Effects of Foliar Spraying with Different Concentrations of Selenium Fertilizer on the Development, Nutrient Absorption, and Quality of Citrus Fruits

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Abstract. Selenium (Se) fertilizer has a good effect on many field crops, but there are few reports on the application of Se fertilizer on citrus. We investigated the effects of 0 mg/L (CK, water treatment), 50 mg/L, 100 mg/L, 150 mg/L, and 200 mg/L sodium selenite aqueous solutions on the growth, nutrition, and fruit quality of 15-year-old citrus unshiu (*Citrus reticulata* Blanco cv. Succosa). The results showed that a low concentration of Se fertilizer promoted the growth and development of the citrus plant, and a high concentration of Se fertilizer was found to slightly inhibit the growth and development of the plant. Among the different treatment groups, 150 mg/L selenium fertilizer showed have the best effect on these evaluated parameters. The results thus suggest that 150 mg/L of Se fertilizer promotes the formation of chlorophyll in the leaves of the test plant and increases the longitudinal and transverse diameter of the fruits and weight of single fruit, significantly enhancing the activity of antioxidant enzymes in the leaves, promoting the absorption of nutrients in the leaves, increasing the contents of total sugar and vitamin, and decreasing the acidity in the fruits and the pericarp thickness. It also promoted the accumulation of the total selenium content in the leaves and fruits and consequently improved the quality of the fruits. These results showed that appropriate concentration of Se treatment can improve the activity of antioxidant enzymes to enhance plant stress resistance, regulate the content of sugar and acid in fruits, and improve the quality of fruits.

Selenium (Se) is an essential element for several human and animal life activities that are shown to have diverse biological functions (Jin et al., 2014). Studies have reported that more than 40 human diseases are linked to Se deficiency or low Se levels in the body (Shi et al., 2010). Se intake from whole foods alone is insufficient to meet the body's normal requirement for Se. Therefore, strengthening the development of Se-rich agricultural products, especially Se-rich fruit and vegetable products, is of great significance. In this way, the Se intake can be increased through daily diet to meet the needs of the population for Se and thus improve the health of the population.

Citrus fruits are one of the most important fruits, ranking first among the four major fruits in the world; their production is mainly distributed across 135 countries and the tropical and subtropical regions (Poles et al., 2020). As the origin and the main producing area of citrus, China ranks first in the

cultivation area and output in the world, with a long history of citrus cultivation (Liu et al., 2019). Meanwhile, the citrus industry plays an important role in the adjustment of the rural industry and alleviation of poverty of farmers (Tripoli et al., 2007). In addition, citrus fruits are rich in vitamin C, folate, dietary fiber, and other bioactive ingredients that are believed to be effective in preventing cancer and other diseases (Ejaz et al., 2006).

Considering the essentiality and importance of Se in human health, citrus is one of the commonly available and consumed fruits. It is therefore of great significance to study the effect of Se on the growth of these fruits and the development of Se-enriched citrus pair. Leaf Se application has been proven to be a convenient, safe, and effective method to increase the crop Se content in a large number of studies (Jing et al., 2017). In addition, related studies have suggested that spraying Se on plant leaves and fruits can effectively improve the Se content in fruits and thereby enhance the dietary absorption capacity of Se. Leaf Se application can increase the content of soluble solids and Se in pear fruits and improve pear quality to a certain extent (Deng et al., 2019). Application of leaf Se fertilizer increases the Se content in blueberries (Liu et al., 2019). However, only a few studies have investigated the effects of exogenous Se on citrus fruits, albeit

the specific relevant details remain unknown. Therefore, the effects of different concentrations of exogenous Se on fruit development and nutrient absorption of 15-year-old citrus unshiu (*Citrus reticulata* Blanco cv. Succosa) were investigated in this study, with the aim of laying a foundation for the development of research activities on Se-rich citrus fruits in the future.

