# EFFECTS OF SELENIUM TREATMENTS ON PHYTOCHEMICAL CHARACTERISTICS OF SALT-STRESSED STRAWBERRY (*FRAGARIA XANANASSA* DUCH CV. RUBYGEM) PLANT

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**Abstract.** In this study, changes in leaf dry matter (LDM), leaf membrane permeability (LMP), leaf relative water content (LRWC), leaf proline (LPC), total chlorophyll (TChl), carotenoid, and fruit total phenol contents (TPC) in strawberry (*Fragaria x ananassa* Duch cv. Rubygem) plants exposed to salt stress were investigated using 1, 5, 10 and 30 µM $SeO_2$ + 100 mM NaCl treatments. Treatment of 30 µM $SeO_2$ + 100 mM NaCl showed a toxic effect on the plants. Among the treatments, 5 µM $SeO_2$ + 100 mM NaCl treatment resulted in the highest LRWC (59.44%), the lowest LDM (20.92%), LMP (22 EC%) and LPC (2.18 µmol/g). Plants treated with 10 µM $SeO_2$ + 100 mM NaCl had the lowest TChl content (0.89 mg/kg). TChl contents were higher with 1 µM $SeO_2$ + 100 mM NaCl (1.23 mg/kg) and 5 µM $SeO_2$ + 100 mM NaCl (1.20 mg/kg) than other $SeO_2$ + NaCl treatments. Although plants treated with 5 µM SeO2 + 100 mM NaCl grew and produced fruit, they had the lowest LDM, LPC, LMP and TPC values, but the highest LRWC values. Treatment of 5 µM $SeO_2$ + 100 mM NaCl gave successful results in improving the physiological and biochemical responses of strawberry plants under salt stress.
**Keywords:** *salinity stress, selenium, strawberry, proline, phenolic compound*

## Introduction

Selenium (Se) is an important micronutrient that plays multiple roles in a wide range of physiological processes and improves crop quality and nutritional value (Tan et al., 2022; Tao et al., 2023). While selenium (Se) causes toxicity when taken in excessive amounts, in low doses it protects plants from various abiotic stresses such as cold, drought, desiccation and metal stress (Gupta and Gupta, 2017)**.** It is an indispensable element for humans and animals. Plants have a fundamental role in Se transfer in the human food chain. In relevant studies, it has been determined that Se plays an antioxidant role, has beneficial effects on plants at low concentrations, promotes plant growth, reduces UV-induced oxidative damage, and improves the recovery of chlorophyll under mild stress conditions, delays senescence, provides resistance to drought and can increase pollen viability (Hartikainen et al., 2000; Combs, 2001; Seppanen et al., 2003; Yao et al., 2009; Hassanuzzaman et al., 2010).

In current environmental conditions, because of climate change and improper agricultural activities throughout their life cycle, plants are exposed to UV a and UV b lights, drought or excessive precipitation, salinity, high or low temperature, heavy metals, pesticides, and many abiotic stress factors that may adversely impact their growth and development. Salinity negatively affects agricultural efficiency by reducing crop quality and productivity worldwide, and various strategies are being emphasized to


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improve the defense abilities of plants against salinity (Ahmad and Anjum, 2023; Rasool et al., 2023).

According to the researchers, the increase in the production of reactive oxygen species (ROS) provided by plants under biotic stress conditions also increases under abiotic stress conditions. In the case of an increase in cellular ROS concentration, the balance between antioxidant defense systems and ROS production is disrupted and plants undergo oxidative stress in the form of chain reactions. The increase in ROS production under stress causes peroxidation of lipids, oxidation of proteins, nucleic acid damage, enzyme inhibition, and thus programmed cell death (PCD) occurs (Mittler, 2002; Hussain et al., 2019; Qin and Huang, 2020). Depending on the effect of PCD stress on the plant, death can be observed either in some tissues and organs or in the whole plant. In these circumstances, economic damage is inevitable for every group that spends labor and input in agriculture.

Strawberry plants are among the most sensitive plant species to salty conditions. The increase in the amount of salt in the environment where strawberry plants grow due to factors such as fertilization, irrigation water quality, etc. causes physiological problems, yield, and quality losses (Akaroğlu and Seferoğlu, 2018). In strawberry plants, salt stress reduces the number of leaves, leaf area, shoot dry weight, number of crowns and ultimately the yield (Sun et al., 2015). The genetic characteristics of strawberry cultivars, the type of salt and certain conditions in the root zone affect the damage threshold. Strawberry plants are sensitive to the accumulation of sodium ($Na^+$) and chlorine ($Cl^-$) ions, and these ions can cause serious damage. Zhang et al. (2021) reported that while leaf damage increased due to increment of $Na^+$ toxicity and oxidative stress in strawberry plants, efficiency of photosynthesis also decreased. The growing environment that is problematic in terms of salt brings about problems such as enzyme activation disorder, nutrient imbalance, membrane dysfunction, disruptions in the general metabolic process, osmotic incompatibility and imbalance in water intake, oxidative stress, and general development failure (Orcutt and Nilsen, 1996; Barroso and Alvarez, 1997; Yıldız et al., 2008; Yılmaz and Kına, 2008; Keutgen and Pawelzik, 2009; Khayyat et al., 2009).

