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CROP PRODUCTION

Influence of sulfur on selenium absorption in strawberry

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ABSTRACT. Selenium (Se) is an essential mineral element for animals and humans, and attention has been devoted to Se biofortification of agricultural crops. Due to the scarcity of studies aimed at guiding the nutritional Se-enrichment on strawberry and the need to understand the interaction between Se and sulfur (S) in this cultivation, the objective of this study was to evaluate the effect of the application of Se and S levels on the nutrition and production of strawberry. Treatments consisted of five levels of Se (0, 0.3, 0.6, 1.2, and 2.4 mg dm^{-3} as sodium selenate) and two levels of S (0 and 60 mg dm^{-3} as gypsum), which were applied in dystroferric Red Latosol soils under greenhouse conditions. The concentration and accumulation of Se are drastically reduced in the shoots and fruits of strawberry with the application of the sulfated fertilization, while absorption of S is maximized with increased levels of Se. Strawberry presents good capacity for nutritional enrichment with Se, which was demonstrated by the accumulation of Se in the fruits, as well as by enhanced fruit yield.

Keywords: *Fragaria* x *ananassa* Duch.; biofortification with selenium; sulfated fertilization; ionic interaction.

Influência do enxofre na absorção de selênio pelo morangueiro

RESUMO. O selênio (Se) é essencial à nutrição de animais e seres humanos, e atenção recente vem sendo dada à biofortificação com Se em cultivos agrícolas. Devido à escassez de estudos que norteiem o enriquecimento nutricional do morangueiro com Se e da necessidade de ampliar a compressão da interação entre o Se e S nesta cultura, o objetivo desta pesquisa foi avaliar o efeito da aplicação de doses de Se e S na nutrição e produção do morangueiro. Os tratamentos foram constituídos de cinco doses de Se (0, 0.3, 0.6, 1.2 e 2.4 mg dm^{-3} como selenato do sódio) e duas doses de S (0 e 60 mg dm^{-3} como gesso agrícola) aplicados em um Latossolo Vermelho distroférrico em condições de casa de vegetação. O teor e acúmulo de Se são reduzidos drasticamente na parte aérea e nos frutos do morangueiro com a aplicação da adubação sulfatada, ao tempo que a absorção de S é maximizada com o aumento das doses de Se. O morangueiro apresenta boa capacidade para o enriquecimento nutricional com Se, demonstrado pelo acúmulo de Se nos frutos e aumento da produção.

Palavras-chave: *Fragaria x ananassa* Duch.; biofortificação com selênio; adubação sulfatada; interação iônica.

Introduction

Selenium (Se) is essential for animals and humans, and its ingestion is associated with risk reduction of numerous diseases, such as cancer (White & Broadley, 2009). However, nearly 1 billion people in the world suffer from Se deficiency due to the consumption of foods with low Se concentration (Rayman, 2012), especially as a result of the low and uneven availability of Se in most soils around world (Kabata-Pendias, 2011). Considering the imbalance of Se contents in soils and that plants are primary sources for the entry of Se into the food chain, agronomic biofortification through the use of concentrated fertilizers is one of the primary alternatives to increase the bioavailability of Se in food (Lyons, 2010).

Although not demonstrated to be essential, the application of low levels of Se has been shown to have beneficial effects on crops. For example, Se application increased antioxidant activity and consequently increased yields (Djanaguiraman, Devi, Shanker, Sheeba, & Bangarusamy, 2005; Ramos et al., 2010). In this sense, biofortification with Se has been the focus of studies in different crops (Ramos et al., 2011; Boldrin et al., 2012; Wang, Wang, Mao, Zhao, & Huang, 2013; Ávila et al., 2014).

Sulfur (S) plays a key role in the nutrition and production of agricultural crops. Considering this element's chemical similarity with Se, S forms tend to compete in such processes as absorption, translocation, and assimilation. For example, when

Se is present as selenate, it enters the same pathway as sulfate, replacing it in the synthesis of such proteins as cystine and methionine (Chang, Van Iersel, Randle, & Sams, 2008; Gupta & Gupta, 2017). Therefore, the S compounds present in the rhizosphere may inhibit Se uptake by plants (White et al., 2004; Schiavon, Pilon, Malagoli, & Pilon-Smits, 2015; Liu et al., 2017), thus influencing levels of these elements in plant tissues. However, despite the benefits that this interaction can exert on the fertilization of crops with Se, studies focused on Brazilian soils are lacking.

