Journal of Food Science and Technology (Iran)

Homepage: www.fsct.modares.ir

Scientific Research

# The role of Selenium in mitigating salt-induced effects on growth, Chlorophyll content, and antioxidant enzyme activity in garlic production

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**ARTICLE INFO**

**Article History:**

Received: 2024/12/3
Accepted: 2025/5/6

**Keywords:**

Morphological traits,
Physiological traits,
Salinity stress,
Hydroponics
.

DOI: **10.22034/FSCT.22.160.187.**

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**ABSTRACT**

This study aimed to evaluate the effect of Se application under salinity conditions on the yield and physiological characteristics of garlic. The experiment was conducted using a completely randomized design with three replications. Treatments included four levels of Se (0, 4, 8, 16 mg $L^{-1}$) and four salinity levels (0, 30, 60, and 90 mM sodium chloride) in a factorial arrangement. Results indicated that low concentrations of Se positively influenced vegetative characteristics. Increasing Se concentration to 8 $mgL^{-1}$ enhanced growth across all studied vegetative traits. Relative water content of the leaves decreased with increasing NaCl concentration compared to the control. Se-treated plants showed increased levels of chlorophyll a, b, total chlorophyll and carotenoids compared to the control. Additionally, superoxide dismutase enzyme activity significantly increased with 8 $mgL^{-1}$ Se treatment at 30 mM sodium chloride compared to the control. Interaction analysis revealed the highest and lowest catalase activity at 8 and 4 $mgL^{-1}$ Se and 90 mM sodium chloride, respectively. Future research should explore the long-term effects of Se application on garlic under varying environmental conditions. Investigating the molecular mechanisms behind Se protective role against salinity stress could provide deeper insights. Additionally, examining the impact of Se on other economically important crops under salinity stress would be valuable.

## 1- Introduction

Soil salinity is a major factor limiting agricultural productivity globally, impacting approximately 7% of the world's land [1]. Enhancing plant tolerance to salinity is therefore essential [2]. Many horticultural crops are particularly sensitive to salinity and can only withstand low levels of salt stress [3]. Plants in a saline environment face two main factors: excessive salts present in the soil, which reduce the soil's osmotic potential and cause a decrease in water uptake and shortage of water in plants [4]. This leads to disruption in cell division, enlargement of cells, and affects all metabolic reactions in plants [5]. The other factor is the excessive amounts of sodium and chloride ions, which reduce the uptake of essential ions such as potassium, calcium, ammonium, and nitrate, as well as decreasing enzyme activity and damaging the membrane structure [6]. These effects result in a decrease in plant metabolic activities, including photosynthesis, and reduce plant growth in saline environments [7]. Due to ionic toxicity and osmotic stress, secondary stresses such as oxidative damage may occur in plants [8]. Salinity stress leads to the formation of Reactive Oxygen Species (ROS) such as superoxide radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl (OH), and singlet oxygen, which cause oxidative damage to lipids, proteins, and nucleic acids [9].

A multitude of strategies have been identified to enhance the resilience of plants to high salt conditions [10]. These strategies include employing water-soluble substances, utilizing nitric oxide, incorporating polyamines, applying brassinosteroids, adding silicon, using melatonin, and introducing Se. While the effect of Se in mitigating environmental stress is well-documented in human and animal studies, its impact on plant stress is less explored [7]. Se is integral to the antioxidant defenses and hormonal regulation in humans and animals, and it similarly contributes to the antioxidant activities in plants [8].

Research indicates that low levels of Se can shield plants from a range of biological challenges, including temperature extremes, water scarcity, saline conditions and toxic metal exposure [11]. The application of Se has been shown to counteract the detrimental effects of salt stress, which typically include stunted plant growth, diminished photosynthetic activity and reduced chlorophyll levels [12]. Investigations into various crops like barley, pumpkin, wheat, cabbage, and rapeseed have revealed that Se, at minimal concentrations, can be advantageous [13]. Recent studies have highlighted the multifaceted role of Se in enhancing plant resilience. For instance, Hasanuzzaman et al. (2020) reported that Se supplementation can improve the antioxidant defense system in plants, thereby mitigating oxidative stress caused by environmental challenges [14]. Additionally, Schiavon et al. (2017) found that Se can enhance the synthesis of sulfur and nitrogen compounds, which are crucial for plant responses to abiotic stress [8]. Moreover, Se has been shown to stimulate root growth and increase water absorption capacity, which is particularly beneficial under drought conditions. According to Sharma et al. [15], Se can enhance the activity of aquaporins, proteins that facilitate water transport across cell membranes, thereby improving plant water status under stress. Furthermore, The role of Se in modulating the uptake and translocation of essential nutrients has been documented, contributing to overall plant health and productivity [16]. Garlic, scientifically known as (*Allium sativum* L.*)* and belonging to the *Alliaceae* family, is a prominent member of the genus *Allium* [17]. It is the second most consumed plant from this genus, after onion, and is highly valued for its rich mineral content [18]. Among plant species, garlic is particularly sensitive to salinity [19]. A two-year study reported that the salinity threshold for garlic is 3.9 $dSm^{-1}$, and at 7.4 $dSm^{-1}$, the yield decreases by 50% [20]. All yield components, including bulb weight and diameter, shoot biomass per unit area, and the percentage of dry matter, which is a major component of bulb quality, decrease with increasing salinity [16].

The primary goal of this study was to assess the impact of Se application on garlic's yield and physiological traits under salinity stress. The objectives included determining the optimal Se concentration for enhancing vegetative growth





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and evaluating the interaction between Se and salinity levels. The study aimed to identify how Se affects chlorophyll content, carotenoids, and enzyme activities such as superoxide dismutase and catalase under different salinity conditions. By analyzing these factors, the research sought to provide insights into Se potential as a mitigative agent against salinity stress in garlic. The findings could inform future agricultural practices and guide further research on the role of Se in improving crop resilience under adverse environmental conditions.

