

Agronomic biofortification of carrot with selenium

Biofortificação agrônômica da cenoura com selênio

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ABSTRACT

The selenium (Se) is essential for human metabolism, but a large part of the world's population has deficiency in this element. This can be reversed by the consumption of biofortified foods, given that plants can efficiently act in controlling excessive and/or accidental consumption of an element that can occur in humans through the use of dietary supplements. The objective of this study was to evaluate the effect of different application forms and sources of Se in the growth, production, nutrition, physical-chemical characteristics, content and accumulation of Se in carrots. The experiment was conducted under greenhouse conditions, using pots containing 4 dm³ of Red-yellow Latosol. A completely randomized design was used in a 2x2x2 factorial scheme (with and without Se application, two sources of Se: selenate and selenite, two forms of application of Se: soil and foliar applications), with five replicates. Foliar application of selenate increased the yield and titratable acidity, reducing root ripening index. Foliar application of selenite increased the content of Se in the shoots and the content of carotenoids in the roots. Both sources of Se (selenate and selenite) and application forms (soil or foliar application) increased their content in the roots. However, the foliar application of selenate was the most effective source and form of application. Therefore, it is possible to increase the contents of Se in the edible part of carrots, favoring the consumption of this element by the population.

Index terms: *Daucus carota* Lam; selenate; selenite; maturation index; carotenoids.

RESUMO

O selênio (Se) é essencial para o metabolismo humano, porém, grande parte da população mundial apresenta deficiência nesse elemento. Isto pode ser revertido pelo consumo de alimentos biofortificados, uma vez que as plantas podem atuar de forma eficiente no controle do consumo excessivo e / ou accidental de um elemento que pode ocorrer nos seres humanos através do uso de suplementos dietéticos. Objetivou-se com o presente estudo avaliar o efeito de formas de aplicação e fontes de Se no crescimento, produção, nutrição, características físico-químicas, teor e acúmulo de Se na cenoura. O experimento foi desenvolvido em casa de vegetação, utilizando vasos com quatro dm³ contendo Latossolo Vermelho Amarelo. Adotou-se o delineamento inteiramente casualizado, em fatorial 2 x 2 x 2 (com e sem aplicação do Se; duas fontes de Se: selenato e selenito; duas formas de aplicação do Se: via solo e foliar), com cinco repetições. O selenato aplicado via foliar aumenta a produção e a acidez titulável e reduz o índice de maturação das raízes. O selenito via foliar aumenta o teor de Se na parte aérea e o teor de carotenoides nas raízes de cenoura. As duas fontes de Se (selenato e selenito) e formas de aplicação (via solo ou foliar) promovem aumento do seu teor nas raízes de cenoura, porém o selenato via foliar é a fonte e forma mais efetiva. Dessa forma, é possível aumentar os teores de Se na parte comestível da cenoura e, assim, favorecer a ingestão desse elemento pela população.

Termos para indexação: *Daucus carota* Lam; selenato; selenito; índice de maturação; carotenoides.

INTRODUCTION

Selenium (Se) is an essential trace element for animals and humans. It acts in the antioxidant system as a key component of selenoproteins and selenoenzymes, with important biological functions (Rayman, 2002). In addition, it can act in the formation of hormones, in DNA synthesis, fertility, reproduction, increasing muscle functions, and delaying the aging process (Suttle, 2010; Cabaraux et al., 2006). Furthermore, there are reports of cancer prevention through the action of selenoenzymes,

which reduce damages caused to the DNA and decreases reactive oxygen species, increasing the production of tumor suppressor proteins (Almondes et al., 2010).

Despite its essential nature, Se deficiency affects hundreds of millions of people in the world, and is relevant in several regions of Brazil, indicating the need to adopt strategies to increasing Se consumption. Plants play a key role in introducing Se into the food chain. Thus, biofortification (increasing the content of a certain element in edible parts of plants) can be an important technique to

increase the consumption of the element by animals and humans.

Although selenium has not been demonstrated to be essential in plants, many studies show that, when supplied in low doses, Se benefits plant growth and production (Kápolna et al., 2009; Ramos et al., 2011; Boldrin et al., 2013; Zhu et al., 2017).

The success of biofortification depends on several factors, specially type of crop, when considering acceptance and consumption by the population. Studies have shown the success of biofortification with Se in lettuce, rice, wheat and broccoli (Ramos et al., 2011; Boldrin et al., 2013; Zembala et al., 2010; Ávila et al., 2014). In this sense, carrot (*Daucus carota* L.), a widely consumed vegetable, renders great potential to this practice, since it presents a high content of carotenoids. These pigments are precursors of vitamin A and have an antioxidant effect in the human body, inhibiting the action of free radicals, which are harmful to cells and are associated with the development of diseases such as cancer, cataracts and arteriosclerosis.