Strawberry (*Fragaria x ananassa* Duch.) has a rich nutritional composition with high levels of micronutrients, vitamin C, and phenolic compounds with anti-inflammatory and antioxidant effects (Giampieri, Alvarez-Suarez, & Battino, 2014). In addition to the beneficial nutrient levels for human health, strawberry is well-accepted by consumers due to such characteristics as flavour and aroma, being consumed *in natura* and processed. In Brazil, strawberry production has increased in recent years, leading small farmers to grow a product of high added value. However, few studies aimed at investigating the nutritional benefits of Se in this culture have been conducted (Zhang, Zhang, & Rui, 2013; Palencia, Martinez, Burducea, Oliveira, & Giralde, 2016).

Due to the lack of information that guides the nutritional enrichment of strawberry with Se and the need to increase the comprehension of the interaction between Se and S in this culture, the objective of this study was to evaluate the effects of the application of levels of Se and S in strawberry production.

Material and method

The experiment was conducted in the greenhouse at the Department of Soil Science of the Federal University of Lavras (UFLA), Minas Gerais State, Brazil from July to November 2014. Figure 1 shows the average temperature and humidity during the study period.

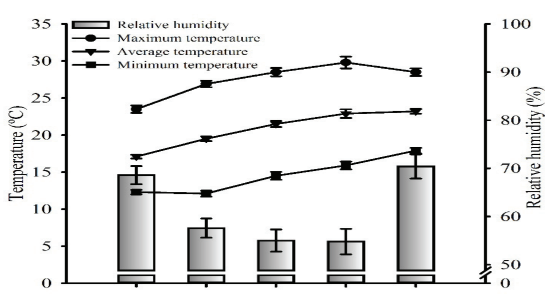

Figure 1. Temperature and relative humidity inside the greenhouse during the experiment period.

Pots with 3 dm^3 of a dystroferric Red Latosol (Embrapa, 2013) were used. Soil samples were collected at 0 – 0.20 m depth in an area of Cerrado native forest, located in Lavras, Minas Gerais State, Brazil. Soil analyses were performed according to the method described by Embrapa (2009) and yielded the following values: pH_{H2O} = 5.6, SOM = 31.4 g kg^{-1}, P = 0.84 mg dm^{-3}, K = 56 mg dm^{-3}, Ca = 1.20 $cmol_c$ dm^{-3}, Mg = 0.20 $cmol_c$ dm^{-3}, Al = 0.50 $cmol_c$ dm^{-3}, H+Al = 6.30 $cmol_c$ dm^{-3}, sum of the bases = 1.54 $cmol_c$ dm^{-3}, CEC at pH 7.0 = 7.84 $cmol_c$ dm^{-3}, base saturation = 20%, aluminium saturation = 22%, Zn = 0.66 mg dm^{-3}, Fe = 37.20 mg dm^{-3}, Mn = 19.96 mg dm^{-3}, Cu = 2.67 mg dm^{-3}, B = 0.43 mg dm^{-3}, S = 13.08 mg dm^{-3}, and Se = 0.1 mg dm^{-3}. Organic matter (OM) was determined by oxidation with $K_2Cr_2O_7$. Exchangeable cations (Ca, Mg, and Al^{3+}) were extracted with 1.0 mol L^{-1} KCl solution. Phosphorus (P), potassium (K) and the available contents of micronutrients: Zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) were extracted with Mehlich-1 solution. Aluminium (Al) was determined by titration with NaOH. P and K were determined by colourimetry and flame photometry, respectively. Calcium and Mg^{2+} along with other micronutrients were determined by atomic absorption spectrometry. Sulfur (S) was determined by turbidimetry. Boron (B) was determined colourimetrically by the curcumin method. The potential acidity (H + Al) was indirectly estimated through the pH values.

The experimental design was completely randomized in a 5 x 2 factorial scheme with three replicates. The treatments consisted of five levels of Se (0, 0.3, 0.6, 1.2, and 2.4 mg dm^{-3}) and two levels of S (0 and 60 mg dm^{-3}) that were applied in the form of sodium selenate (Na_2SeO_4) and gypsum, respectively. The levels of Se used were based on the studies of Boldrin et al. (2012) and Ramos et al. (2012) on tropical soils. Sodium selenate was chosen due to its greater mobility within plants compared to sodium selenite, as well as its competitiveness with S in the process of absorption by plants, which is the focus of this study.

