 2016). However, the concentration obtained in this study is high enough to meet the recommended daily intake in the United States (55 µg d⁻¹ Se) and in the United Kingdom (60-75 µg d⁻¹ Se) (WHITE and BROADLEY, 2005), assuming that 16 to 23 g FW of biofortified strawberries (2-3 fruits) are consumed and that 92%-95% of the weight of these fruits is water. On the other hand, eating 8.2 g d⁻¹ – the per capita consumption (LUCIER et al., 2006) – of strawberries with Se concentrations found in this study would contribute 0.026 mg Se, approximately half of the daily intake recommended in the United States. The Se concentration found in the vegetative organs of strawberries corroborate other results indicating that as the Se concentration in the medium increases, the greater the concentration in plant tissues (SCHIAVON et al., 2016; DA SILVA et al., 2017).

Concentrations of other minerals in plants

Changes in the concentrations of some elements are probably one of the first observable signs of Se presence in plants (PAZURKIEWICZ-KOCOT et al., 2003). Modifications in the absorption and accumulation of different elements in strawberry plants could be due to an alteration in the absorption path or a change in the permeability coefficient of plasma membranes (PAZURKIEWICZ-KOCOT et al., 2003). The result can be damage to the membrane, triggering oxidative stress and production of reactive oxygen species (ROS) (HARTIKAINEN et al., 2000), caused either by excess Se or by Se's inhibitory effect on Zn concentration (FARGAŠOVÁ et al., 2006). It is known that ROS production induces membrane depolarization, which modifies the flow of ions, including chloride and potassium efflux and calcium influx (DEMIDCHIK, 2014). The slight increases in Ca and Na, as well as the decreases in K and Zn, observed in this study could possibly be due to these ions' involvement in the regulation of cell membrane potential and turgor (PAZURKIEWICZ-KOCOT et al., 2003). On the other hand, the increase in Na can also be attributed to the application of 2 and 4 ml L⁻¹ Se as sodium selenite, which contributed 1.16 and 2.32 mg L⁻¹ of Na, respectively. However, these results differ from those found in lettuce plants, in which Na concentration decreased in plants treated with up to approximately 3.15 mg L⁻¹ Se as sodium selenite (DA SILVA et al., 2017). For the second sampling, K was possibly replaced by Na, resulting in reduced K concentrations in the roots, crowns, and leaves (BENTON, 2012).

Similar results have been documented in corn plants treated with approximately 4 mg L⁻¹ Se in nutrient solution, in which K content

Tab. 4: Spearman correlation coefficients obtained from the relationships between growth and mineral variables and concentrations of Se present in different plant organs, evaluated at 98 DAT.

		[Se]						[Se]			
		Root	Crown	Leaves	Fruit			Root	Crown	Leaves	Fruit
Root	P	-0.14	-0.27	-0.30	-0.18	Crown	P	-0.18	-0.55	-0.50	-0.35
	Ca	0.43	-0.01	0.03	0.29		Ca	0.18	0.88 *	0.70 *	0.42
	K	-0.67	-0.88 *	-0.80 *	-0.82 *		K	-0.31	-0.68	-0.73 *	-0.49
	Mg	-0.04	-0.49	-0.47	-0.16		Mg	-0.41	0.01	0.03	-0.21
	Na	0.63	0.48	0.45	0.64		Na	0.42	0.79 *	0.78 *	0.54
	Fe	-0.10	0.66	0.78 *	0.08		Fe	0.59	-0.05	0.06	0.34
	Zn	-0.43	-0.79 *	-0.82 **	-0.48		Zn	-0.68	-0.83 *	-0.93 **	-0.73 *
	Cu	0.57	0.70 *	0.85 **	0.44		Cu	-0.45	0.16	0.22	-0.13
	Mn	0.57	0.92 **	0.92 **	0.62		Mn	-0.64	-0.28	-0.43	-0.48
	Se	1	0.52	0.52	0.82 *		Se	0.52	1	0.90 *	0.73 *
	RDW	0.20	-0.32	-0.22	-0.18		PSC	0.32	-0.23	-0.18	-0.03
	RFW	-0.59	-0.81 **	-0.79 *	-0.79 *		PFC	0.28	-0.23	-0.27	-0.03
Leaves	P	-0.67	-0.79 *	-0.83 **	-0.69	Fruit	P	0.72 *	0.84 **	0.73 *	0.89 **
	Ca	0.16	-0.46	-0.46	0.04		Ca	0.24	-0.19	0.19	-0.11
	K	-0.59	-0.97 **	-0.90	-0.76 *		K	-0.65	-0.72 *	-0.67	-0.58
	Mg	-0.28	-0.61	-0.63	-0.35		Mg	-0.91 **	-0.44	-0.29	-0.77 *
	Na	0.81 **	0.47	0.42	0.71 *		Na	0.83 **	0.49	0.54	0.68
	Fe	0.53	0.01	0.08	0.25		Fe	0.30	-0.25	0.00	0.08
	Zn	-0.65	-0.90 **	-0.88 **	-0.84 **		Zn	-0.27	-0.55	-0.50	-0.35
	Cu	-0.81 **	-0.47	-0.47	-0.69		Cu	-0.72 *	-0.50	-0.43	-0.62
	Mn	-0.14	0.49	0.39	0.16		Mn	-0.53	-0.81 **	-0.66	-0.64
	Se	0.52	0.90 *	1	0.60		Se	0.82 *	0.73 *	0.60	1
	DWL	0.02	-0.77 *	-0.78 *	-0.27		FDW	0.23	0.15	-0.05	0.22
	FWL	-0.60	-0.97 **	-0.85 **	-0.60		FFW	0.63	0.45	0.20	0.65