Research has already shown that carrots are highly efficient in absorbing and metabolizing Se, when supplied at low doses via the leaf (Kápolna et al., 2009) and soil (Bañuelos et al., 2015; Smoleń et al., 2016). However, there are few studies focusing on this specie.

When considering biofortification programs with Se, it is important to consider the chemical form of Se that is to be applied, as well as the form of application. Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are the inorganic forms of the element with the highest potential for absorption and bioaccumulation by plants. Selenite has a strong interaction with the clay fraction of a large portion of the soils cultivated in tropical and subtropical regions, therefore, less availability when compared to selenate (Ylärinta, 1984; Martínez et al., 2006; Li; McGrath; Zhao, 2008). Moreover, after absorption by the roots, selenate presents greater mobility in the xylem, whereas selenite is rapidly assimilated into organic compounds with low mobility (Li; McGrath; Zhao, 2008). On the other hand, when applied via the leaf, selenite tends to accumulate more in the aerial part than selenate, as verified by Ylärinta (1984), when studying *Phleum pratense* L.

Therefore, considering the essentiality of Se to humans, and the importance of carrot in the global food scenario, this study aimed at evaluating the effect of different forms of application and sources of Se in the production of plant dry mass and fresh roots, physico-chemical characteristics and carotenoid concentration in

roots, as well as in the macro and micronutrient contents in the shoot and roots of carrots.

MATERIAL AND METHODS

Conduction of the experiment and experimental design

The experiment was conducted under greenhouse conditions at the Department of Soil Science at the Universidade Federal de Lavras, using 4 kg pots, filled with dystrophic Red-Yellow Latosol (LVAd) collected in the 0-20 cm layer. Chemical and physical soil characterization was performed according to the methods described by Embrapa (1997), presenting the following values: pH in water: 4.8; P-mehlich-1: 1.1 mg dm^{-3} ; P-resine: 26.6 mg dm^{-3} ; K: 32 mg dm^{-3} ; Ca: 0.3 cmol dm^{-3} ; Mg: 0.1 cmol dm^{-3} ; Al: 0.6 cmol dm^{-3} ; H + Al: 4.5 cmol dm^{-3} ; SB: 0.5 cmol dm^{-3} ; SOM: 1.6%; V: 9.6%; t: 1.1 cmol dm^{-3} ; T: 5.0 cmol dm^{-3} ; m: 55.5%; S: 9.9 mg dm^{-3} ; Zn: 0.5 mg dm^{-3} ; B: 0.2 mg dm^{-3} ; Fe: 41.6 mg dm^{-3} ; Mn: 4.1 mg dm^{-3} ; Cu: 0.5 mg dm^{-3} ; sand: 740 g kg^{-1} ; silt: 30 g kg^{-1} ; clay: 230 g kg^{-1} . The natural content of Se in the soil, analyzed according to Ramos et al. (2010), was of 0.065 mg dm^{-3} .

The experiment was conducted in a completely randomized design, using five replicates, with 2x2x2 factorial scheme, considering the presence and absence of Se; two sources of Se (sodium selenate - Na_2SeO_4 and sodium selenite - Na_2SeO_3), and two forms of application (soil and foliar application at the doses of 1.0 mg dm^{-3} and 50 $\mu\text{m L}^{-1}$, respectively). Each experimental unit consisted of six plants per pot. The concentration of Se in foliar application was chosen within the range suggested by Kápolna et al. (2009) for carrot, and the same applied by Boldrin et al. (2013) in rice.

Based on the soil chemical analysis, liming was performed aiming to increase base saturation to 70%, using dolomitic limestone (CaO: 37%, MgO: 15% and RTNP: 85%). The soil was incubated for 30 days, with humidity close to 60% of the total pore volume. Planting and cover fertilization were performed according to Malavolta (1980): 300 mg dm^{-3} of N and 150 mg dm^{-3} of K (divided into three applications); 200 mg dm^{-3} of P; 50 mg dm^{-3} of S; 1.5 mg dm^{-3} of Cu; 5 mg dm^{-3} of Zn and 0.1 mg dm^{-3} of Mo per pot. The application of Se in the soil was carried out together with the fertilization of planting.

After fertilization, a total of 15 carrot seeds (*Daucus carota* L. cv Brasília) were sown in each vase and cut to six seedlings at 14 days after emergence. The foliar application of Se was applied 45 days after sowing (DAS), in the morning (between 8:30 and 9:30 h), using

a CO₂ pressurized sprayer with a constant pressure of 2.8 kgf cm⁻², evenly distributing the solution on all leaves.