Liming materials were applied based on the soil chemical analysis to raise base saturation up to 80% with the application of calcium carbonate and magnesium carbonate. Soil samples were homogenized and incubated for 15 days at 60% of field capacity. After the incubation period, fertilization was performed with the application of S and Se according to the treatments along with 466 mg dm^{-3} of triple superphosphate and 200 mg dm^{-3} of potassium chloride in each pot.

Each pot received one Albion cultivar strawberry seedling (*Fragaria* L.). During the growing period, plants were fertilized with mineral N at the level of 111 mg dm^{-3} of urea, which was performed at 30, 60, 90, 120, and 150 days after transplanting. Micronutrient fertilization consisted of applying 1 mg dm^{-3} of B as boric acid every 20 days after the beginning of flowering. Soil moisture was monitored daily and maintained close to the field capacity.

After 194 days, the fruits were harvested weekly and weighed. Total production was calculated using the sum of all harvests throughout the experimental period. At the end of the harvest, the fruits and shoots of the plants were oven dried with forced ventilation at 65°C for 72 hours, after which they were weighed, ground, and analysed for S and Se concentrations.

Sulfur concentration was obtained by the turbidimetric method according to Embrapa (2009). Samples were digested according to the 3051A method, described by the United States Environmental Protection Agency (USEPA, 1998). The quantification of Se in the extracts was performed by electrothermal atomization in graphite furnace atomic absorption spectrometry. The White Clover certified material (BCR402, Institute for Reference Materials and Measurements, Geel, Belgium) was used for quality control of the results.

Accumulation of nutrients was evaluated by relating the concentrations of S and Se found in fruits and shoots with their respective dry masses. The Se/S ratio in fruits and shoots was also calculated.

Data were submitted to analysis of variance and polynomial regression using Sisvar 5.3 statistical software (Ferreira, 2014).

Result

The concentration, accumulation, and Se/S ratio in shoots and fruits, as well as the production of strawberry fruits, were influenced by the interaction between the Se and S levels ($p < 0.05$). In general, there was a significant reduction of these variables in the treatments with application of S as a function of the levels of Se (Figures 2, 3, 4, and 5).

The maximum concentration of Se was 51.90 mg kg^{-1} and 28.62 mg kg^{-1} in shoots and fruits, respectively, for treatments without S application and 12.06 and 8.88 mg kg^{-1}, respectively, in the treatments with S application (Figure 2A and B). This result indicates that S has an inhibitory effect on the uptake of Se by the crop, decreasing the levels of Se in the shoots and in the strawberry fruits by approximately 76 and 69%, respectively.