## 2-Materials and Methods

This research was conducted in a greenhouse at the University of Tabriz, Iran, starting on September 30, 2019, and concluding on May 25, 2020. Data analysis and report finalization were completed by May 20, 2023. It is important to acknowledge that the dataset was gathered by the article's first author, Rozita Khademi Astaneh, at the university's greenhouse. This ensures proper credit for the data collection effort. The dataset comprises solely observational data of the plants, with results extracted in the university's lab. The consent procedure was approved by the University of Tabriz's ethics committee, ensuring compliance with all ethical guidelines and requirements for research involving human participant

Within the greenhouse, temperatures were maintained at 32°C during the day and 18°C at night. Natural variations in light and day length were observed, with relative humidity fluctuating between 23% and 65%. The research utilized garlic cloves sourced in February from the Maryanaj area in Hamedan Province, known for producing the 'Hamedan white' garlic variant. The experimental setup followed a factorial arrangement within a completely randomized design, consisting of three replications. After a chilling period, individual garlic cloves, still attached to their basal plates, were submerged in Se solutions at varying concentrations (0, 4, 8, 16 mg $L^{-1}$) for 26 hours. Following this immersion, the cloves were transferred to plastic containers filled with perlite, with three cloves planted per container. Throughout the study, the plants were manually watered daily with 800 mL of a modified Hoagland nutrient solution. Salinity stress was gradually introduced to the plants, which had reached the six-leaf stage, over a span of 10 days using nutrient solutions at four different salinity concentrations (0, 30, 60, 90 mM). This stress application continued for a total of 40 days.

Morphological parameters measured included the leaf count at the end of vegetative growth, the emergence of the final leaf, stem length and girth, the number of bulbs, and the dimensions of the bulbs post-harvest. The weight of the bulbs and roots was determined using a scale accurate to 0.1 gr. For dry weight assessment, the samples were oven-dried at 70°C, with roots dried for 48 hours and bulbs for 72 hours.

### Determination of relative water content

Relative leaf water content was measured according to the method proposed by Ritchie et al. (1990) [21]. Leaf samples were taken using scissors from the mature leaves of all experimental treatments and their fresh weight was measured using an accurate balance. The samples were then placed in distilled water and stored at 4°C for 24 hours. After this period, the fresh weight of the leaves was measured again and the leaves were placed in an oven at 70°C for another 24 hours to determine their dry weight. The RWC is calculated by:

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \qquad (1)$$

Where, FW is the weight of fresh sample, TW is the weight of turgid sample and DW is the weight of dry sample (all units is gr).

### Chlorophyll and carotenoids determination

To assess the levels of chlorophyll in leaves (specifically chlorophyll a, chlorophyll b and total chlorophyll content), the procedure outlined by Arnon (1949) was employed [22]. This involved pulverizing 1 gr of fresh leaf tissue with liquid nitrogen into a fine powder, then mixing it with 20 mL of 80% acetone. The mixture was passed through Whatman No.2 filter paper to obtain a clear solution. Additional washings of the mortar, pestle and residual plant matter were conducted with 10 mL of 80% acetone and the combined filtrate was made up to a final volume





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of 20 mL. The quantification of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids was based on measuring the absorbance of light at 645 nm and 663 nm for chlorophylls and at 470 nm for carotenoids, using a Spekol 1500 Analytik Jena AG spectrophotometer, comparing the readings against a control of 80% acetone.

### Superoxide dismutase activity

The enzymatic function of superoxide dismutase was evaluated by its capacity to inhibit the photoreduction of Nitroblue Tetrazolium (NBT). The assay mixture for measuring this enzyme's activity included 50 mM potassium phosphate buffer at pH 7.0, 1.0 mM ethylenediaminetetraacetic acid (EDTA), 12 mM methionine, 75 µM nitroblue tetrazolium, 2 µM riboflavin, 50 mM sodium carbonate and the enzyme sample. This mixture was exposed to a 40-watt light source, positioned 30 centimeters away to initiate the reaction. Enzyme activity was quantified as the amount of enzyme required to achieve a 50% reduction in NBT photoreduction at an absorbance of 560 nanometers, relative to controls lacking the enzyme. Activity levels were expressed in enzyme units per milligram of protein content.

### Catalase activity

Catalase enzyme activity was assessed using the technique established by Aebi [23]. The assay involved a reaction solution comprising 50 mM potassium phosphate buffer (pH 7.0), 10 mM hydrogen peroxide and distilled water. This mixture, combined with the enzyme sample, was introduced into a 2-milliliter quartz cuvette. Catalase activity was quantified by the decomposition rate of hydrogen peroxide, expressed in micromoles per minute per milligram of protein, measured at an absorbance of 240 nanometers using a spectrophotometer.

Statistical analysis was performed using SPSS software, where the average results of the different treatments were evaluated through Duncan's multiple range test, with significance determined at both 1% and 5% levels. Also, the results were visually represented through charts created with Excel software.

## 3-Results

As indicated in **Table 1**, variance analysis revealed that Se application had a notable impact on stem thickness, bulb count per plot and both the fresh and dry mass of bulbs and roots, with statistical significance at the 1% level. Conversely, the influence on bulb size was not statistically significant. Salinity levels significantly affected all measured growth parameters, except for bulb size, with a 5% significance threshold. Also, the combined influence of salinity and Se was significant across all growth parameters at the 1% level.