Growth, root production and nutrient contents

At the end of the experiment, plants were separated in aerial part and roots. The roots were washed and weighed to determine fresh mass production. Two roots of each plot were separated for physico-chemical analysis: soluble solids (SS), titratable acidity (TA), maturation index (MI), pH and carotenoid contents.

The remaining roots and aerial parts of the plants were dried in a forced air circulation oven at 65 °C for 72 h until constant weight, and the aerial dry mass (ADM) was determined. The parts were ground in a Willey type mill with 1.0 mm (20 mesh) mesh sieves, digested, determining the contents of macro and micronutrients according to the methodology described by Malavolta, Vitti and Oliveira (1997).

Physico-chemical characteristics and carotenoid content of roots

The determination of soluble solids (°Brix) (SS) and pH in fresh root samples was done according to the methodology proposed by AOAC (2010), the titratable acidity (TA) was obtained according to the IAL method (2008) and the maturation index (MI) was obtained by the relationship between soluble solids and titratable acidity, according to Tressler and Joslyn (1961). Carotenoids were determined according to the methodology proposed by Rodriguez-Amaya (2001).

Determination of Se contents

For the determination of Se contents, 0.5 g of dry tissues (shoot and roots) were digested in 6 mL of a mixture of nitric acid (HNO₃ - ≥ 65%) and perchloric acid (HClO₄ - 69.72%) in a 2:1 ratio. The digestion was performed in a digestion block for 2 h; (initial temperature 50 °C) until complete digestion, the temperature was gradually increased (50 °C every 30 min) for 2 h until reaching a final temperature of 200 °C. Ten milliliters of deionized water was added after cooling.

A sample with a standard reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) of known content (6.7 mg kg⁻¹) was included in each digestion battery for quality control. The mean recovery for Se in this reference material was of 89.4% (n=4). The extracts were analyzed by atomic absorption spectroscopy with electro-thermal atomization in a graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada).

Statistical analysis

The normality of the data was analyzed using the Anderson-Darling test and the homoscedasticity of the data was verified with the variance equation test (or Leven's test). After the data were submitted to analysis of variance and the significance was verified by the F test, the Scott-Knott test was performed at a level of 5% of probability (p<0.05), using the statistical program SISVAR (Ferreira, 2011). Graphs were built using the Sigma Plot 12.5 software (Systat Software, Inc., San Jose, CA, USA).

RESULTS AND DISCUSSION

Production of dry shoot mass and fresh root mass

The production of dry shoot mass (DSM) was significantly affected (p≤0.05) by both the presence and the form of Se application, as well as by the interaction between these factors, while the production of fresh roots was altered by the forms and sources of Se, and by the interaction of these factors (Figure 1). However, when Se was applied on the leaves, there was an increase of 14% in relation to the control, and of 16% in relation to soil application (Figure 1A).

Regarding the production of fresh roots, there was no influence of Se application via soil, regardless of the source (Figure 1B). On the other hand, foliar application of selenate increased root yield by 73% in relation to the control, and by 70% when compared to soil application. No difference was observed between selenite and control.

Although Se is not considered essential for plants, several studies have shown that, in low concentrations, this element has beneficial effect over growth and stress tolerance, by increasing the antioxidant capacity of plants through the activation of enzymes superoxide dismutase, catalase, glutathione peroxidase, decreasing reactive oxygen species and lipid peroxidation (Djanaguiraman et al., 2005; Xue; Hartikainen; Piironen, 2001). However, agricultural crops are sensitive to high concentrations of Se in their tissue, with sensitivity varying among plant species (Lyons et al., 2005), requiring care when establishing doses of Se.

Physical-chemical characteristics and carotenoid contents in the roots

The physical-chemical characteristics of carrot roots were significantly affected (p≤0.05) by the application forms and sources of Se, as well as by the interaction of these factors (Figure 2).