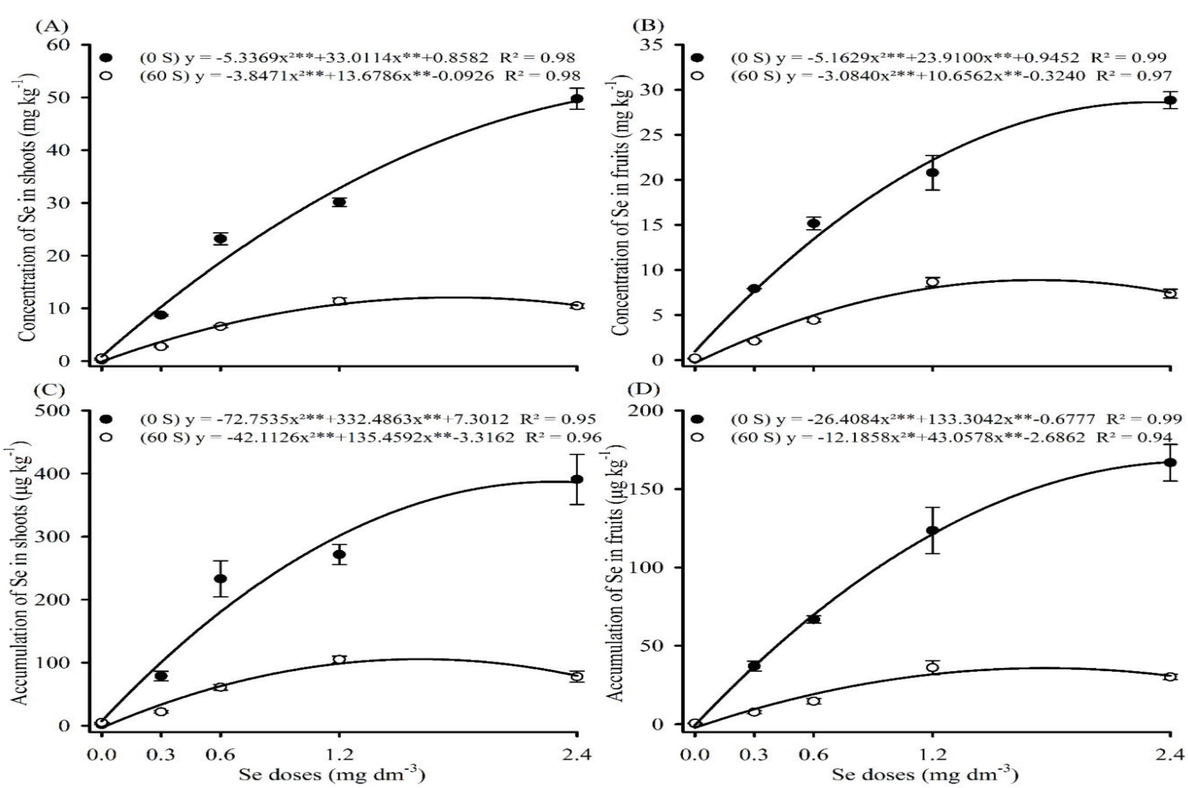

Figure 2. Concentration (A e B) and accumulation (C e D) of Se in shoots and fruits of strawberry as a function of Se and S levels applied. *, ** Siginifcant at $p < 0.05$ and $p < 0.01$.

In the absence of sulfated fertilization, the maximum accumulation of Se in shoots and fruits was 387 and 168 μg kg^{-1} of Se at the levels of 2.2 and 2.5 mg dm^{-3} of Se, respectively; whereas in the presence of sulfated fertilization, 105.6 and 35 μg kg^{-1} of Se at the levels of 1.6 and 1.7 mg dm^{-3} of Se were observed (Figure 2C and D).

Sulfur concentrations in shoots and fruits of strawberry increased linearly as a function of the levels of Se for the treatments without S application, representing an increase of 61% and 57% with the application of the highest level of Se in relation to the zero level for shoots and fruits, respectively (Figure 3A and B). With the application of 60 mg dm^{-3} of S, the S concentrations were reduced in the shoots and fruits as a function of the levels of Se (Figure 3A and B).

The accumulation of S in the shoots presented a quadratic response for the levels of Se in the treatment without S. The greatest accumulation was 7.6 mg kg^{-1} at the level of 2.02 of Se. However, no significant adjustment was obtained for Se with the sulfated fertilization (Figure 3C). For both levels of S, the accumulation of S in the fruits presented a linear increase for the levels of Se with maximum accumulation of 4.9 and 2.74 mg kg^{-1} for the highest Se concentration (Figure 3D).

The Se/S ratio in shoots and fruits presented a quadratic adjustment for both levels of S. It was observed that the highest values of this ratio are found in the absence of the sulfated fertilization with values of 52 and 38 for the levels of 2.05 and 1.88 mg dm^{-3} of Se, respectively (Figure 4).