* = significant ($P < 0.05$); ** significant ($P < 0.01$). RFW= root fresh weight, RDW= root dry weight, CFW= crown fresh weight, CDW= crown dry weight, FWL= fresh weight of leaves, DWL= dry weight of leaves, FFW= fruit fresh weight, FDW= fruit dry weight.

decreased and Ca increased in both the shoots (HAWRYLAK-NOWAK, 2008) and roots; additionally, Na increased in the leaves, in which turgor was also observed (PAZURKIEWICZ-KOCOT et al., 2003). This study's results differ from those reported in lettuce plants treated with 3 mg L⁻¹ Se-selenite; in that study, Ca concentration decreased (RIOS et al., 2013), whereas in plants treated with approximately 9.4 mg L⁻¹ Se-selenite, K concentration increased (RIOS et al., 2013). In the same way, increases in K in tomato plants treated with 2 mg L⁻¹ Se-selenite have also been recorded (CASTILLO-GODINA et al., 2016). Mg also plays a protective role in maintaining the integrity of plant tissue (WILKINSON et al., 1990) as a Se tolerance mechanism (FENG et al., 2009). Therefore, an increase of this ion would be expected; however, in this study, Mg decreased in the roots. In other studies, conflicting results were found in strawberry roots (MIMMO et al., 2017) and in tomato roots and leaves (SCHIAVON et al., 2013; CASTILLO-GODINA et al., 2016), whereas in corn plants (HAWRYLAK-NOWAK, 2008) and tomato leaves (SCHIAVON et al., 2013), no significant effects associated with Se treatments were found.

Se has also been found to modify the transport or absorption of other elements (DJANAGUIRAMAN et al., 2005; PILON-SMITS et al., 2009). Depending on the Se concentration, Se will cause synergistic or antagonistic effects, such as those observed in this study for P concentration in different organs. The antagonistic effect of Se-selenite on P observed in the leaves is probably the result of competition between these ions (HOPPER and PARKER, 1999), since both are absorbed through phosphate transporters (ZHAO et al., 2010). This antagonistic effect has also been reported in lettuce plants treated with

Se levels ranging from approximately 1.58 to 9.5 mg L⁻¹ (RIOS et al., 2013). The synergistic effects observed in fruits between P and Se have also been reported in lettuce plants treated with Se-selenite at concentrations of approximately 1.97 mg L⁻¹ (DA SILVA et al., 2017) and approximately 0.79 mg L⁻¹ (RIOS et al., 2013), and in corn plants when Se increases from approximately 0.4 to 4 mg L⁻¹ (HAWRYLAK-NOWAK, 2008). The decrease of P in leaves and its increase in fruits is hard to explain but probably was due to some translocation process of this element associated with fruit growth or maturation.