Materials and Methods

These experiments were conducted in the citrus garden of Zhejiang Citrus Research Institute, and 15-year-old citrus unshiu (*Citrus reticulata* Blanco cv. Succosa) served as the experimental material. The soil fertility of the test site was as follows: pH 5.14; organic matter, 40.6 g/kg; hydrolytic nitrogen, 302.4 mg/kg; available phosphorus, 495 mg/kg; available potassium, 270 mg/kg; exchangeable calcium, 2.07 cmol/kg; exchangeable magnesium, 0.53 cmol/kg.

A single-factor randomized block design was used in the experiment, and Na₂SeO₃ was diluted to the following five gradients: 1) 0 mg/L (CK), 2) 50 mg/L, 3) 100 mg/L, 4) 150 mg/L, and 5) 200 mg/L. Each treatment was repeated five times. This experiment was performed from 2019 to 2020, and the spraying treatment was conducted once every 10 d, totaling three times, after the flower withering period of oranges. Different treatments were implemented after 4:00 PM.

After fruit ripening, the leaves and fruits in each treatment group were collected on 20 Nov. During this time, the third leaves from the top of the fruit branch from the middle of the peripheral branches of the tree crown were picked. Normal development of fruits without any diseases or infestation in the middle of the tree were sampled. The samples were immediately brought back to the laboratory for pretreatment after collection.

The concentration of total Se in the leaves and sarcocarp was determined after HNO₃-HClO₄ (4:1) digestion at 180 °C temperature. Then, the digestive solution was restored to 2 mL with 6 mol/L HCl, followed by cooling and filtering. Hydride Generation Atomic Fluorescence Spectrometry-2100 (AFS-2100; Beijing Tiantai Instrument Co. Ltd., Beijing, China) was used to determine the concentration of Se in the filtrate (Liu et al., 2017). The content of soluble solids (TSS) in the fruits was determined using a hand saccharimeter. The soluble sugar content was determined using the anthrone sulphate method (Eskandari and Gineau, 2020). The content of vitamin C (VC) was determined using the 2, 6-dichloroindigophenol titration method (Svehla et al., 1963). The titratable acid content was determined using the sodium hydroxide neutralization titration method (Basavaiah and Prameela, 2004). The activity of peroxidase (POD) was determined by the principle that H₂O₂ could oxidize guaiacol to brown product under the catalysis of peroxidase (Lurie et al., 1997). The activity of superoxide dismutase (SOD) was determined by the nitrogen blue tetrazole method

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(Beauchamp and Fridovich, 1971). The activity of malondialdehyde (MDA) was determined by thiobarbituric acid method (Draper and Hadley, 1990). Leaf chlorophyll content (SPAD) was measured using the chlorophyll content meter CCM-200 plus.

Data were statistically analyzed with one-factor of variance on the basis of SAS (v8.1; SAS Institute, Inc., Cary, NC), and the Duncan's multiple range test at $P \leq 0.05$ was used to compare the significant differences between treatments.

Results

The application of exogenous Se showed a certain effect on the growth of citrus (Table 1). Our study revealed that only 150 mg/L of exogenous Se treatment increased the longitudinal diameter of the citrus fruits by 9.15%, significantly, and 150 and 200 mg/L of exogenous Se treatment dramatically increased the transverse diameter of the citrus fruits by 9.99% and 10.13%, respectively. However, no significant difference was noted between the results of other treatments and CK. Moreover, exogenous Se application could significantly increase the fruit weight, with 150 mg/L concentration treatment showing the most obvious effect. In addition, the synthesis of chlorophyll was markedly promoted by treatment with 100, 150, and 200 mg/L of exogenous Se treatment, showing increased chlorophyll content by 39.3%, 52.8%, and 52.3%, respectively,

Table 1. Effects of selenium fertilizer with different concentration on physiology of citrus.