**Table 1**. Variance analysis of Se and salinity effects on vegetative characteristics of garlic plants (number of leaves, stem diameter, bulb diameter and height, number of cloves per bulb, fresh and dry weight of bulb and root)"

| | | Mean of squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | bulb | | root | |
| Sources of variation | df | Number of leave | Stem diameter (mm) | Garlic bulb diameter (mm) | Garlic stem diameter (mm) | number of cloves per bulb | DW(gr) | FW(gr) | DW(gr) | FW(gr) |
| Se | 3 | 18.5* | 8.969** | 5.062ns | 8.969** | 38.809** | 43.141** | 1109.416** | 4.061** | 179.796** |
| Salinity | 3 | 75.167** | 8.377** | 8.606* | 8.377** | 30.483** | 26.352** | 1773.046** | 12.170** | 643.040** |
| Se× Salinity | 9 | 48.267** | 6.736** | 25.138** | 6.736** | 27.038** | 29.178** | 1061.755** | 6.486** | 536.670** |
| Error | 32 | 164.667 | 7.555 | 120.593 | 7.555 | 22.653 | 140.795 | 823.677 | 7.818 | 669.062 |

**, * and ns: Significantly difference at the 1 and 5 % of probability levels and non-significantly difference, respectively.





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According to the results of **Table 2**, the treatment with 16 $mgL^{-1}$ Se had the highest number of leaves. The treatment with 8 $mgL^{-1}$ Se had the highest bulb diameter and number of bulbs per plot. The treatment with 4 $mgL^{-1}$ Se showed the highest fresh and dry weight of roots. The treatment with 16 $mgL^{-1}$ Se had the highest dry weight and length of bulbs at sodium chloride concentrations of 90 mM and 60 mM, respectively and the highest fresh weight of bulbs in non-saline conditions.

**Table 2-** Comprehensive parameters of garlic plants grown under various Se and salinity (NaCl) treatments

| Selected biometric parameters | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | | Number of leave | Stem diameter (mm) | Garlic bulb diameter (mm) | Garlic bulb height (mm) | Number of cloves per bulb | Bulb | | Root | |
| Se (Se) ($mg L^{-1}$) | NaCl (mM) | | | | | | FW(gr) | DW(gr) | FW(gr) | DW(gr) |
| 0 | 0 | $43.66^{cd}$ | $7.65^{f}$ | $45.06^{bcd}$ | $36.5^{ab}$ | $15.06^{ab}$ | $61.8^{def}$ | $21.4^{de}$ | $8.94^{ghi}$ | $6.1^{cdefg}$ |
| | 30 | $46.66^{cd}$ | $8.35^{e}$ | $45.96^{abc}$ | $33.47^{bcd}$ | $9.1^{f}$ | $53.9^{fg}$ | $20.3^{de}$ | $9.67^{efg}$ | $5.2^{gh}$ |
| | 60 | $41^{d}$ | $7.18^{f}$ | $45.78^{abcd}$ | $33.84^{bcd}$ | $10.5^{ef}$ | $83.5^{bc}$ | $22.9^{bcde}$ | $10^{def}$ | $7.7^{b}$ |
| | 90 | $42^{d}$ | $8.63^{de}$ | $43.56^{bcde}$ | $36.68^{ab}$ | $12.06^{cd}$ | $64.8^{de}$ | $14.7^{f}$ | $7.7^{j}$ | $6.2^{cdef}$ |
| 4 | 0 | $43^{d}$ | $8.69^{de}$ | $44.99^{bcd}$ | $36.09^{ab}$ | $9.4^{f}$ | $70.1^{d}$ | $24.1^{abcd}$ | $10.53^{de}$ | $8.02^{b}$ |
| | 30 | $44^{cb}$ | $9.49^{e}$ | $46.76^{ab}$ | $36.71^{ab}$ | $12.2^{c}$ | $100.9^{a}$ | $25.7^{abc}$ | $11.7^{c}$ | $9.91^{a}$ |
| | 60 | $50.33^{ab}$ | $7.3^{f}$ | $40.67^{e}$ | $32.23^{d}$ | $9.2^{f}$ | $66.9^{d}$ | $22.2^{cde}$ | $9.88^{def}$ | $6.6^{c}$ |
| | 90 | $45^{cd}$ | $5.63^{g}$ | $42.06^{de}$ | $26.91^{e}$ | $10.63^{def}$ | $56.4^{efg}$ | $20.5^{de}$ | $9.51^{fgh}$ | $5.5^{efgh}$ |
| 8 | 0 | $49^{b}$ | $11.88^{a}$ | $42.2^{de}$ | $36.05^{ab}$ | $16.2^{a}$ | $78.6^{c}$ | $26.5^{ab}$ | $14.42^{a}$ | $8.07^{b}$ |
| | 30 | $47.33^{bc}$ | $9.61^{c}$ | $48.97^{a}$ | $35.93^{abc}$ | $16.53^{a}$ | $70.4^{d}$ | $26.4^{ab}$ | $11.97^{c}$ | $6.6^{c}$ |
| | 60 | $42^{d}$ | $7.43^{f}$ | $42.78^{cde}$ | $33.11^{ab}$ | $14.3^{b}$ | $62.2^{def}$ | $19.4^{e}$ | $8.41^{ij}$ | $5.6^{defg}$ |
| | 90 | $37^{e}$ | $9.26^{cd}$ | $42.56^{cde}$ | $32.16^{d}$ | $6.96^{g}$ | $50.8^{g}$ | $21.8^{cde}$ | $10.7^{d}$ | $4.6^{h}$ |
| 16 | 0 | $43.33^{cd}$ | $10.55^{b}$ | $47.23^{ab}$ | $34.9^{bcd}$ | $14.6^{b}$ | $89.95^{b}$ | $22.7^{bcde}$ | $12.9^{7b}$ | $9.24^{a}$ |
| | 30 | $53.3^{a}$ | $7.8^{f}$ | $37.36^{e}$ | $33.94^{bcd}$ | $16.3^{a}$ | $63.9^{de}$ | $23.8^{abcd}$ | $8.71^{h}$i | $6.43^{cde}$ |
| | 60 | $42.66^{d}$ | $9.36^{c}$ | $46.91^{ab}$ | $39.25^{a}$ | $15^{ab}$ | $67.1^{d}$ | $22.5^{bcde}$ | $10.59^{de}$ | $6.56^{cd}$ |
| | 90 | $41^{d}$ | $9.29^{cd}$ | $43.91^{bcde}$ | $32.3^{cd}$ | $11.6^{cde}$ | $61.6^{def}$ | $26.9^{a}$ | $7.5^{k}$ | $5.3^{fgh}$ |
| Salinity treatment (S) | | ** | ** | * | ** | ** | ** | ** | ** | ** |
| Se treatment (SNP) | | * | ** | NS | * | ** | ** | ** | ** | ** |