Another metabolic pathway modified by Se is cellular redox balance variation (DJANAGUIRAMAN et al., 2005; PILON-SMITS et al., 2009), which involves antioxidant synthesis associated with the reduction of ROS levels (FENG et al., 2013). The change probably occurs due to modifications in the concentrations of some microelements used as cofactors for superoxide dismutase enzymes (Fe, Mn, Cu, and Zn), the peroxidase enzyme (Fe), the catalase enzyme (Fe), and enzymes involved in the biosynthesis pathway for chlorophyll (Fe) (FENG et al., 2013). Depending on Se concentration, a pro-oxidant or antioxidant response can be induced, modifying gene expression (FLOHÉ et al., 2000) and changing the transcripts' abundance and post-transcriptional regulation of different proteins (SCHIAVON et al., 2015), among which some transport proteins can be found. This could partially explain the variability in the concentration of some elements assessed in plants. It has been reported that high Se concentrations induced the highest Fe absorption, while low Se concentrations reduced Fe absorption (FENG and WEI, 2012). However, this effect on Fe is not a constant, since FARGAŠOVÁ et al. (2006) found that the absorption

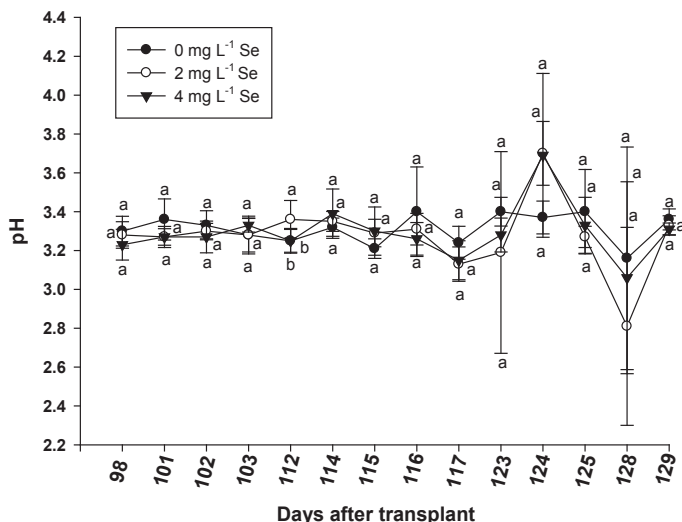


Fig. 4: pH values over time of strawberry fruits harvested from plants treated with different concentrations of Se in nutrient solution. Averages with different letters were statistically different according to LSD ($\alpha \leq 0.05$).

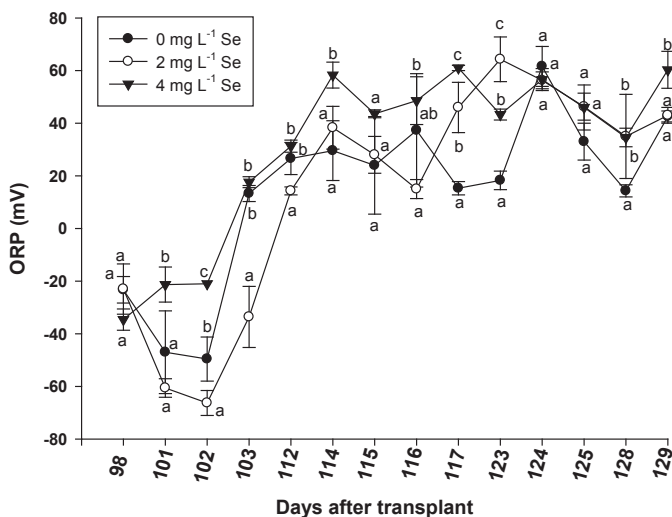


Fig. 5: Oxidation-reduction potential (ORP) values over time of strawberry fruits harvested from plants treated with different concentrations of Se in nutrient solution. Averages with different letters were statistically different according to LSD ($\alpha \leq 0.05$).

of elements such as Zn and Fe is inhibited by increasing Se levels. Furthermore, LONGCHAMP et al. (2016) detected a 30% decrease in Zn content in the leaves and stems of corn plants submitted to 1 mg L⁻¹ Se-selenite, while at 0.01 mg L⁻¹ concentrations, Fe concentration in the roots increased; no significant effects were found for Zn, Cu, and Mn. DA SILVA et al. (2017) also demonstrated that treatment with selenite decreased the concentrations of Cu and Fe.