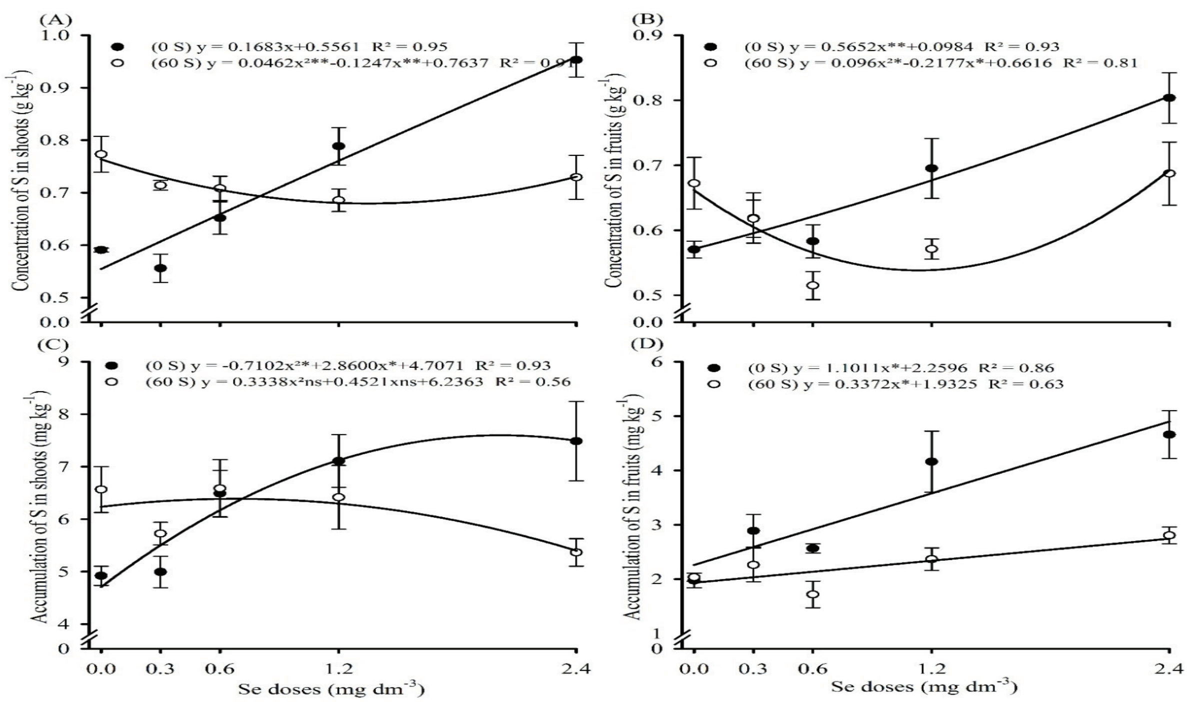

Figure 3. Concentration (A e B) and accumulation (C e D) of S in shoots and fruits of strawberry as a function of Se and S levels applied. *, ** Siginifcant at $p < 0.05$ and $p < 0.01$.

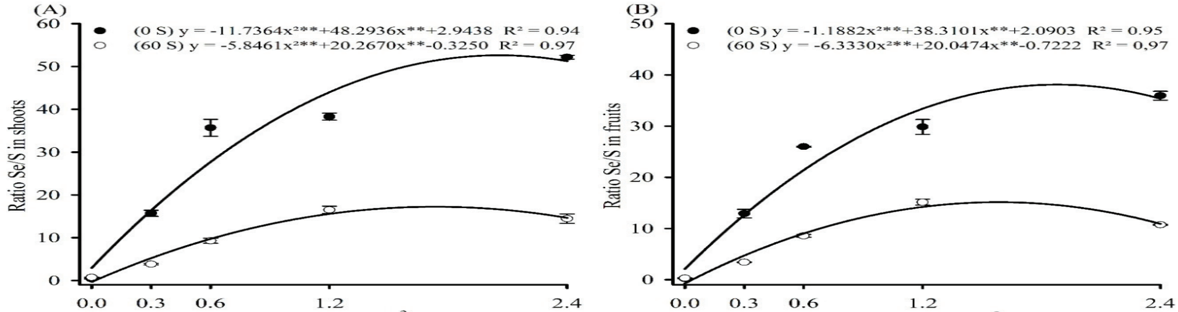

Figure 4. Se/S ratio in shoots (A) and fruits (B) of strawberry as a function of Se and S levels applied.*, ** Siginifcant at $p < 0.05$ and $p < 0.01$.

The yield of strawberry fruits increased by 40% with the application of Se in the absence of sulfated fertilization. The production of 63.4 g pot^{-1} at the level of 2.1 mg dm^{-3} of Se in the absence of sulfated fertilization compared to the production of 38.2 g pot^{-1} at the level 1.4 mg dm^{-3} of Se with application of S (Figure 5) demonstrates that in low dosages, Se does not only add nutritional value to the fruits but also has the potential to increase crop yield.

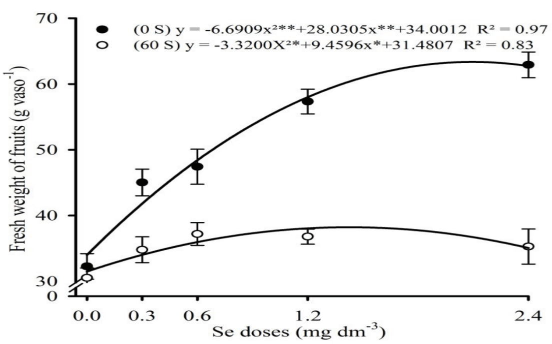

Figure 5. Yield of fresh weight strawberry fruits as a function of Se and S levels applied. *, ** Siginifcant at $p < 0.05$ and $p < 0.01$.

