



RESEARCH ARTICLE

The Effect of Iodine on Rocket (*Eruca sativa* L.) Plant Under Salt StressHande Dizkara • Ertan Yıldırım • Melek Ekinci[✉]

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ARTICLE INFO

Article History

Received: 19.07.2025

Accepted: 11.08.2025

First Published: 30.09.2025

Keywords

Biofortification

Iodine

Rocket

Salinity



ABSTRACT

The main objective of this study was to investigate how iodine biofortification (potassium iodate (KIO₃)) applied to rocket affects the physiological and biochemical responses of the plant under salt stress. Rocket (*Eruca sativa* L.) seeds were used as material and the applications were started when the seeds germinated and the plants had 2-3 leaves. 50 (T1) and 100 (T2) µM KIO₃ applications were applied to the leaves of the plants by spraying and also by irrigation to the soil. Salt stress was applied by applying water prepared with 100 mM NaCl to the plant as irrigation from the soil. The effects of the applications on some plant morphological and physiological properties were investigated. In the study, salinity significantly reduced plant growth and development in rocket. Iodine application alleviated this damage and supported growth in rocket against salt stress. The plant height, stem diameter, leaf number and leaf area decreased by 29%, 27%, 22% and 56%, respectively, under salt stress, while iodine applications reduced this decrease. Electrical conductivity (EC) increased with salinity, while leaf water relative content (LRWC) decreased in rocket. This effect of salt was low in iodine application. Plant fresh weight, root fresh weight, plant dry weight and root dry weight decreased by 55%, 13%, 20% and 10% in the control group with salinity, and iodine application mitigated this decrease. The chlorophyll content, root fresh and dry weight of plants, especially those under salt stress, increased with iodine application. Hydrogen peroxide (H₂O₂), malondialdehyde (MDA), proline, sucrose and antioxidant enzyme activities, which increased with salinity, also increased with iodine application. Iodine supports plant development and is also effective in salt stress tolerance of rocket plants.

Please cite this paper as follows:

Dizkara, H., Yıldırım, E., & Ekinci, M. (2025). The effect of iodine on rocket (*Eruca sativa* L.) plant under salt stress. *Journal of Agricultural Production*, 6(3), 148-156. <https://doi.org/10.56430/japro.1746259>

1. Introduction

Environmental factors such as climate and soil to which plants are exposed have a great effect on quality and yield. For efficient cultivation, optimum environmental requirements of plants must be encountered. Any deviation from optimum requirements of the plant creates stress for that plant (Levitt, 1990). Abiotic stresses caused by environmental factors (low and high temperature, drought, salinity, heavy metals, radiation, nutrient deficiency, etc.) cause significant problems especially in agricultural production. Plants respond to these stress conditions with physiological and metabolic changes in a

way that their growth and development are minimally damaged (Kalefetoğlu & Ekmekçi, 2005).

Salinity, which limits plant production especially in arid and semiarid regions and affects the entire metabolism of the plant, including its morphology and anatomy, is among the most important abiotic stress factors (Ashraf & Foolad, 2007; Çirka et al., 2022; Levitt, 1990). Salt stress is defined as the presence of various salts in soil or water at concentrations that can inhibit plant growth and causes large areas to become unavailable for agriculture. Approximately 6% of the land on the earth's surface and 20% of irrigated areas face salinity problems, and it is reported that these values may increase by 50% in the next 20

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years (Hasanuzzaman et al., 2013). Salt is an important stress in growth and development by negatively affecting the current growth and generation transfer processes of plants and negatively affecting the current physiological cycle. Photosynthesis capacity, one of the most important physiological processes, decreases depending on the type, duration, age and species of plant (Najar et al., 2019). Increasing salt concentration in the soil solution increases the osmotic potential of the water, and since plants close their stomata to maintain water balance, growth, yield and quality decrease as a result of the decline in biochemical events such as respiration and photosynthesis. Salt accumulated around the root affects the stomatal conductance of CO₂ in the leaf by affecting the osmotic potential, cell volume and water amounts in the cells, photosynthesis and meristematic tissues of the plant (Arif et al., 2020; Munns et al., 2000; Munns, 2002). Soil salinity affects plant growth by restricting the uptake of water by the roots due to the high osmotic pressure of the soil moisture in the root zone due to the high concentration of soluble salts. Salinity also interferes with the balanced absorption of essential nutrient ions by plants (Tester & Devenport, 2003). Increased Na concentration disrupts cell membrane functions and intracellular ion balance due to decreased metabolic activity, leading to cell growth arrest and death (Ashraf & Bashir, 2003; Ashraf & Harris 2005; Rus et al., 2001). In addition, increased Na⁺ and Cl⁻ disrupt the cytosolic structure of the cytoplasm and the metabolic cycle of organelles within the cell (Ali et al., 2024; Serrano et al., 1999; Zhu, 2001).