The main source of selenium (Se) is the plants (Sucu and Yağcı, 2023). Se, as a micro element, can be used against environmental stress in plants. It has a protective effect against many abiotic stress factors in plants (Karimi et al., 2020; Rasool et al., 2023). It regulates the intake of elements and antioxidant activities in plants under stress. It can regulate or repair the damaged photosynthetic system to rebalance electron transfer (Feng et al., 2013). Se, which has a vital role in the activity of the glutathione peroxidase (GPX) enzyme (Lobanov et al., 2008), activates antioxidative detoxification systems (Hasanuzzaman and Fujita, 2011) and improves photosynthesis by reducing the negative effects of salt (Diao et al., 2014).

The concentration of Se in agricultural soils is highly variable and ranges from 0.01 mg $kg^{-1}$ to 10 mg $kg^{-1}$ in seleniferous areas. When Se is added to the growing substrate, it has been found to enhance plant growth. Studies aimed at biologically enhancing plants with Se are based on the application of Se fertilizers (Mimmo et al., 2017). Selenate or selenite can be used as selenium sources. Doses of Se routinely used in studies under hydroponic conditions to counteract environmental stress (for both $Se^{6+}$ and $Se^{4+}$) are usually less than 1 mg $L^{-1}$ (Feng et al., 2013).

Selenium can be applied using four different techniques: seed dressing, seed soaking, soil and foliar application. However, soil and foliar application are the easiest (Millan et. al., 2021). Yin et. al. (2019) reported that root application of Se significantly


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increased Se accumulation, photosynthetic rate, biomass accumulation and tolerance to cadmium stress in rice compared to foliar application. Under the same growth conditions, it is seen that most of the scientific studies carried out to understand which of the different doses of Se application can be more beneficial against abiotic stress caused by high salt concentration in the root zone of strawberry plants and why, have been carried out in the form of foliar application. However, there are few studies on the application of Se -as selenate- as a supplement to the nutrient solution against abiotic stress caused by high salinity in the root zone of strawberry and how the plant responds to this stress in soilless culture.

The aim of this study is to determine the effects of selenium treatments on phytochemical characteristics (membrane permeability, relative water, chlorophyll, carotenoid, proline and total phenol contents in strawberry (*Fragaria x ananassa* Duch cv. Rubygem) plants exposed to salt stress.

## Materials and methods

### *Plants materials and growing system*

The research was carried out in a PE covered tunnel greenhouse with 60 $m^2$ floor area, side, and roof ventilation, located in the application garden of Aydın Adnan Menderes University Sultanhisar Vocational School (Aydın, Türkiye). Frigo seedlings of strawberry (*Fragaria x ananassa* Duch cv. Rubygem) were used in the experiment. They were grown in a hydroponic pot system consisting of a nutrient tank and 10 pots, each pot having a volume of 6.5 liters. It was ensured that the water drained from the pots was collected in the tank again. These pots were filled with a mixture of peat + perlite (1:1 v/v). The water and fertilizer requirements of the plants were met with the nutrient solution. Salt and Se were added to the nutrient solution.

### *Plant nutrition solutions and treatments*

Plant nutrition solutions were prepared in two different formulations to be used during vegetative and generative development periods (Adak, 2010) (*Table 1*). $KNO_3$, $NH_4NO_3$, $Ca(NO_3)_2$, $KH_2PO_4$, $MgSO_4.7H_2O$, Fe-EDDHA (6% Fe), $MnSO_4$, $H_2O$ (32% Mn), $ZnSO_4.7H_2O$ (23% Zn), $CuSO_4.5H_2O$ (25% Cu), $H_3BO_3$ (17% B) and $Na_2MoO_4.2H_2O$ (39% Mo) fertilizers were used to prepare nutrient solutions (Luts et al., 1996). After strawberry plants formed the $6^{th}$ leaf, NaCl supplement was made by adding to the nutrient solution. Se was added to nutrient solution as sodium selenate ($Na_2SO_4$). As Se and salt source, 100 mM doses of NaCl and 1, 5, 10 and 30 µM doses of Se were used (Kacar and Inal, 2008).

### *Processes and applications*

During the vegetative period from November 18 to January 27, which is the first flowering date on strawberry plants, the relevant nutrient solution was applied once every 2 days for 15 min. On January 27, the old leaves of the plants were removed as in farmer practice. The plants could not be treated with salt and selenium until this date. As a result of the observations, Rubygam strawberry cultivar has not bloomed in vegetative development period. To avoid plant losses due to the days when the temperature drops to -6°C from time to time in November-December and the effects of salt and selenium treatments, treatments were left to the period when the plant came out of rest.