Discussion

Plants have different capacities to accumulate Se in tissues, and in general, low levels of Se can have positive effects on nutrition and productive yield of agricultural crops. In the present study, we observed beneficial effects of Se fertilization on strawberry crops, which were reflected by the significant accumulation of Se in shoots and fruits, and increased fruit production (Figures 2 and 5). A positive response to Se application in strawberry was verified by Palencia et al. (2016), who observed an increase in growth and fruit quality when selenite and selenate was applied in hydroponic cultivation.

These results indicate a drastic reduction in the bioconcentration of Se in strawberry with the application of 60 mg dm^{-3} of S, which was approximately 3-5 times lower than in the treatment without sulfated fertilization (Figure 2). This result can be explained by the finding that Se, primarily in the form of selenate, is actively absorbed by sulfate transporters such that selenate uptake by plants can be reduced by the presence of sulfate in the rhizosphere (White et al., 2004; Cabannes, Buchner, Broadley, & Hawkesford, 2011; Shinmachi et al., 2010).

The competitive inhibition of S has been verified in several studies, such as significant reductions in the root uptake of selenate in the presence of S for *Brassica napus* L. and *Allium sativum* L. under field and hydroponic conditions (Liu et al., 2017; Cheng et al., 2016). It is also worth mentioning that the reduction in the absorption of Se by the application of S, besides the physiological effect, can be related to chemical conversions of Se in the soil to forms that are less available to plants. Liu et al. (2014) observed that the application of S can lower the pH and increase soil organic matter contents, promoting the conversion of Se soluble in fractions with low availability, such as Fe and Mn bound to organic matter. In general, strawberry's ability to accumulate Se in its fruits, despite competitive inhibition with the S, is an important feature for the species to be considered within biofortification programmes.

Conversely, the results show a synergistic effect between Se and S. The absorption of S increased by approximately 60% with the application of Se; however, this effect is limited to low S contents in the soil. The application of selenate imitates the starvation of S to activate the expression of sulfate transporters, thereby stimulating the absorption and accumulation of S in strawberry (Schiavon, Pilon, Malagoli, & Pilon-Smits, 2015). Boldrin et al. (2016), when evaluating levels and sources of Se in seven wheat strains, observed that treatment with 10 μM selenate promoted accumulation of S in the shoots of all examined wheat strains. Similarly, Hawrylak-Nowak (2013) and Guerrero, Llugany, Palacios, and Valiente (2014) also observed significant accumulations of S in lettuce and wheat, respectively, when treated with levels of selenate in hydroponic cultivation.

The considerable accumulation of Se in strawberry fruits represents a potential transfer of this element to humans, helping to mitigate malnutrition. The recommendation for ingestion of Se in adults is 30-70 μg day^{-1} with a maximum level tolerance of 400 μg day^{-1} according to the United States Department of Agriculture (USDA, 2003).

Despite the increasing production and consumption of strawberries in Brazil, there is a great shortage of accurate information about their consumption *per capita*. However, considering the average *per capita* consumption of fresh strawberries in the United States, which in 2015 was 3.50 kg $year^{-1}$ (USDA, 2016), in conjunction with the results of this study, the content of Se in strawberry fruits may contribute to the daily intake of Se in approximately 27.3 and 7.1 μg per day in treatment without and with sulfated fertilization, respectively, with the application of 2.3 mg dm^{-3} of Se.

Therefore, in conditions where the level of Se in the soil is restrictive, as in the Brazilian Latosols, the balanced management of the sulfated fertilization in the biofortification process of Se crops is of great importance, considering the great influence of the S on the accumulation of Se Plants.

Conclusion

The application of S in conjunction with Se significantly reduces the production and the concentrations of Se in strawberry shoots and fruits.

In conditions of low supply of S, the fertilization with S provided greater absorption and accumulation of S in both shoots and fruits.

Strawberry has good capacity for nutritional enrichment with Se, which was demonstrated by the accumulation of Se in the fruits and increase of the fruit production.

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