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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| S×Se | ** | ** | ** | ** | ** | ** | ** | ** | ** |

The averages of the traits, which have the same letters in each column, have no significant difference at the 5% probability level.

The application of Se resulted in an increase in stem diameter, with the highest stem diameter observed in the treatment with 8 mgL$^{-1}$ Se under non-saline conditions. Stem height was affected by salinity treatment and increased with the concentration of sodium chloride in non-Se conditions compared to the control. The highest stem height was recorded at a sodium chloride concentration of 60 mM and with the treatment of 8 mgL$^{-1}$ Se.

**Relative Water Content (RWC)**

The simple and interactive effects of salinity and Se were significant at the 1% probability level on the RWC of leaves (**Table 3**). As shown in **Fig 1**, with increasing salt concentration, the RWC of leaves decreased compared to the control plant. In non-saline conditions, the application of 16 mgL$^{-1}$Se significantly increased the RWC of garlic plant leaves compared to the control.

The simple and interactive effects of salinity and Se were significant at the 1% probability level on the RWC of leaves (**Table 3**). As shown in **Fig 1**, increasing salt concentration led to a decrease in the RWC of leaves compared to the control plants. Under non-saline conditions, the application of 16 mgL$^{-1}$ Se significantly increased the RWC of garlic plant leaves compared to the control.

**Table 3**. Variance analysis of Se and salinity effects on RWC, Chlorophyll content, carotenoids, and activities of SOD and CAT enzymes in garlic plants

| Source of variation | df | Mean of squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | RWC | Chl a | Chl b | Total Chl | Carotenoid | SOD | CAT |
| Se | 3 | 92.50** | 0.025** | 0.097** | 0.228** | 0.020** | 226.228** | 91.308** |
| Salinity | 3 | 212.944** | 0.30** | 0.150** | 0.403** | 0.018** | 53.917** | 60.972** |
| Se×Salinity | 9 | 174.689** | 0.016** | 0.105** | 0.183** | 0.015** | 114.213** | 73.226** |
| Error | 32 | 45.333 | 0.024 | 0.018 | 0.076 | 0.001 | 116.309 | 0.000218 |

**, * and ns: Significantly difference at the 1 and 5 % of probability levels and non-significantly difference, respectively.

The lowest RWC of leaves was first observed in the treatment with 4 mgL$^{-1}$ Se without salinity stress, followed by the highest sodium chloride concentration (90 mM) without Se treatment. The highest RWC was observed in the treatment with 8 mgL$^{-1}$ Se and 30 mM sodium chloride concentration, which did not differ significantly from the 60 mM sodium chloride concentration (**Fig 1**).



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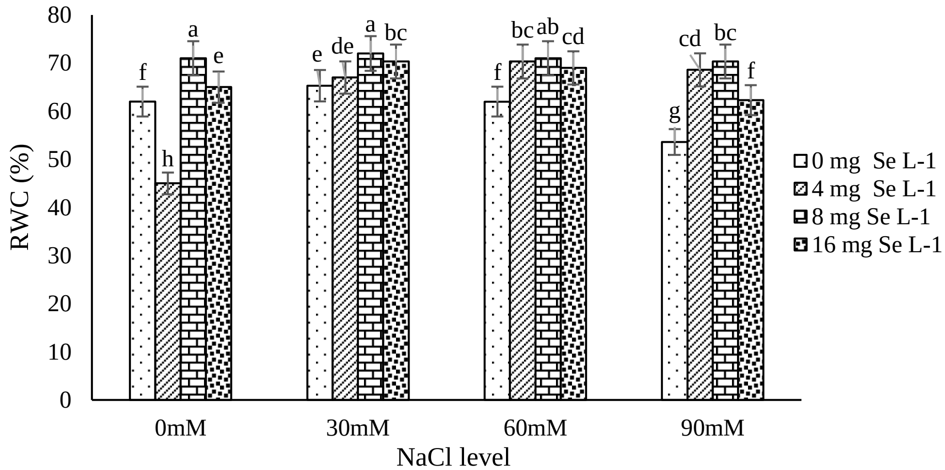


**Fig.1.** Impact of Se and sodium chloride on the RWC of garlic leaves

Chlorophyll content

Chlorophyll a

The study demonstrated that both the individual and combined impacts of salinity stress and Se supplementation had a statistically significant influence on chlorophyll a levels with a less than 1% probability of random occurrence. An inverse relationship was observed between chlorophyll a content and increasing levels of sodium chloride in samples not treated with Se. Conversely, in samples without salinity stress, chlorophyll a initially increased with Se supplementation but decreased with higher Se doses. The highest chlorophyll a concentration was found in samples treated with 4 $mgL^{-1}$ Se and 30 mM sodium chloride, while the lowest concentration was observed in samples treated with 16 $mgL^{-1}$ Se at a sodium chloride level of 60 mM (**Fig 2**).