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***Table 1.*** *Formulation of plant nutrition solutions (PNS) prepared for vegetative and generative development period (Adak, 2010)*

| Vegetative development period | | Generative development period | |
|---|---|---|---|
| **Nutrients** | **Amount ($L^{-1}$)** | **Nutrients** | **Amount ($L^{-1}$)** |
| $NO_3^-$ | 11.5 mmol | $NO_3^-$ | 11 mmol |
| $H_2PO_4^-$ | 1.5 mmol | $H_2PO_4^-$ | 1.5 mmol |
| $SO_4^-$ | 1.5 mmol | $SO_4^-$ | 1.5 mmol |
| $NH_4$ + | 0.5 mmol | $K^+$ | 5.5 mmol |
| $K^+$ | 3.5 mmol | $Ca^{++}$ | 3.5 mmol |
| $Ca^{++}$ | 4.5 mmol | $Mg^{++}$ | 1.5 mmol |
| $Mg^{++}$ | 1.5 mmol | Fe | 20 µmol |
| Fe | 20 µmol | Mn | 20 µmol |
| Mn | 20 µmol | Zn | 10 µmol |
| Zn | 10 µmol | B | 12 µmol |
| B | 12 µmol | Cu | 0.75 µmol |
| Cu | 0.75 µmol | Mo | 0.5 µmol |
| Mo | 0.5 µmol | | |

Since the research also aimed to obtain flowering and fruit, the formation of new leaves in the plant was waited for salt and selenium treatments. Following removal of old leaves and the formation of new leaves, the nutrient solution recommended for generative period was started to be applied once every 2 days for 15 min. During this period, NaCl treatment to plants was also started. Selenium treatment was started 1 week after NaCl treatment (Turhan and Eriş, 2007). The application steps of the trial are presented in *Table 2* along with their dates.

***Table 2.*** *The application steps of the trial*

| Date | Applications |
|---|---|
| 22 August | Planting refrigerated seedlings in plastic cups |
| 23 September | Planting tube seedlings obtained from refrigerated seedlings in pots in the greenhouse |
| 18 November | Starting to apply the nutrient solution of the vegetative development period once every 2 days for 15 min |
| 27 January | Removing old leaves from plants |
| 06 February | Starting to apply the nutrient solution of the generative development period once every 2 days for 15 min |
| 22 February | Treatment with NaCl |
| 01 March | Treatment with selenium |
| 17 March | Collecting leaf samples for membrane permeability and leaf relative water content analysis |
| 19 April | Collecting leaf samples for leaf fresh and dry weight, % dry matter content, proline, and chlorophyll analyses |
| 19 April | Collecting fruit samples for total phenolic content analysis |

### *Leaf fresh weight (g), leaf dry weight (g) and leaf dry matter (%)*

The leaves collected from healthy plants in each pot were harvested and placed in plastic, self-locking bags at 8:30-9:00 in the morning**.** Fresh weights (LFW) of the leaf


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samples were recorded**.** After the samples were kept in the oven at 65°C for 48 h**,** their dry weight**s** (LDW) were determined. Leaf dry matter content (LDM) was calculated according to the formula below to *Equation 1:* (Kacar and Inal, 2008).

$$\text{Leaf dry matter content (LDM \%)} = (\text{LFW} / \text{LDW}) \times 100 \quad \text{(Eq.1)}$$

LFW: fresh weight, LDW: dry weight

### *Leaf relative water content (%)*

Leaf relative water content (LRWC) was determined according to Kumar et al. (2013). 0.5 g of fresh leaf sample was kept in 100 ml distilled water for 4 h. At the end of this period, the turgid weights (TW) of the leaf samples was determined and then the samples was kept in the oven at 65°C for 48 h. The dry weight (DW) of the samples was determined after 48 h. Relative water content was calculated according to *Equation 2*:

$$\text{Leaf relative water content (LRWC\%)} = (\text{LFW} - \text{LDW}) / (\text{TW} - \text{LDW}) \times 100 \quad \text{(Eq.2)}$$

LFW: fresh weight, LDW: dry weight, TW: turgid weight

### *Leaf membrane permeability (EC%)*

Membrane permeability (LMP) was determined according to Lutts et al. (1996). 20 discs, 1 cm in size, were taken from the leaves. After washing with pure water they were placed in dark glass bottles. 10 ml of pure water was added to them. After 24 h, the solution in the bottles was measured with an EC meter (EC1 value). After being kept in the autoclave at 120°C for 20 min, the EC values in the room were measured (EC2). The obtained values were calculated as membrane permeability (%EC) at defoliation using *Equation 3*:

$$\text{Leaf membrane permeability (\%EC)} = (\text{EC1} / \text{EC2}) \times 100 \quad \text{(Eq.3)}$$