Treatment	Longitudinal (mm)	Transverse (mm)	Wt (g)	SPAD
CK	39.76 $\pm$ 1.61 b	50.84 $\pm$ 2.88 b	61.80 $\pm$ 1.74 c	96.26 $\pm$ 11.20 c
50 mg/L	40.83 $\pm$ 2.28 ab	51.96 $\pm$ 2.74 ab	65.96 $\pm$ 0.50 b	103.85 $\pm$ 9.02 c
100 mg/L	42.07 $\pm$ 2.45 ab	52.84 $\pm$ 3.74 ab	68.04 $\pm$ 1.69 b	134.14 $\pm$ 11.88 b
150 mg/L	43.40 $\pm$ 3.37 a	55.92 $\pm$ 4.92 a	70.70 $\pm$ 1.24 a	147.07 $\pm$ 13.96 a
200 mg/L	42.99 $\pm$ 1.65 ab	55.99 $\pm$ 2.55 a	67.64 $\pm$ 1.74 b	146.63 $\pm$ 19.78 a

CK means 0 mg/L. Mean $\pm$ SE (n = 5). Means within each column followed by different letters are significantly different by Duncan's test at $P \leq 0.05$.

SPAD = leaf chlorophyll content.

when compared with the corresponding CK treatment. However, 50 mg/L treatment could promote chlorophyll synthesis to only a small extent, albeit no significant difference was noted in the outcomes.

Through the determination of related antioxidant enzyme content in the leaves (Fig. 1), we found that the application of exogenous Se fertilizer with different concentrations effectively increased the activities of SOD and POD and the content of soluble sugar in the leaves. In addition, with an increase in the exogenous Se fertilizer concentration, the activities of SOD and POD first showed an increasing trend and then showed a decreasing trend. We noted that among all treatments, 150 mg/L of exogenous Se fertilizer had the most significant effect on the activity of SOD and POD in the plant leaves. In addition, the higher the concentration of exogenous Se, the greater was the content of soluble sugar in the leaves. However, the MDA content in the leaves was

remarkably reduced when exogenous Se was applied. Moreover, the MDA content in the leaves decreased with an increase in the exogenous Se fertilizer concentration.

The application of exogenous Se fertilizer showed an important effect on the content of mineral elements in the leaves (Table 2). When compared with the effects of CK treatment, the contents of N and P showed an increasing trend at first and then showed a decreasing trend, reaching the peak when the concentration of exogenous Se fertilizer was 100 to 150 mg/L, whereas the content of K in the leaves increased with an increase in the Se concentration. As for the medium element Ca and Mg, when compared with the CK treatment results, 50 and 100 mg/L Se fertilizer concentrations could significantly increase the Ca content of the leaves by 3.34% and 16.38%, respectively. The content of Mg in the leaves first increased and then decreased with an increase in the exogenous Se concentration.

Fig. 1. Effects of selenium fertilizer with different concentrations on superoxide dismutase (SOD) (A), peroxidase (POD) (B), malondialdehyde (MDA) (C), and soluble sugar (D). CK means 0 mg/L. Data (means $\pm$ SE, n = 5) followed by the different letters among treatments indicate significant differences at $P \leq 0.05$.

Table 2. Effects of selenium fertilizer with different concentrations on the contents of large and medium elements in citrus leaves.

Treatment	N (g/kg)	P (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)
CK	9.77 ± 0.05 e	0.55 ± 0.04 c	5.31 ± 0.03 e	8.09 ± 0.03 c	0.46 ± 0.02 d
50 mg/L	11.55 ± 0.03 c	0.67 ± 0.03 b	5.52 ± 0.02 d	8.36 ± 0.05 b	0.67 ± 0.02 b
100 mg/L	12.26 ± 0.02 b	0.77 ± 0.01 a	5.94 ± 0.05 c	9.38 ± 0.04 a	0.78 ± 0.02 a
150 mg/L	12.44 ± 0.10 a	0.73 ± 0.01 a	6.12 ± 0.10 b	8.08 ± 0.04 c	0.64 ± 0.02 b
200 mg/L	11.34 ± 0.06 d	0.63 ± 0.04 b	6.50 ± 0.04 a	7.77 ± 0.05 d	0.51 ± 0.03 c

CK means 0 mg/L. Mean ± SE (n = 5). Means within each column followed by different letters are significantly different by Duncan's test at $P \leq 0.05$.