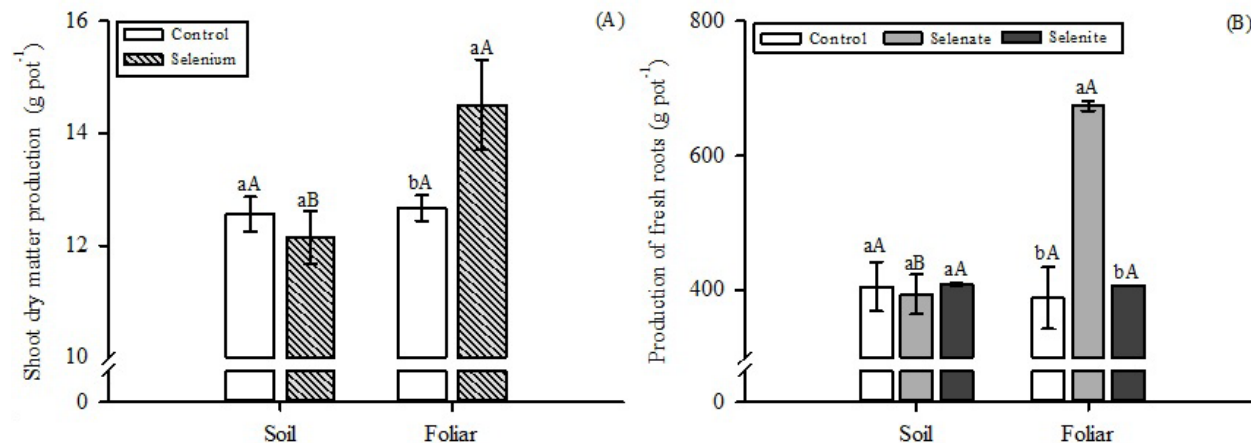


Figure 1: Production of dry shoot mass (A) and fresh roots (B) of carrot plants according to the sources and application of Se. (A): uppercase letters compare the presence and absence of Se in each form of application and upper and lower case letters compare the forms of application of Se in the presence or absence of Se. Same letters mean they do not differ from each other (F test, $p < 0.05$); (B): lowercase letters compare the sources of Se in each form of application and uppercase letters compare the application forms of Se in each source. Same letters mean they do not differ from each other (Scott-Knott test, $p < 0.05$). Vertical bar indicates the standard error of the mean ($n=5$).

Soluble solids (SS) contents were increased by the application of selenate via soil (35%) and via foliar (7%), while, with selenite, the effect occurred only for foliar application, with a 23% increase in relation to the control (Figure 2A). Selenate applied via soil was more efficient than its foliar supply while the opposite was observed for selenite.

Soluble solids influence the taste of the products and are represented by acids, salts, vitamins, amino acids, sugars and other substances important for food quality. Therefore, the use of Se improved the flavor of the carrot, which also occurred in pear fruits when applying the element in the form of selenate (Pezzarossa et al., 2012). The knowledge of the influence of Se in characteristics such as SS ($^{\circ}$ Brix) of vegetable crops is important because the consumer has preference for products with higher levels of SS, thus greater sweetness, especially when destined for *in natura* consumption or in salads, as is the case of carrots (Nascimento; Cardoso; Coccozza, 2014).

Increased titratable acidity (TA) was detected when Se was applied in the soil by both sources. An increase in TA was detected when applying Se via soil for both sources. This also occurred when applying selenite via foliar, with more pronounced effect when applying selenite via soil (Figure 2B). Similar behavior was observed for root pH (Figure 2C).

The TA and pH of vegetables crops are related to the food quality, given that they are linked to their flavor and aroma, which are very important for consumer acceptance. The pH values found in this study are within those expected for carrots, since this culture is classified as a low acid food. Silva et al. (2016) found a similar pH value (6.0) when studying carrots.

In relation to the maturation index (MI), when Se was supplied via soil, there was a reduction of 54% when selenate was applied, and of 64% when selenite was used, in relation to that obtained in the control plants (Figure 2D). On the other hand, foliar application of selenate reduced MI by approximately 45%, while selenite did not differ from control.

The MI is inversely proportional to the titratable acidity, which is related to the fact that the organic acids are used during maturation process, either through respiration or transformation into sugars (Lombardi; Moraes; Camelatto, 2000; Matarazzo et al., 2013). In a research conducted with tomatoes, Zhu et al. (2017) observed lower fruit maturation rates when plants were treated with 1 mg L⁻¹ of Se in the form of selenite. The authors related this behavior to the interference of Se in the production of ethylene. In addition, the maturation delay of the fruits can be related to the antioxidant effect of the element, as evidenced by Zhu et al. (2016) in tomato plants grown in the presence of selenate.