### *Chlorophyl content (mg/kg)*

Chlorophyll and carotenoid concentrations were determined according to Lichtenthaler and Wellburn (1983). Fresh leaf samples of the plants (100–200 mg) were homogenized with 15 ml of 80% (vol/vol) acetone and filtered using white band filter paper. In the resulting extraction, absorbance values of total chlorophyll at 652 nm, chlorophyll a at 663 nm, chlorophyll b at 645 nm and carotenoids at 470 nm were measured in a UV spectrophotometer (UV-160 A UV-Visable Recording Spectrophotometer Shimadzu, Australia). Calculations were made using *Equations 4, 5, 6 and 7:*

$$\text{Total chlorophyll} = \text{A652} \times 27.8 / \text{mg sample weight} \quad \text{(Eq.4)}$$

$$\text{Chlorophyll a (Cl a)} = (11.75 \times \text{A663} - 2.35 \times \text{A645}) \times 20 / \text{mg sample weight} \quad \text{(Eq.5)}$$

$$\text{Chlorophyll b (Cl b)} = (18.61 \times \text{A645} - 3.96 \times \text{A663}) \times 20 / \text{mg sample weight} \quad \text{(Eq.6)}$$

$$\text{Carotenoid} = (1000 \times \text{A470} - 2.27 \times \text{Kla} - 81.4 \times \text{Klb}) / 227) \times 20 / \text{mg sample weight} \quad \text{(Eq.7)}$$

A: measured absorbance value


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### *Leaf proline content (µmol/g sample)*

Leaf proline content (LPC) was determined referring to Bates et al. (1973). 2 ml of acid ninhydrin was added on 0.5 g of dry plant material. Preparation of acid ninhydrin solution: It was prepared by shaking 1.25 g of ninhydrin with 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid until dissolved. The reagent, which remained stable for 24 h, was preserved in the cold (+4°C). 2 ml of glacial acetic acid was added, and it was incubated for 1 h at 100°C. It was then kept in an ice bath until cooling. The reaction mixture was then extracted with 4 ml of toluene. Toluene was aspirated from the aqueous phase and cooled at room temperature, and then the absorbance values were determined by UV spectrophotometer (CE 5502 UV spectrophotometer) at a wavelength of 520 nm. The standards containing 0.1, 0.2, 0.3, 0.4 µmol/proline were prepared. Proline amounts were calculated with the help of the drawn standard curve (*Equation 8*):

$$\mu g \text{ proline / ml toluene) / 115.5 } \mu mol) / (g \text{ sample) / 5) = } \mu mol \text{ proline/g fw} \quad \text{(Eq.8)}$$

### *Total phenolic content (mg GAE/100 g fw)*

Total phenolic content (TPC) of fruits was determined according to Yıldız et al. (2014). Fruit samples were extracted thrice in 80% aqueous methanol (pH 2.0) by shaking at room temperature for 90 min. Supernatants were centrifuged, filtered and the volume of each of these samples was made up to 50 ml with the solvent. The extracts were stored at −20°C for further analysis. The total amount of phenol was determined according to the Folin–Ciocalteu procedure. Gallic acid was used as a standard for comparison and the results are expressed in milligrams of gallic acid equivalent per 100 g sample (mg GAE/100 g fw).

### *Water quality analysis*

The values related to the quality analysis of the water used in the experiment were presented in *Table 3*. It was performed in the laboratory of Aydin Adnan Menderes University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition.

***Table 3.*** *Values of evaporation residue, dissolved substance, pH, EC, temporary hardness, total hardness, and SAR in the control group water sample*

| Evaporation residue (mg/L) | Dissolved substances (mg/L) | pH | EC (µS/cm) | Temporary hardness (German) | Total hardness (German) | SAR (me/L) | Type |
|---|---|---|---|---|---|---|---|
| 580 | 360 | 7.45 | 348 | 21.28 | 19.06 | 0.11 | $C_2S_1$ |

SAR: sodium adsorption range EC: electrical conductivity

### *Statistical analysis*

An experiment based on a completely randomized design with three replicates was used. The data of the characteristics (*Table 4*) were analyzed with one-way analysis of variance (ANOVA) using the statistical package programs SPSS 25. Statistical differences between the averages were computed at the 5% significance level by the LSD multiple comparison method.


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***Table 4.*** *Description and unit of characteristics*

| Symbol | Characteristics |
|---|---|
| LFW | Leaf fresh weight (g) |
| LDW | Leaf dry weight (g) |
| LDM | Leaf dry matter (%) |
| LRWC | Leaf relative water content (%) |
| LPC | Leaf membrane permeability (EC%) |
| LMP | Leaf proline content (µmol/g) |
| Chl-a | Chlorophyll a (mg/kg) |
| Chl-b | Chlorophyll b (mg/kg) |
| TChl | Total chlorophyll (mg/kg) |
| TPC | Total phenolic content (mg/100 g) |

## Results

Plants treated with 30 µM $SeO_2$ + 100 mM NaCl were severely damaged (*Fig. 1*), so analyzes could not be performed on these plants. It was observed that leaf burns increased in those plants one week after the treatment and plant deaths occurred in a short time. Therefore, analyzes could not be performed on these plants. Plants exposed to other treatments survived (*Fig. 2*). A very small amount of fruit was obtained from 100 mM NaCl, 1 µM $SeO_2$ + 100 mM NaCl and 10 µM $SeO_2$ + 100 mM NaCl treatments, but the fruit quality was quite low. After the control, 5 µM $SeO_2$ + 100 mM NaCl treatment was clearly more successful than other treatments in terms of both plant development and fruit quality and quantity (*Figs. 2* and *3*). Plants treated with 1 µM $SeO_2$ + 100 mM NaCl, 10 µM $SeO_2$ + 100 mM NaCl and 100 mM NaCl also produced a small amount of fruit, but their fruits were quite inadequate in quality.