We determined the element contents of sarcocarp (Table 3) and found that the contents of N, P, and K in the sarcocarp increased significantly under Se fertilizer treatment. We found that, with an increase in the exogenous Se fertilizer concentration, the effect of exogenous Se fertilizer on the contents of N, P, and K in sarcocarp was stronger. The application of Se fertilizer could decrease the contents of Ca and Mg in sarcocarp compared with the CK treatment, whereas a gradual decrease in Ca and Mg contents was observed with an increase in the Se fertilizer concentration.

The application of exogenous Se fertilizer also showed a certain effect on the content of Se in leaves and sarcocarp (Fig. 2). The results suggested that the application of leaf Se fertilizer at any concentration could significantly increase the total Se content in the leaves and sarcocarp. The higher the leaf Se fertilizer concentration, the greater was the total leaf Se content. However, with an increase in the Se fertilizer concentration, the total fruit Se content first showed an increasing trend and then became stable.

Different concentrations of exogenous Se fertilizer were found to have obvious effects on the quality of citrus fruits (Table 4). Compared with the results of CK treatment, treatment with exogenous Se fertilizer significantly reduced the fruit peel thickness, with the effect of 200 mg/L treatment being the most obvious, as it showed 14.57% decrease. On the other hand, the treatments with 50, 100, and 150 mg/L concentrations effectively reduced the pericarp thickness by 9.06%, 10.63%, and 9.84%, respectively, albeit no significant difference was observed among the results of the three treatments. Similarly, different concentrations of exogenous Se fertilizer significantly reduced the content of total acid in sarcocarp, albeit no significant difference was observed among the results of the different treatments. On the other hand, the application of exogenous Se fertilizer at the concentrations of 50, 100, 150, and 200 mg/L distinctly increase the contents of TSS, total sugar, reducing sugar, and VC in the

sarcocarp. Compared with the CK results, the percentage content of TSS increased by 10.68%, 13.88%, 18.16%, and 23.3%, respectively. The corresponding increase in the total sugar content was 5.83%, 8.33%, 14.17%, and 12.04%; the reducing sugar content was increased by 16.61%, 20.22%, 26.35%, and 18.05%, respectively; and the content of VC was increased by 10.17%, 9.12%, 15.29%, and 13.49%, respectively.

Discussion

The assimilates of photosynthesis are the material basis for plant cell activities, and photosynthates accumulation directly affects a series of activities including plant growth and development (Matsoukas et al., 2012). Studies have shown that Se can be used to increase plant productivity by improving photosynthesis. For instance, the application of Se in rice was shown to have a positive effect on photosynthesis, resulting in increased yield (Zhang et al., 2014). Moreover, previous experiments on blueberry plants with different concentrations of Se have shown that the use of appropriate concentrations of Se could promote the growth of blueberry fruit in both transverse and longitudinal diameters, whereas a high concentration of Se was found to inhibit the increase in the weight of blueberry fruits (Wang et al., 2018). In this study, the chlorophyll content increased gradually with an increase in the exogenous Se concentration, reaching a stable level with 150 to 200 mg/L concentration. In this concentration range, the longitudinal and transverse diameters of fruits as well as the weight of a single fruit were also increased, indicating that the application of appropriate exogenous Se fertilizer could have positive effects on the improvement of citrus fruit yield.

Peroxisation in living organisms is known to be caused mainly by reactive oxygen species (ROS) and their derivatives of lipid peroxidation reaction (Wang et al., 2016). Antioxidant enzymes work against these

peroxidation reactions, and by using redox reactions, they convert peroxides into less toxic or harmless substances. Once Se enters an organism, it catalyzes the decomposition of ROS and simultaneously produces ROS. Studies have shown that the influence of Se in an organism is related to its content. Findings have revealed that Se at lower concentrations can decrease lipid peroxidation and increase the activity of antioxidative enzymes (Ardebili et al., 2014; Xu et al., 2014). In this study, the contents of SOD and POD in the leaves first increased and then decreased with increasing concentration of Se fertilizer, which may be because Se can directly remove ROS through the nonenzymatic mechanism under low Se concentration (Hartikainen et al., 2000). Moreover, this treatment stimulated the synthesis of sugar and increased the content of soluble sugar in the leaves. However, a high concentration of Se showed a certain toxic effect on plants, possible by causing peroxidation reaction in the treated plants (Moller et al., 2007). In the process of lipid peroxidation, more ROS is produced, although the antioxidant enzymes in the leaves cannot decompose excess ROS, and the excessive accumulation of ROS can damage lipids, proteins, nucleic acids, and other biological macromolecules to further increase the MDA content (Moller et al., 2007). An experiment by Qing et al. (2015) showed that the addition of a small amount of Se to the growth medium could reduce the membrane lipid peroxidation of leaves and increase the activity of antioxidant enzymes; this finding is consistent with those of the present study. These results together indicate that an appropriate Se concentration can effectively improve the antioxidant capacity of citrus leaves.