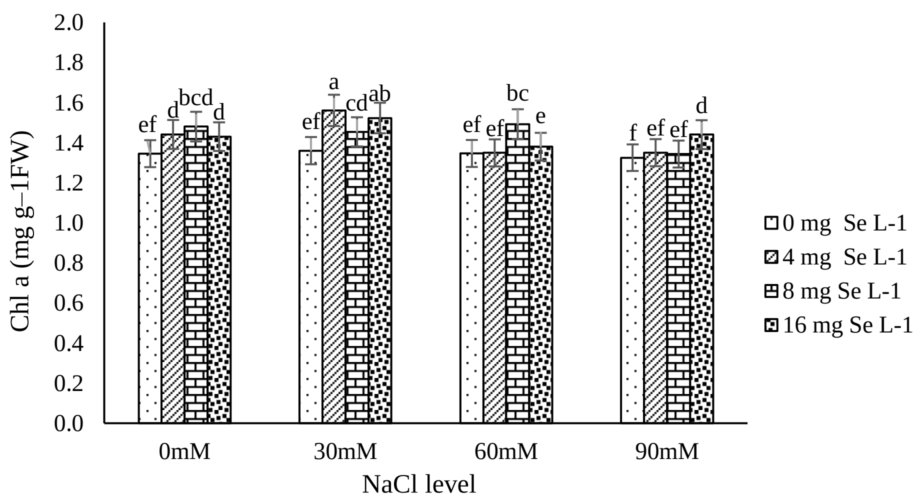


**Fig.2.** Impact of Se and sodium chloride on chlorophyll a content in garlic leaves

Chlorophyll b

The experimental data, analyzed through variance, indicated that both the individual and combined effects of salinity and Se had a statistically significant impact on chlorophyll b levels at the 1% probability level, as shown in **Table 3**. An upward trend in chlorophyll b content was observed with increasing Se levels in





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environments free of salinity. Conversely, in the absence of Se, an increase in sodium chloride led to a reduction in chlorophyll b levels. The highest chlorophyll b content was found in the treatment with 4 mgL$^{-1}$ Se at a 30 mM sodium chloride concentration, while the lowest content was observed in the treatments with 0 and 4 mgL$^{-1}$ Se at a 90 mM sodium chloride concentration (**Fig 3**).

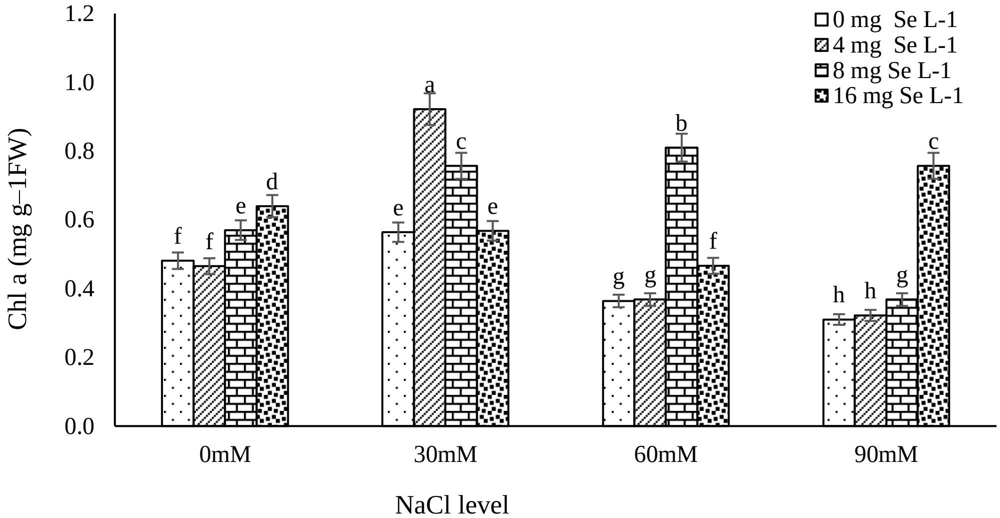


**Fig.3.** Impact of Se and sodium chloride on chlorophyll b content in garlic leaves

Total Chlorophyll

The simple effects of salinity stress and Se treatment, as well as their interactive effects, were statistically significant at the 1% probability level on total chlorophyll content (**Table 3**). Se treatment in non-saline conditions led to a significant increase in total chlorophyll content. In the absence of Se treatment, total chlorophyll content decreased with increasing salinity levels. The interactive effects showed that the highest total chlorophyll content was recorded in the treatment with 4 mgL$^{-1}$ Se and 30 mM sodium chloride concentration. The lowest total chlorophyll content was observed in the treatments with 0, 4, and 8 mgL$^{-1}$ Se at a 90 mM sodium chloride concentration (**Fig 4**).