***Figure 1.*** *Strawberry plants two weeks after $SeO_2$ + NaCl treatments*


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***Figure 2.*** *Strawberry fruits obtained from control (left) and 5 µM $SeO_2$ + 100 mM NaCl (right) treatment*


***Figure 3.*** *Strawberry plants before (above) and after (below) the experiment*

Data on treatments are presented in *Tables 5, 6, 7,* and *Figures 4* and *5*. Leaf fresh weight (LFW), leaf dry weight (LDW) and leaf dry matter (LDM) differed statistically


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by treatments ($p < 0.05$). The control plants had the highest LFW (13.57 g) and LDW (2.77 g). The differences among the LDW values of control, 10 μM $SeO_2$ + 100 mM NaCl and 100 mM NaCl treatments were statistically insignificant. 1 μM $SeO_2$ + 100 mM NaCl treatment resulted in the lowest LFW (8.15 g) and LDW (2.14 g). Plants treated with 5 μM $SeO_2$ + 100 mM NaCl had the highest LFW (11.18 g) and LDM (20.92%) after control plants. LDM was determined between 20.43% (control) and 31.76% (10 μM $SeO_2$ + 100 mM NaCl treatment) (*Table 5*).

***Table 5.*** *Changes in leaf fresh weight (g), leaf dry weight (g) and leaf dry matter (%) in strawberry (Fragaria x ananassa Duch cv. Rubygem) plants treated with selenium and salt*

| Treatment | LFW (g) | LDW (g) | LDM (%) |
|---|---|---|---|
| Control | 13.57 a | 2.77 a | 20.43 c |
| 1 μM $SeO_2$ + 100 mM NaCl | 8.15 c | 2.14 c | 26.67 b |
| 5 μM $SeO_2$ + 100 mM NaCl | 11.18 b | 2.34 bc | 20.92 c |
| 10 μM $SeO_2$ + 100 mM NaCl | 8.28 c | 2.61 ab | 31.76 a |
| 30 μM $SeO_2$ + 100 mM NaCl | - | - | - |
| 100 mM NaCl | 9.71 bc | 2.57 ab | 26.85 b |
| Significance | *** | ** | *** |
| LSD (0.05) | 1.59 | 0.33 | 1.35 |

LFW: Leaf fresh weight (g), LDW: Leaf dry weight (g), LDM: Leaf dry matter (%). Different lowercase letters indicate significant differences ($p < 0.05$) between means in the same column

Leaf relative water content (LRWC) and leaf membrane permeability (LMP) differed statistically by treatments ($p < 0.05$). 5 μM $SeO_2$ + 100 mM NaCl treatment resulted in the highest LRWC (59.44%). LRWC was recorded between 20.78% (1 μM $SeO_2$ + 100 mM NaCl treatment) and 59.44%. The difference between the LRWC values of control and 100 mM NaCl treatment were statistically insignificant. LMP changed between 16.84 EC% (control) and 82.90 EC% (1 μM $SeO_2$ + 100 mM NaCl treatment). Plants treated with 5 μM $SeO_2$ + 100 mM NaCl had the lowest LMP (22 EC%) after control plants (*Table 6*).

***Table 6.*** *Changes in leaf membrane permeability (EC%) and leaf relative water content (%) in strawberry (Fragaria x ananassa Duch cv. Rubygem) plants treated with selenium and salt*

| Treatment | LRWC (%) | LMP (EC%) |
|---|---|---|
| Control | 26.19 b | 16.84 d |
| 1 μM $SeO_2$ + 100 mM NaCl | 20.78 c | 82.90 a |
| 5 μM $SeO_2$ + 100 mM NaCl | 59.44 a | 22.00 c |
| 10 μM $SeO_2$ + 100 mM NaCl | 22.42 c | 82.38 a |
| 30 μM $SeO_2$ + 100 mM NaCl | - | - |
| 100 mM NaCl | 28.40 b | 75.03 b |
| Significance | *** | *** |
| LSD (0.05) | 2.95 | 3.74 |

LRWC: Leaf relative water content (%), LMP: Leaf membrane permeability (EC%). Different lowercase letters indicate significant differences ($p < 0.05$) between means in the same column


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Contents of chlorophyll a (Chl-a), chlorophyll b (Chl-a), total chlorophyll (TChl) and carotenoid differed statistically by treatments ($p < 0.05$). The differences between the Chl-a, Chl-b, TChl and carotenoid values of control and 100 mM NaCl treatment were statistically insignificant. The control plants (0.92 mg/kg) and plants treated with 100 mM NaCl (0.99 mg/kg) had the highest Chl-a contents. Although strawberry plants treated with 5 µM $SeO_2$ + 100 mM NaCl had the lowest Chl-b content (0.31 mg/kg), they contained 1.23 mg/kg of TChl. TChl content was determined between 0.89 mg/kg (10 µM $SeO_2$ + 100 mM NaCl treatment) and 1.51 mg/kg (control). The control and 100 mM NaCl treatments contained higher TChl and carotenoid than other treatments. Plants treated with 1 and 5 µM $SeO_2$ + 100 mM NaCl contained 0.370 mg/kg and 0.365 mg/kg carotenoid, respectively (*Table 7*).