Mineral elements are essential nutrients in plant life activities, and they participate in various metabolic processes in plants. Exploration of their accumulation is deemed essential to understand the growth mechanism of plants (Gill et al., 2013). In this study, the application of exogenous Se showed different effects on the leaf and fruit Se content. The present study also showed that the application of a low concentration of Se fertilizer could promote the absorption of N, P, K, Ca, and Mg contents in the leaves, whereas their absorption was inhibited under a high concentration of Se fertilizer application. The same conclusion was confirmed for tea (Qin et al., 2019) and pear (Liu et al., 2015) plants, possibly because an increase in the Se concentration causes oxidative and toxic stress simultaneously in the plants, thereby damaging the integrity of plant cell membranes and reducing its selective transmittance (Feng et al., 2009). However, in this study, the treatment with a high-concentration Se fertilizer (150–200 mg/L) did not inhibit the uptake of K by leaves, although the specific reasons for this finding remain to be explored. Different from the results for the treated leaves, the application of exogenous Se could significantly increase the contents of N, P, and K in sarcocarp, without showing any inhibitory effect. However, it reduced the contents of

Table 3. Effects of selenium fertilizer with different concentrations on the contents of large and medium elements in sarcocarp.

Treatment	N (g/kg)	P (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)
CK	1.21 ± 0.03 e	0.04 ± 0.01 e	0.48 ± 0.02 d	0.27 ± 0.01 a	0.13 ± 0.03 a
50 mg/L	1.30 ± 0.02 d	0.06 ± 0.01 d	0.75 ± 0.01 c	0.22 ± 0.02 b	0.10 ± 0.02 a
100 mg/L	1.41 ± 0.03 c	0.08 ± 0.01 c	0.73 ± 0.01 c	0.07 ± 0.01 c	0.04 ± 0.01 b
150 mg/L	1.47 ± 0.02 b	0.14 ± 0.01 b	1.52 ± 0.02 b	0.06 ± 0.01 cd	0.05 ± 0.01 b
200 mg/L	1.61 ± 0.03 a	0.17 ± 0.01 a	1.63 ± 0.01 a	0.04 ± 0.02 d	0.04 ± 0.01 b

CK means 0 mg/L. Mean ± SE (n = 5). Means within each column followed by different letters are significantly different by Duncan's test at $P \leq 0.05$.

Fig. 2. Effects of different concentrations of selenium fertilizer on total selenium content in leaves (A) and sarcocarp (B). CK means 0 mg/L. Data (means $\pm$ SE, $n = 5$) followed by the different letters among treatments indicate significant differences at $P \leq 0.05$.

Table 4. Effects of different concentrations of selenium fertilizer on citrus fruit quality.