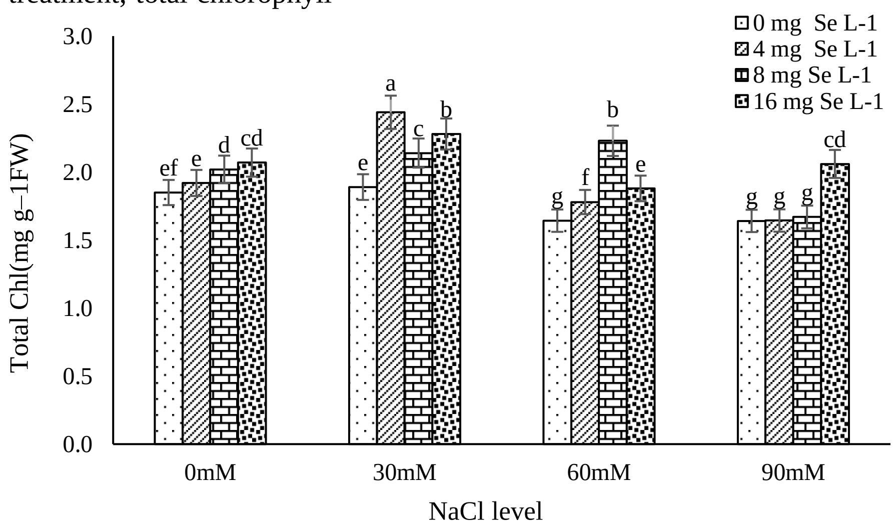


**Fig.4.** Impact of Se and sodium chloride on total chlorophyll content in garlic leaves





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Carotenoid

The simple effects of salinity stress, Se treatment and their interactive effects were statistically significant at the 1% probability level on leaf carotenoids (**Table 3**). Increasing sodium chloride concentration led to a decrease in carotenoid content. The application of Se in non-saline conditions significantly increased carotenoid content (**Fig 5**). The interactive effects showed a significant increase in carotenoid content with the application of Se (4, 8, and 16 $mgL^{-1}$) at different salinity levels. The highest carotenoid content was recorded at 30, 60, and 90 mM sodium chloride concentrations with the application of 4, 8, and 16 $mgL^{-1}$ Se, respectively (**Fig 5**).

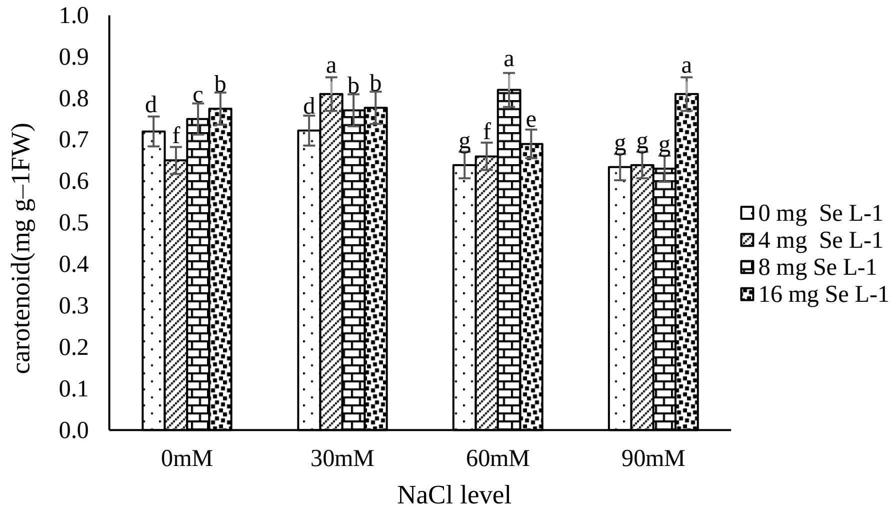


**Fig.5.** Carotenoid content in garlic under salinity stress with various Se treatment levels"

Superoxide dismutase activity

The interactive effects of salinity and Se were statistically significant at the 1% probability level on Superoxide Dismutase (SOD) activity (**Table 3**). Based on the results of the simple effects, SOD activity increased with rising sodium chloride concentrations and Se levels. The highest SOD activity was observed in the treatment without Se at a 60 mM sodium chloride concentration. Considering the interactive effects of salinity and Se, the application of 8 $mgL^{-1}$ Se at 30 mM sodium chloride concentration significantly increased SOD activity compared to the control (**Fig 6**).





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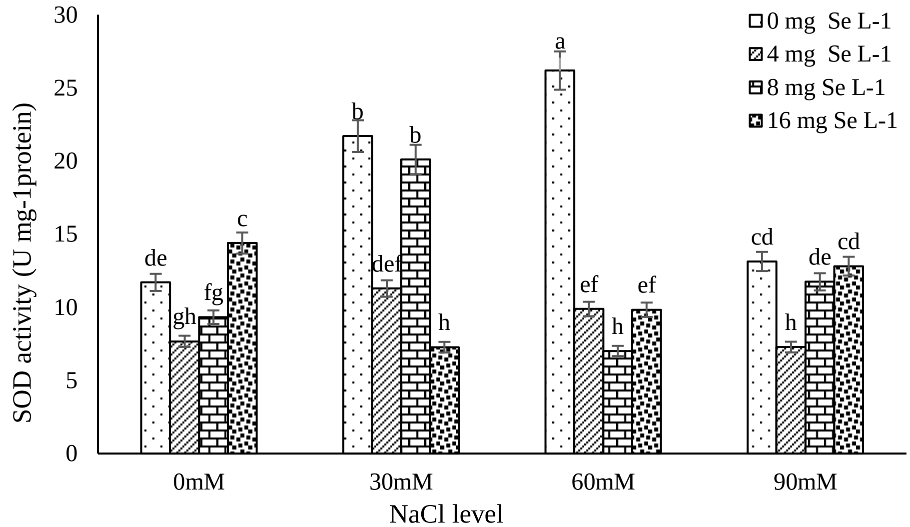


**Fig.6.** Superoxide dismutase enzyme activity in garlic under salinity stress with various Se treatment levels

Catalase activity

The interactive effects of salinity and Se were statistically significant at the 1% probability level on Catalase (CAT) activity (**Table 3**). CAT activity increased with rising Se concentrations. Salinity stress also significantly increased CAT activity, with the highest activity observed at a 60 mM sodium chloride concentration without Se treatment (**Fig 7**). According to the results of the interactive effects, the highest CAT activity was recorded in the treatment with 8 $mgL^{-1}$ Se and 90 mM sodium chloride, while the lowest activity was observed in the treatment with 4 $mgL^{-1}$ Se and 90 mM sodium chloride.