***Table 7.*** *Changes in chlorophyll, and carotenoid contents in leaves of strawberry (Fragaria x ananassa Duch cv. Rubygem) plants treated with Se and salt*

| Treatment | Chl-a (mg/kg) | Chl-b (mg/kg) | TChl (mg/kg) | Carotenoid (mg/kg) |
|---|---|---|---|---|
| Control | 0.92 a | 0.43 a | 1.51 a | 0.456 a |
| 1 µM $SeO_2$ + 100 mM NaCl | 0.68 b | 0.39 a | 1.23 b | 0.370 b |
| 5 µM $SeO_2$ + 100 mM NaCl | 0.73 b | 0.31 b | 1.20 b | 0.365 b |
| 10 µM $SeO_2$ + 100 mM NaCl | 0.37 c | 0.41 a | 0.89 c | 0.255 c |
| 30 µM $SeO_2$ + 100 mM NaCl | - | - | - | - |
| 100 mM NaCl | 0.99 a | 0.42 a | 1.56 a | 0.440 a |
| Significance | *** | *** | *** | *** |
| LSD (0.05) | 0.11 | 0.06 | 0.19 | 0.054 |

Chl-a: Chlorophyll a (mg/kg), Chl-b: Chlorophyll b (mg/kg), TChl: Total chlorophyll (mg/kg). Different lowercase letters indicate significant differences ($p < 0.05$) between means in the same column

On the other hand, LPC and fruit TPC differed statistically by treatments ($p < 0.05$). LPC varied from 1.88 µmol/g (control) to 9.15 µmol/g (100 mM NaCl treatment). Strawberry plants treated with 5 µM $SeO_2$ + 100 mM NaCl also had the lowest LPC (2.18 µmol/g) after control plants (*Fig. 4*). The lowest TPC in strawberry fruit was determined with the treatment of 5 µmol $SeO_2$ + 100 mM NaCl (178.7 mg/100 g), and the highest TPC was recorded with the treatment of 1 µmol $SeO_2$ + 100 mM NaCl (247.2 mg/100 g) (*Fig. 5*).

## Discussion

Plants exposed to salt stress develop various tolerance strategies in response. Increasing the activities of non-enzymatic antioxidants and antioxidant enzymes that scavenge ROS (Reactive Oxygen Species), inducing osmolyte biosynthesis and various plant growth regulators, altering the pathway of photosynthesis, regulating gene expression and ion uptake through the SOS pathway, and activating stress-related genes to promote the synthesis of transcription factors and production of stress proteins are considered among important tolerance strategies (Yılmaz et al., 2011).

Salt stress in strawberry plants leads to a decrease in the number of leaves, leaf area, shoot dry weight, crown number, and ultimately yield (Sun et al., 2015). Due to the increase in Na + toxicity and oxidative stress in strawberry plants, leaf damage


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increases and photosynthesis efficiency decreases (Zhang et al., 2021). Plants have different capacities to accumulate Se within their tissues, and in general, low Se levels can have a positive impact on the nutrition and yield of agricultural crops (Santiago et al., 2018). Se is harmful to plants at high concentrations, it can be beneficial at low concentrations (Germ et al., 2007). In our research, 30 µM $SeO_2$ + 100 mM NaCl treatment showed a clearly toxic effect on plants. As a matter of fact, it caused increased leaf burns on plants in a short time and plant deaths one week after the treatment.