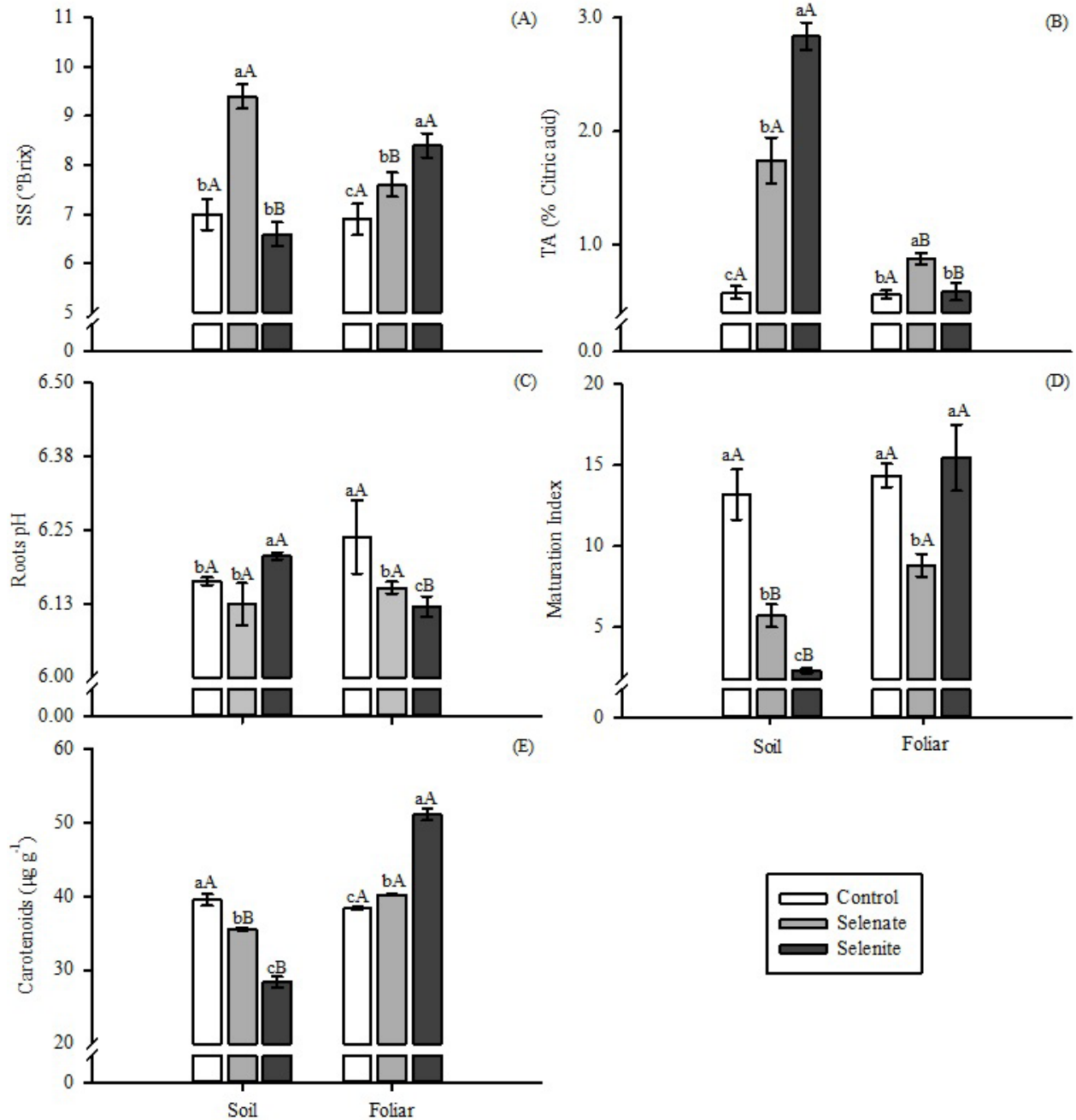


Figure 2: Soluble solids (A), titratable acidity (B), pH (C), maturation index (D) and carotenoid content (E) in carrot roots according to sources and application of Se. Lowercase letters compare sources (Scott-Knott's test, $p < 0.05$) in each application form, and uppercase letters compare application forms (F test, $p < 0.05$) in each source. Same letters mean they do not differ from each other (F test, $p < 0.05$). Vertical bar indicates the standard error of the mean ($n=5$).

Carotenoid content in the roots was affected by the interaction between the forms of application and the sources of Se (Figure 2E). The application of Se in the soil reduced the levels of carotenoids for both sources. On the other hand, foliar application promoted increases of 7 and 33% for selenate and selenite, respectively, in relation to the control. Several factors may influence carotenoid content, among which Rodríguez-Amaya (2001) highlights maturation degree.

Furthermore, carotenoids are compounds with a protective function in plants, acting against possible oxidative damages. Selenium presents a higher phytotoxicity when compared to selenate (Ríos et al., 2010; Guerrero et al., 2014; González-Morales et al., 2017). Therefore, an increase in carotenoid content was observed when foliar selenite was applied. When applied in the soil, this source of Se did not influence carotenoids. Thus, the increase in

carotenoid production when foliar selenite was applied may be related to the natural protection process of plants against a possible phytotoxicity caused by selenite (Figure 2E).