Treatment	Peel thickness (mm)	Total acid (g/100 mL)	TSS (%)	Total sugar (g/100 g)	Reducing sugar (g/100 g)	VC (mg/100 g)
CK	2.54 $\pm$ 0.08 a	0.52 $\pm$ 0.04 a	10.3 $\pm$ 0.26 d	10.80 $\pm$ 0.17 c	2.77 $\pm$ 0.21 c	18.97 $\pm$ 0.32 b
50 mg/L	2.31 $\pm$ 0.08 b	0.42 $\pm$ 0.02 b	11.40 $\pm$ 0.20 c	11.43 $\pm$ 0.15 b	3.23 $\pm$ 0.15 b	20.90 $\pm$ 0.70 a
100 mg/L	2.27 $\pm$ 0.02 b	0.41 $\pm$ 0.01 b	11.73 $\pm$ 0.21 c	11.70 $\pm$ 0.10 b	3.33 $\pm$ 0.25 b	20.70 $\pm$ 0.36 a
150 mg/L	2.29 $\pm$ 0.05 b	0.45 $\pm$ 0.04 b	12.17 $\pm$ 0.21 b	12.33 $\pm$ 0.15 a	3.50 $\pm$ 0.10 a	21.87 $\pm$ 0.42 a
200 mg/L	2.17 $\pm$ 0.03 c	0.43 $\pm$ 0.04 b	12.70 $\pm$ 0.10 a	12.10 $\pm$ 0.26 a	3.27 $\pm$ 0.15 b	21.53 $\pm$ 1.75 a

CK means 0 mg/L. Mean $\pm$ SE ($n = 5$). Means within each column followed by different letters are significantly different by Duncan's test at $P \leq 0.05$.

TSS = total soluble solids; VC = vitamin C.

Ca and Mg in fruits; these results are consistent with those of Du et al. (2020), which indicated that a high-concentration Se fertilizer inhibits the absorption of Ca and Mg nutrients in watermelon, and this may be related to differences in the structure of leaves and fruits.

Se has various biological functions, and it is an essential trace element for human life activities (Lyons et al., 2005). The intake of Se-rich foods can make up for the deficiency of Se in human nutrition. Jing et al. (2017) reported that spraying water-soluble sodium selenite (Na_2SeO_3) on the leaf surface could effectively increase the total Se content in the resultant leaves and fruits. In this study, the application of exogenous Se on the leaves not only increased the Se content in the leaves but also increased the total Se content in the fruits, which reached the national standard of 10 to 50 $\mu\text{g/kg}$ Se-rich fruits. Similar results have also been reported in peaches, wherein the application of Se fertilizer to the foliar surface increased the Se content in the leaves and sarcocarp (Beatrice et al., 2012). These results suggest that the application of exogenous Se significantly improves the Se content in citrus fruits. As a nonessential element in plants, Se showed a positive effect on the quality of plant products. Some studies have reported the effects of Se on different horticultural crops. For instance, Zhu et al. (2017) suggested that leaf Se application could improve the concentration of soluble sugar, vitamin C, soluble protein, and soluble solid solution but reduce the concentration of organic acids, thereby improving the quality

of table grapes. According to a study by Zhao et al. (2013), foliar spraying with an appropriate amount of Se could improve the hardness of pear and jujube; increase the contents of vitamins, soluble protein, and soluble sugar in the fruit; and reduce the content of organic acids. However, excessive spraying (>300 mg/L) significantly reduced the fruit quality. In this study, the application of exogenous Se was found to decrease the peel thickness and total acid content of citrus fruits, whereas the contents of soluble solids, total sugars, reducing sugars, and VC in the fruits were prominently increased, which had a positive overall effect on the improvement of citrus fruit quality. These differences in the effects may be attributed to the effect of exogenous Se on the fruit quality depending on the species type (Deng et al., 2019).

Spraying of Se on the leaves obviously improves certain agronomic traits, antioxidant enzyme activities, and quality of citrus plant, although the effect depends mainly on the concentration of Se used in the treatment. The treatment with the Se fertilizer concentration of 150 mg/L showed an obvious improvement in the yield and quality of the citrus fruits and leaves, and the finding can serve as a reference to guide future research and development of Se-enriched citrus plants. Moreover, the activity of antioxidant enzymes in the leaves was enhanced and the adaptation of citrus plant to adversity was improved with this treatment. However, a high concentration (200 mg/L) of Se fertilizer showed a toxic effect on the growth and development of citrus fruits and leaves,

suggesting that a high Se concentration is not conducive to the growth and development of citrus fruits and leaves and to the improvement of their quality. In summary, the application of Se fertilizer with appropriate concentration on leaf surface had important and positive effects on the growth promotion and quality improvement of citrus.

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