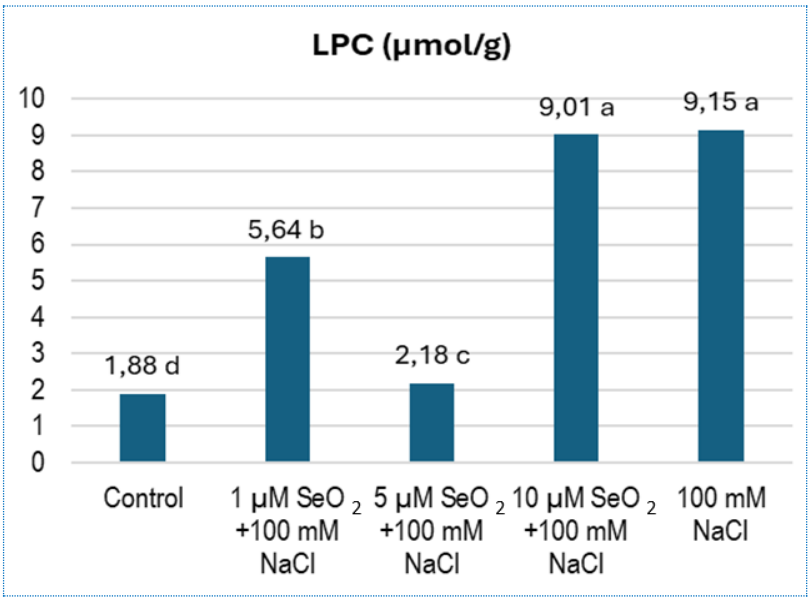


***Figure 4.*** *Leaf proline contents (LPC, µmol/g) of strawberry plants treated with $SeO_2$ + NaCl. Different lowercase letters indicate significant differences ($p < 0.05$) between means*

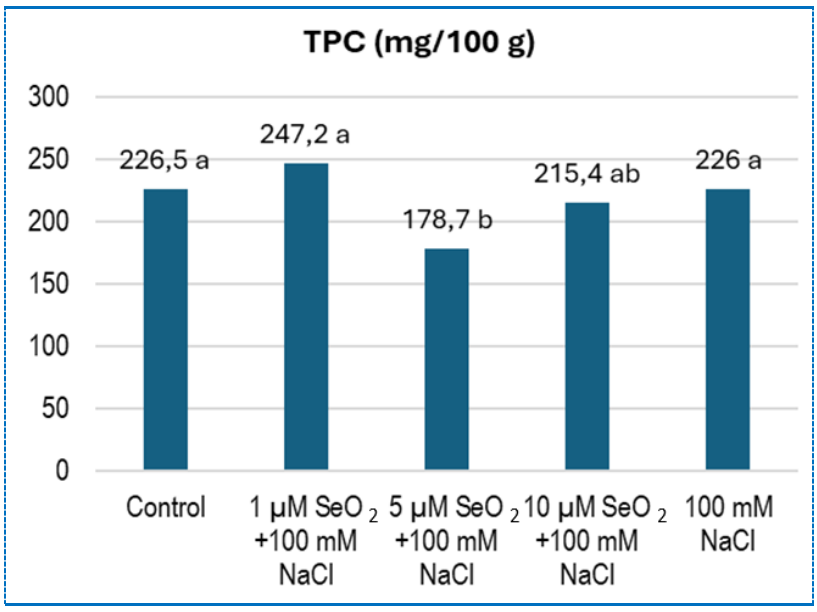


***Figure 5.*** *Total phenolic contents (TPC, mg/100 g) of fruits in strawberry plants treated with $SeO_2$ + NaCl. Different lowercase letters indicate significant differences ($p < 0.05$) between means*


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Se treatments at low concentrations to plants under NaCl stress can improve many physiological and biochemical parameters (Alsamadany et al., 2023; Rasool et al., 2023). Low dose of 5 μM Se treatment in bean plant (*Phaseolus vulgaris* L.) improved shoot and root fresh weight, chlorophyll, carotenoid, and relative water contents of the plant (Farag et al., 2022). Reporting that Se treatments affected the amount of leaf dry matter in lettuce plant, Xue et al. (2001) determined that high doses of Se (1.0 mg/kg) applied to the soil had a toxic effect on plants. In this research, LFW, LDW and LDM were significantly influenced by $SeO_2$ + NaCl treatments. After the control (20.43%), treatment of 5 μM $SeO_2$ + 100 mM NaCl was noted with the lowest LDM rate (20.92%).

Many researchers reported that Se treatments to plants under NaCl stress affect the relative water content, leaf membrane permeability and leaf chlorophyll contents (Rasool et al., 2023; Lutts et al., 1996; Alsamadany et al., 2023; Farag et al., 2022; Xue et al., 2001). Excessive amounts of soluble salts in the soil solution reduce water utilization by plants (Yılmaz et al., 2011). As the salt concentration in the solution environment of the root zone increases, the water use of the plant decreases. Salinity reduces the relative water content of the leaves (Poury et al., 2023). Se treatments at low concentrations are beneficial in preventing RWC reduction under salinity stress (Hawrylak-Nowak, 2009; Elkelish et al., 2019). Selenium treatment improved the relative water content in tomato leaves under salt stress, increasing it up to 65% (Avcu et al., 2013). In our study, plants treated with 5 μM $SeO_2$ + 100 mM NaCl had the highest LRWC (59.44%). Treatment of 5 μM $SeO_2$ + 100 mM NaCl increased RWC approximately 2 times compared to 100 mM NaCl. Therefore, this treatment significantly improved the LRWC compared to other treatments. Lutts et al. (1996) reported that NaCl increased membrane permeability in the leaves of five rice varieties with different salt tolerance. In this study, plants treated with 1 (82.90%) and 10 μM $SeO_2$ + 100 mM NaCl (82.38%) had the highest LMP, followed by 100 mM NaCl (75.03%). However, plants treated with 5 μM $SeO_2$ + 100 mM NaCl had the lowest LMP (22%) after the control plants (16.84%). Treatment of 5 μM $SeO_2$ + 100 mM NaCl resulted in a significant decrease in EC% values. This result was considered remarkable in terms of plant growth and development.