Nutrient contents

The contents of potassium (K) and iron (Fe) in the shoots, and of manganese (Mn) and iron (Fe) in the roots, were significantly affected ($p \leq 0.05$) by the interaction between sources and forms of application of Se (Figure 3). The other nutrients were not influenced by the treatments.

Selenate application in the soil caused a reduction of 26% of K when compared to the control, whereas selenite treatment did not differ from the control. On the other hand, the supply of Se via foliar did not affect the contents of K in the aerial part of the plants, regardless of the source used (Figure 3A).

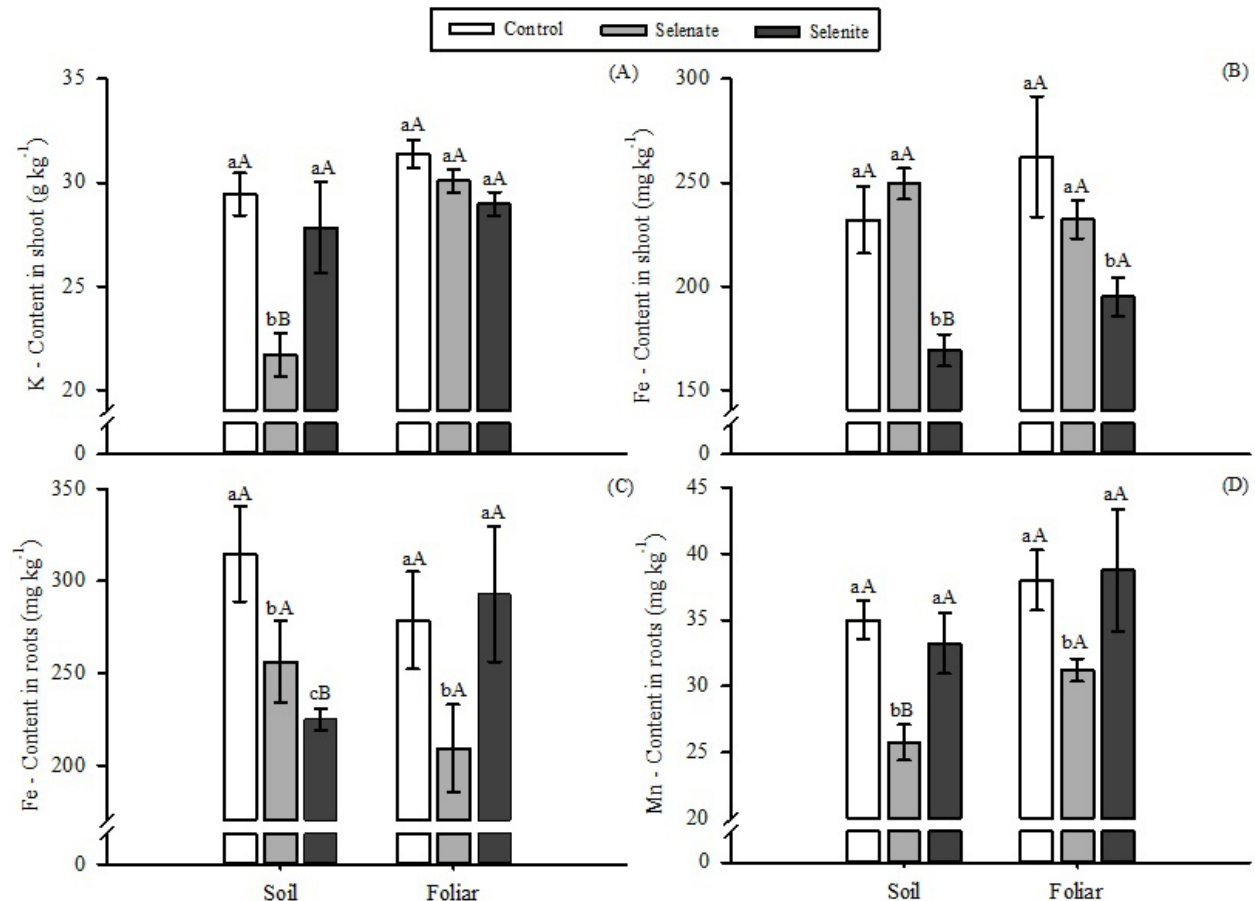


Figure 3: Contents of K and Fe (A and B) in the shoots and of Fe and Mn (C and D) in the roots, from different Se sources and forms of application. Lowercase letters compare sources (Scott-Knott's test, $p < 0.05$) in each application form and uppercase letters compare application forms (F test, $p < 0.05$) in each source. Same letters mean they do not differ from each other. Vertical bar indicates the standard error of the mean ($n=5$).