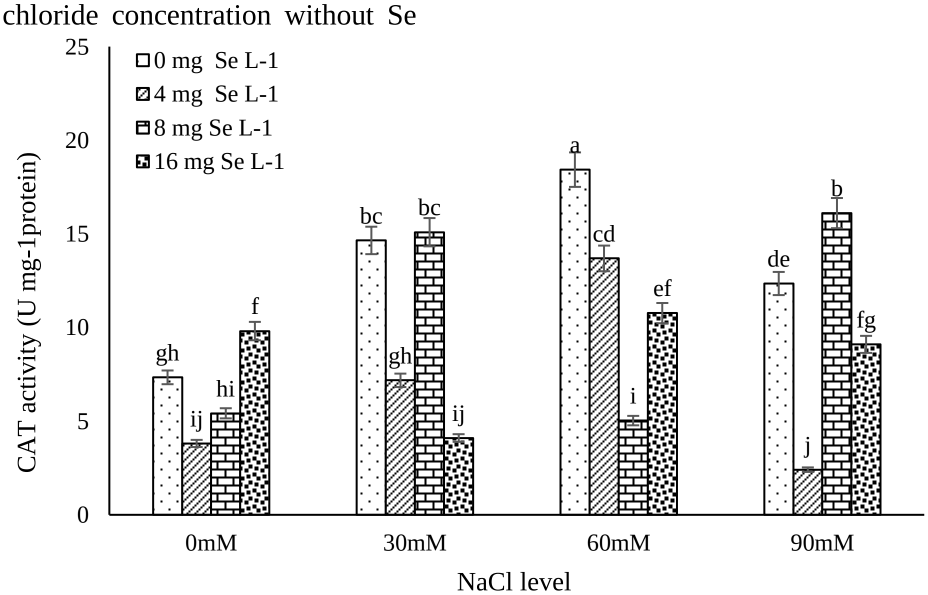


**Fig.7.** Catalase enzyme activity in garlic under salinity stress with various Se treatment levels

## 4-Discussion

The results of this study demonstrated that Se application at low concentrations, positively influenced growth characteristics. Specifically, increasing Se concentration up to 8 $mgL^{-1}$ enhanced all growth traits studied, but higher concentrations led to a decline. These findings




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align with recent studies on cucumber and watermelon under saline stress [4-6]. The higher growth characteristics observed at the 8 $mgL^{-1}$ treatment may be due to increased leaf area, which in turn provides more assimilates for plant growth.

Se application up to a certain level also increased the dry weight of the stem, consistent with findings in other crops [8]. This increase in Se content enhances plant performance by protecting chlorophyll [24]. Plants possess antioxidant enzymes such as SOD and CAT to combat ROS. This increase in Se content enhances plant performance by protecting chlorophyll [25]. Se increases the activity of these two enzymes at low concentrations, which could potentially increase leaf production in plants through increased antioxidant activity. Additionally, selenium's growth-promoting effects may result from increased starch accumulation in chloroplasts and cell protection [26]. Se application in the form of selenate and selenite has been shown to be beneficial for plants under saline stress [6, 27]. The significant differences in leaf water content between Se-treated plants indicate varying effects on water uptake or loss through stomata and the ability to regulate osmotic pressure to maintain tissue turgor [14]. Se stimulates root growth and increases water absorption capacity, leading to higher leaf water content [28]. Additionally, Schiavon et al. (2017) noted that Se can enhance plant growth and stress tolerance by inducing the synthesis of sulfur and nitrogen compounds, which are vital for plant responses to abiotic stress [8]. Khan et al. (2023) further emphasized that Se, even in trace amounts, can significantly improve plant growth and development by acting as an antioxidant and stimulator [25].

The observed decline in chlorophyll levels under saline conditions is likely due to a shift in nitrogen metabolism, favoring proline production over chlorophyll. However, Se introduction under these conditions increases chlorophyll levels, suggesting Se alleviates damage to the photosynthetic apparatus and cell membranes [29]. Studies have shown that Se-treated plants exhibit higher levels of chlorophyll a, b, and total chlorophyll compared to untreated plants [30]. Excessive Se, however, can reduce chlorophyll, carotenoid, and xanthophyll levels, indicating a delicate balance in the effects of Se on plant physiology [4].

Carotenoids play a crucial role as biological antioxidants, protecting plant tissues. Se application increases chlorophyll and carotenoid contents, consistent with recent findings [5]. This increase may be due to the inhibition of ROS by antioxidant enzymes [14]. Se can improve the strength of cellular organelles and the membrane system in leaf cells under salinity stress. One of the most significant biochemical changes in plants under stress is the production of ROS and hydroxyl radicals in chloroplasts and mitochondria. ROS are produced during vital processes such as photorespiration, photosynthesis, and respiration, disrupting normal plant metabolism and potentially leading to cell death. Plants have various protective mechanisms to eliminate or reduce ROS, including antioxidant enzyme systems. Higher levels of antioxidants in plants correlate with greater resistance to oxidative damage [31]. One of the most significant biochemical changes that occur in plants under stress is the production of ROS and hydroxyl radicals in chloroplasts and mitochondria [32]. ROS are produced during vital processes such as photorespiration, photosynthesis and respiration and can disrupt normal plant metabolism, ultimately leading to cell death through water decomposition and electron transfer disorders. Plants have various protective mechanisms to eliminate or reduce ROS, which are effective at different stress levels [31]. Antioxidant enzyme systems are one of these protective mechanisms. Plants with higher levels of antioxidants exhibit greater resistance to oxidative damage. The activities of SOD and CAT enzymes under Se and salinity treatments are shown in **Figs 6 and 7**.