In this study, leaf chlorophyll and carotenoid contents of strawberry plants were significantly influenced by $SeO_2$ + NaCl treatments. In our research, some unexpected decreases in photosynthetic pigment content were determined with $SeO_2$ + NaCl treatments. However, Hawrylak-Nowak (2009) reported that 5 and 10 μM Se applications increased photosynthetic pigments in cucumber leaves exposed to salt stress. While Chl-a, Chl-b and TChl and carotenoids were determined at the highest level in control and 100 mM NaCl treatments, they were detected at the lowest level in 10 μM $SeO_2$ + 100 mM NaCl treatments. They showed a slight decrease in $SeO_2$ + NaCl treatments compared to control and NaCl treatments. Additionally, TChl and carotenoid losses were not at high levels with 5 μM $SeO_2$ + 100 mM NaCl application. Parida et al. (2002) determined that the total chlorophyll content in water-grown mangrove (*Bruguiera parviflora)* plants increased for two weeks after 100 mM NaCl treatment but decreased slowly for 45 days at 400 mM. Heidari et al. (2014) also reported that NaCl application increased the chlorophyll content in sunflower lines. Misra et al. (1997) concluded that the increase in chlorophyll content under salt stress may be due to an increase in the number of chloroplasts in stressed leaves.

Salinity increases proline content in the leaves (Poury et al., 2023). Rising in proline content under salt stress protects the plant from oxidative damage by preserving enzyme


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mechanisms (Mahajan and Tuteja, 2005; Tiika et al., 2023). In saline conditions, high proline levels play a role in protecting the structure of membranes and proteins in the plant and maintaining high water potential (Mohamed et al., 2007). Accumulation of proline is one of the natural ways to adapt to stress conditions, and the accumulation of proline indicates that the plant is under severe stress (Ahmad and Jhon, 2005). In this study, LPC values of strawberry plants were significantly impacted by $SeO_2$ + NaCl treatments. As expected, LPC was determined to be highest at 100 mM NaCl treatment. Plants 5 µM $SeO_2$ + 100 mM NaCl treatment accumulated the lowest LPC (2.18 µmol/g) after control (*Fig. 4*), thus they were least affected by salt-stress conditions.

On the other hand, flower and fruit formations occurred in strawberry plants treated with 1, 5 and 10 µmol $SeO_2$ + 100 mM NaCl and 100 mM NaCl. Strawberry plants treated with 1 and 10 µmol $SeO_2$ + 100 mM NaCl and 100 mM NaCl produced very few and poor fruits in terms of quality characteristic and quantity. Although of poor quality, the amount of fruit in the 100 mM NaCl treatment was slightly higher than 1 and 10 µmol $SeO_2$ + 100 mM NaCl treatments. Fruit quantity and visual quality in plants treated with 5 µmol $SeO_2$ + 100 mM NaCl were similar to those of control plants (*Fig. 2*).

According to studies, total phenolic substance amounts increase under salt stress (Noreen and Ashraf, 2009; Tetiktabanlar et al., 2020). Under abiotic or biotic stress conditions such as salinity, plants increase the synthesis of phenolic compounds that play an important role in scavenging free radicals (Ksouri et al., 2007; Neves et al., 2010). In our study, the fruits of plants treated with 5 µmol $SeO_2$ + 100 mM NaCl synthesized TPC at the lowest level compared to those of all other treatments, including the control. This finding showed that this treatment reduced TPC in fruits.

Ramos et al. (2010) found that low doses of Se had an antioxidant effect and increased plant growth in lettuce plants, while high doses of Se had a yield-reducing effect. Therefore, optimal Se supplementation offers promising potential for use against relatively high ambient NaCl levels (Seppanen et al., 2003)**.** In this study, while 5 µM $SeO_2$ + 100 mM NaCl treatment played an antioxidant role in strawberry plants, it reduced the stress caused by salt and gave successful results in terms of plant growth and fruit formation. This treatment allowed the plants to continue growing and bearing fruit despite salt stress.

## Conclusions

In this study, strawberry plants (*Fragaria x ananassa Duch*. cv. Rubygem) treated with 5 µM $SeO_2$ + 100 mM NaCl gave successful results in improving the physiological and biochemical responses of plants under salt stress. They were able to grow and produce quality fruit despite a small amount of photosynthetic pigment reductions due to salinity stress. They had the lowest leaf dry matter (%), leaf proline content (µmol/g) and leaf membrane permeability (EC%) and total phenolic content (mg/100 g), but the highest leaf relative water content (%) values. All these findings were remarkable. However, it is recommended to continue studies on reducing salinity stress in strawberries by using 2, 3, 4 µM Se doses, examining more parameters and including yield and fruit quality characteristics.

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