For the second sampling, additional changes in the concentrations of elements were evident in different plant organs (Fig. 1). The changes undoubtedly occurred because the longer exposure time to the Se solution (38 days elapsed between the first and second sampling dates) increased the concentration of Se, since it is known that the concentration of this element in plant tissues is a function of its availability in the growing medium (BECVORT-AZCURRA et al., 2012). The results of foliar analyses suggest that Se application in nutrient solution at concentrations of 2 and 4 mg L⁻¹ was not associated with adverse effects on macronutrient concentrations, which remained

above the sufficiency ranges (BENTON, 2012). The same pattern was repeated for micronutrients, except for Mn, which decreased to concentrations below the sufficiency ranges in treated and untreated plants. In a study of strawberry plants, Se-selenate application in concentrations of 10 and 100 μ M (approximately 0.79 and 7.90 mg L⁻¹) did not significantly modify the concentrations of P, K, Ca, Mg, S, Fe, and Mn in the roots, shoots, and fruits (MIMMO et al., 2017). Moreover, in cucumber plants, selenite and selenate application in lower concentrations than those used in this study (<10 μ M, or approximately 0.79 mg L⁻¹) did not substantially modify the concentrations of N, P, K, Mg, Ca, and S (HAWRYLAK-NOWAK et al., 2015). In tomato plants, treatment with Se-selenite at concentrations of 2 and 5 mg L⁻¹ did not interfere negatively with the accumulation of N, P, K, Ca, and Mg in the stems, leaves, and fruits (CASTILLO-GODINA et al., 2016). In lettuce plants treated with Se-selenite at concentrations of 5 and 10 mg L⁻¹, the concentrations of N, P, K, Ca, Mg, Na, Zn, Mn, and Cu were not substantially modified (LÓPEZ-GUTIÉRREZ et al., 2015).

pH and ORP in fruit dynamics

For the duration of the experiment, the pH of the fruit pulp was maintained at an average value of 3.32 for the three treatments, a value considered as acceptable in strawberry fruits (ROUDEILLAC and TRAJKOVSKI, 2004; PÉREZ DE CAMACARO et al., 2005).

Low ORP values obtained in the first 12 days of harvest indicated that Se applied at 2 mg L⁻¹ had an impact on the reducing capacity of the fruit pulp. The ORP values mentioned indicate the antioxidant capacity – that is, the capacity of the system under analysis to give electrons in comparison with a hydrogen electrode (BENAVIDES-MENDOZA et al., 2002). The lower the ORP value, the higher the capacity to release electrons to function as an antioxidant.

This transient response to Se application could be caused by the capacity of this metalloid to induce oxidative stress, the magnitude of which depends on Se concentration. At low concentrations, it can be an activator of the plants’ antioxidant system (KONG et al., 2005), as seemed to occur with treatment at 2 mg L⁻¹ during the first 122 days. In contrast, at high concentrations, it functions as a pro-oxidant agent (HARTIKAINEN et al., 2000), a situation that undoubtedly occurred when Se was applied at a concentration of 4 mg L⁻¹, as well as after 122 days when applied at a concentration of 2 mg L⁻¹. These results indicate that if the purpose of Se application is to increase the antioxidant state of the fruits, the applied concentration should be less than 2 mg L⁻¹; otherwise, Se should be applied intermittently (for example, once a week) instead of continuously, as was done in this study.

Se application as sodium selenite in nutrient solution at a concentration of 2 mg L⁻¹ was determined to be a proper enrichment technique for strawberry fruits. Plant biomass decreased when Se was applied at concentrations of 2 and 4 mg L⁻¹, but fruit production was not diminished. Mineral concentrations in different plant organs were both positively and negatively associated with Se application, but the concentrations did not decrease below the recommended ranges. Neither pH nor ORP in fruit pulp were modified by Se treatments, and a positive effect was observed on the antioxidant status of the fruits in the first days of harvest when treated at 2 mg L⁻¹.

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