Antioxidant enzymes protect membranes, proteins, and macromolecules against oxidative damage caused by reactive oxygen species and contribute to the resistance and stability of plants under environmental stresses such as salinity [32]. Salinity stress, similar to other environmental stresses, leads to the accumulation of ROS in cells. Plants have an efficient defense





[ DOI: 10.22034/FSCT.22.160.187 ]

system consisting of antioxidant enzymes to combat the oxidative stress caused. Antioxidant enzymes protect membranes, proteins and macromolecules against oxidative damage caused by ROS and contribute to the resistance and stability of plants under environmental stresses such as salinity [33]. Therefore, the antioxidant capacity of plants is directly related to their tolerance to stress [8]. Sairam et al. [34] reported an increase in the activity of the SOD enzyme in plants under salinity stress compared to the control. The findings of Ashraf and Ali (2008) suggest that the presence of saline conditions enhances the action of the antioxidant enzyme SOD in mustard plant foliage. This heightened enzyme activity could be attributed to an accelerated synthesis of DNA and proteins within the plant tissues [31]. Additionally, when Se is administered to plants subjected to saline environments, there is a notable rise in the activity of the SOD enzyme [15].

## 5-Conclusion

The application of Se significantly enhanced the weight of fresh shoots, dry root weight, number of shoots per plant, and shoot diameter and length compared to control plants. Se at low concentrations positively influenced growth characteristics, with all traits improving up to a concentration of 8 $mgL^{-1}$, beyond which they declined. Increased salinity levels reduced the relative water content of leaves, but Se treatment at 8 $mgL^{-1}$improved water content at 30 and 90 mM sodium chloride levels. Se regulated plant water status by stimulating root growth and enhancing water absorption capacity, thereby increasing leaf water content. It also protected chloroplast enzymes, leading to higher chlorophyll and photosynthetic pigment levels. Under salinity stress, the activity of superoxide dismutase and catalase enzymes increased, with Se treatment further boosting antioxidant enzyme activity. This increase may be attributed to enhanced DNA and protein synthesis in tissues. Future research should investigate the long-term effects of Se application on garlic under diverse environmental conditions. Exploring the molecular mechanisms behind Se's protective role against salinity stress could provide deeper insights. Additionally, examining the impact of Se on other economically important crops under salinity stress would be valuable. Field trials to validate these findings under real-world agricultural conditions are recommended.

## 6-Acknowledgement

The authors would like to thank the editor in chief and the anonymous referees for their valuable suggestions and useful comments that improved the paper content substantially. This study was supported by a grant from University of Tabriz, Iran. The authors are grateful for the support provided by University of Tabriz.

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[ DOI: 10.22034/FSCT.22.160.187 ]

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# نقش سلنیوم در کاهش اثرات ناشی از نمک بر رشد، محتوای کلروفیل و فعالیت آنزیم آنتی‌اکسیدانی گیاه سیر

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گروه باغبانی دانشگاه تبریز

**اطلاعات مقاله**

**تاریخ های مقاله :**

**تاریخ دریافت:** ۱۴۰۳/۰۹/۱۳

**تاریخ پذیرش:** ۱۴۰۴/۰۲/۱۶

**کلمات کلیدی:**

صفات مورفولوژیکی،

صفات فیزیولوژیکی،

تنش شوری،

هیدروپونیک

**DOI: 10.22034/FSCT.22.160.187.**

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**چکیده**

هدف این مطالعه ارزیابی اثر کاربرد Se در شرایط شوری بر عملکرد و ویژگی‌های فیزیولوژیکی سیر بود. این آزمایش در قالب طرح کاملا تصادفی با سه تکرار انجام شد. تیمارها شامل چهار سطح ۰ ، ۴ ، ۸ ، ۱۶ میلی‌گرم سلنیوم و چهار سطح نمک (۰ ، ۳۰ ، ۶۰ و ۹۰ میلی متر کلرید سدیم) بود. نتایج نشان داد که غلظت‌های پایین Se به طور مثبت بر ویژگی‌های گیاهی سیر تأثیر می‌گذارد. افزایش غلظت Se به ۸ میلی‌گرم بر لیتر، رشد را در تمام صفات رویشی مورد مطالعه افزایش داد. محتوای آب نسبی برگ‌ها با افزایش غلظت NaCl در مقایسه با تیمار شاهد، کاهش یافت. گیاهان تحت تیمار Se در مقایسه با شاهد، سطح کلروفیل a ، b و کاروتنوئیدها را افزایش دادند. علاوه بر این، فعالیت آنزیم سوپراکسید دیسموتاز با مصرف ۸ میلی‌گرم بر لیتر Se و ۳۰ میلی موس در مقایسه با کنترل به طور قابل توجهی افزایش یافت. نتایج این مطالعه نشان داد که بالاترین و پایین‌ترین فعالیت کاتالاز در ۸ و ۴میلی‌گرم بر لیتر Se و ۹۰ میلی موس کلرید سدیم است. تحقیقات آینده باید اثرات بلند مدت استفاده از Se را بر سیر در شرایط محیطی متفاوت بررسی کند. بررسی مکانیسم‌های مولکولی نقش محافظتی Se در برابر استرس شوری می‌تواند بینش عمیق‌تری را فراهم کند. همچنین، بررسی تاثیر Se بر سایر محصولات مهم اقتصادی تحت استرس شوری قابل پیشنهاد است.