Drahonovský et al. (2016) detected reduction in K content in wild plant species when applied 1 ppm of Selenium, compared to control plants. However, there are few studies justifying the possible reduction of K when selenate is applied in plants. In this sense, Filek et al. (2010) relate the lower K content to the role of this ion in the osmotic regulation of cells, as well as may be related to an excess of ion input in the cells. That can also be applied to carrot plants.

The application of Se as selenite promoted a reduction in the content of Fe in the aerial part of the plants in both forms of application. The greatest reduction was observed for soil application (Figure 3B). The application of both forms of Se in the soil, and as selenate in foliar application, reduced the content of Fe in the roots (Figure 3C). For wheat grown in nutrient solution, Guerrero et al. (2014) observed that, when plants were submitted to different concentrations of Se (selenate and selenite), there was a reduction in Fe content in the aerial part and in the roots. For rapeseed, Zembala et al. (2010) detected a reduction in Fe content in the roots when Se was applied in the soil as selenate.

The application of selenite by both application forms reduced Mn levels in the roots (Figure 3D). Zembala et al. (2010), in a study conducted with rapeseed, found similar patterns. For selenite, the forms of application did not affect the contents of Mn in the roots.

Content and accumulation of Se

The levels of Se in the shoot and roots, and the accumulation of Se in the aerial part of carrot plants, were significantly influenced ($p \leq 0.05$) by the sources and forms of application of Se, as well as by the interaction between factors (Figure 4). There was an increase in Se content in the shoots (Figure 4A) and roots (Figure 4B) regardless of the form or source of Se application.

When Se was supplied via soil, the highest Se content was observed for selenite. However, the opposite was obtained when Se was applied via foliar (Figure 4A). In the roots (Figure 4B), the concentration was always higher when selenate was applied, either via soil or foliar application. This pattern was found for species such as wheat, rice, cabbage, tomato, and carrot (Li; McGrath; Zhao, 2008; Boldrin et al., 2013, Avila et al., 2014; Zhu et al., 2016; Kápolna et al., 2009).

When compared to the control treatment, the presence of Se as selenate promoted an increase of Se 2028 and 3570 times higher in the roots when applied to soil and leaves, respectively (Figure 4B). Similar results were observed by Kápolna et al. (2009), in a study conducted

with foliar application of Se in the form of selenate in carrot plants.

The highest accumulation of Se in the shoots was observed with foliar application of selenite (Figure 4C), which is similar to the findings of previous studies (Cartes; Gianfreda; Mora, 2005; Sors; Ellis; Salt, 2005; Boldrin et al., 2013). In general, foliar application promoted greater accumulation of Se in the aerial part regardless of the source used. When selenite is applied to soil, it tends to accumulate in a greater proportion in the roots (Boldrin et al., 2013, Guerrero et al., 2014; Smoleń et al., 2016), and when supplied via foliar, it accumulates more in the shoots.

In the interior of the plants, differences in the mobility of selenate and selenite have been reported, and it has been proven that the transport of selenate to shoot is faster than that of selenite (Zhang et al., 2003). After absorption by the roots, selenite is rapidly converted into organic forms, such as selenomethionine (Zayed; Lytle; Terry, 1998), and stored in the roots. Thus, it can be assumed that the same occurs when selenite is applied via foliar, accumulating in the aerial part of the plant.

Furthermore, studies conducted under field conditions and greenhouse have demonstrated the higher efficiency of selenate for Se biofortification when compared to selenite (Cartes; Gianfreda; Mora, 2005; Boldrin et al., 2013). This was also demonstrated in hydroponic cultivation (Ramos et al., 2011).

The most efficient way for Se absorption and metabolism in the human organism is the organic form found in vegetables, which makes them the most suitable products for the insertion of Se and other essential elements in the human food chain. Plants act efficiently in controlling excessive and/or accidental consumption of Se, which may occur through the use of dietary supplements that have the inorganic form of the element.

In this context, Se biofortification of carrot appears as an alternative to increase the intake of this element by the population, since an adult human is expected to ingest at least 70 μg per day, with a maximum tolerable limit of 400 μg (USDA, 2012).

From the results of this study, by consuming 50 g of fresh carrot that has been biofortified with selenite, applied through foliar application, which was the highest content of Se (14 mg kg^{-1} of dry mass), and considering the humidity of the carrot of 85%, the daily intake of the element would be of 105 μg . Although this consumption is above the minimum daily requirement, the quantity is within the range of tolerable daily consumption.

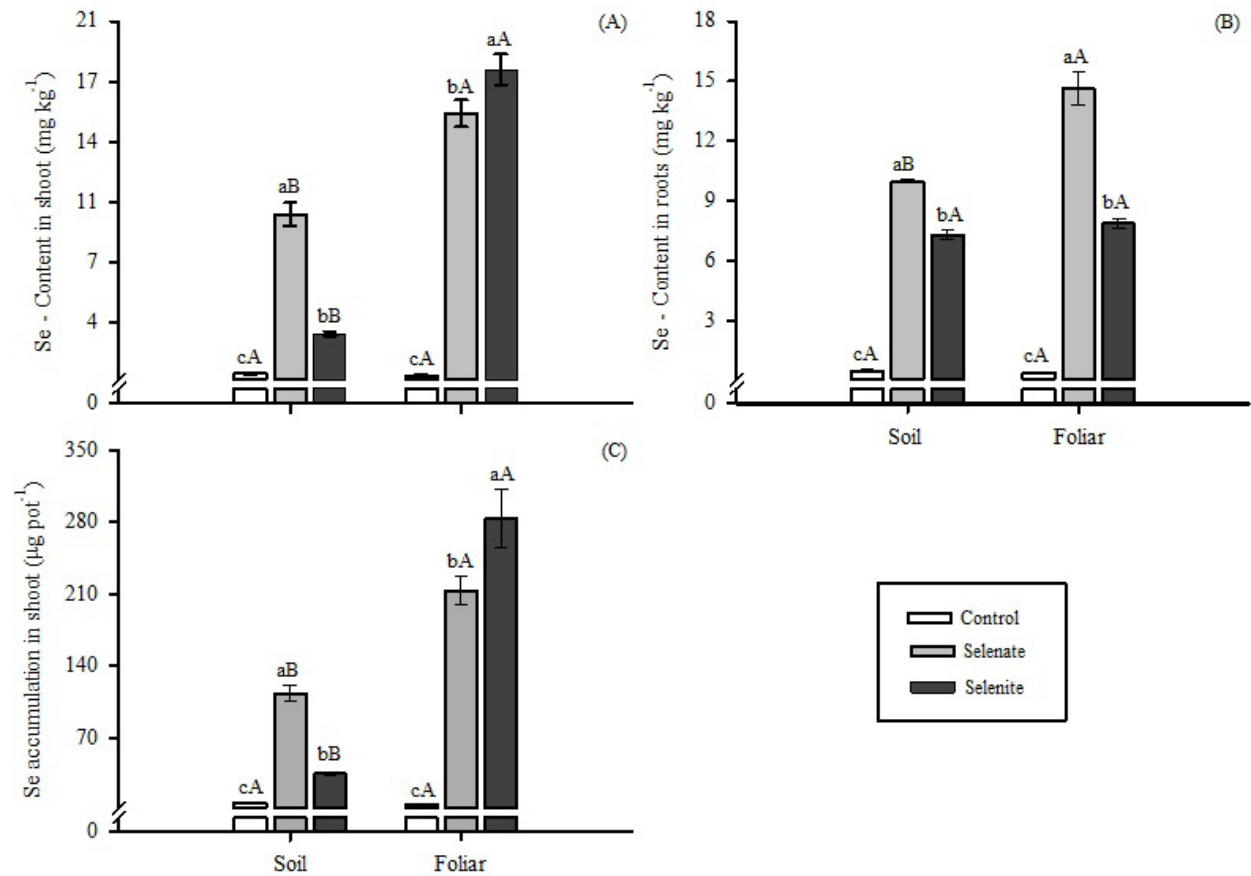


Figure 4: Se contents in the shoots (A) and roots (B), and Se accumulation in the aerial part (C) of carrot plants, according to the sources and application of Se. Lowercase letters compare sources (Scott-Knott's test, $p < 0.05$) in each application form and uppercase letters compare application forms (F test, $p < 0.05$) in each source. Same letters mean they do not differ from each other. Vertical bar indicates the standard error of the mean ($n=5$).

Although the results of carrot biofortification have been favorable, new studies regarding the application of the element to the crop under field conditions are still required, and further sensory tests must be done to determine if the changes in pH and maturation index caused by Se application are perceivable by consumers.

CONCLUSIONS

Foliar application of Se contributes to increase the production of dry mass of carrot plants. Foliar selenate increases yield, titratable acidity and reduces root maturation. When supplied via soil, selenate reduces the content of K in the shoot and the levels of Mn in the roots. When selenite is supplied via foliar application, the content

and accumulation of Se in the aerial part of the plants is increased, promoting higher contents of carotenoids and reducing the pH of the carrot roots. The application of Se, both through soil and foliar means, of both sources, increases its content in carrot roots. The most effective form of Se biofortification is foliar application as selenate.

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