Various organic and inorganic applications are used to increase tolerance to stress conditions. One of these is the iodine application, which has been emphasized especially in recent years. It has been determined that iodine, which is generally not required during the plant growth process, increases plant adaptation to stresses such as drought and salinity (Fuentes et al., 2022; Leyva et al., 2011; Shalaby, 2025; Sodaiezhadeh et al., 2020). Iodine is an element used for biofortification in plant growth (Dávila-Rangel et al., 2019). Indeed, it has been determined that exogenous application of iodine as biofortification enhances biomass and antioxidant capacity by stimulating the biosynthesis and accumulation of phenols (Blasco et al., 2008). It has been reported that iodine increases biomass production in plants by regulating the expression of various genes, activity and structure of proteins, maintains ROS homeostasis and improves plant defense responses (Riyazuddin et al., 2023).

One of the leafy vegetables, rocket (arugula, *Eruca sativa* L.), whose leaves are consumed, originates from the Mediterranean basin (Vural et al., 2000). Especially known in Mediterranean countries and the world, rocket is a vegetable that is found in nature in the wild and has been cultivated. It is usually produced for its leaves, and its harvest is done in the first year (Eşiyok, 2012). Although rocket, whose leaves are consumed, has a bitter taste and aroma, its high nutritional

content in terms of health also makes its consumption attractive (Barillari et al., 2005). It shows that rocket contains important minerals as well as proteins and vitamins that are beneficial for human nutrition, and it is important in nutrition due to its high fiber content (Barlas et al., 2011).

In addition to areas with salinity problems, the short growing season of rocket, combined with the intensive irrigation and fertilization used in cultivation, can cause salt accumulation in the soil. Therefore, salinity negatively impact rocket's growth and development (Kaya, 2021). The main objective of this study was to investigate how iodine biofortification (KIO₃) applied to rocket affects the physiological and biochemical responses of the plant under salt stress. The changes caused by iodine application in plant growth, development and yield, how it affects the negative effects caused by salt stress and whether the resistance to salt stress increases were examined.

2. Materials and Methods

In this study, rocket (*Eruca sativa* L.) seeds from the Brassicaceae family were used as material. In the study conducted in a semi-controlled (20±5°C temperature, 50±5% moisture) glass greenhouse, seeds were planted in pots (1.5 L) prepared with a mixture of garden soil:peat:sand (3:1:1, v:v:v).

The study was established with two salt levels (0 and 100 mM NaCl), three iodine doses (0, 50 and 100 µM KIO₃), three replications, 6 pots in each replication, totaling 108 pots. Initially, seeds were sown equally in each pot, and after the seeds germinated and emerged to the soil surface, thinning was done so that 10 plants remained in each pot. The applications were started when the seeds germinated and the plants had 2-3 leaves. 50 (T1) and 100 (T2) µM KIO₃ applications were applied to the leaves of the plants by spraying and also by irrigation to the soil in order to ensure that plants can uptake both from the leaves and roots. Salt stress was applied by applying water prepared with 100 mM NaCl to the plant as irrigation from the soil. Salt stress application was applied by increasing gradually. The effect of the applications was determined by examining various morphological and physiological parameters after approximately 30 days. In this study, the effect of the applications was examined in terms of plant height, stem diameter, number of leaves, leaf area, plant fresh weight, plant dry weight, root fresh weight, root dry weight, leaf area, EC (Shams et al., 2019), LRWC (Shams et al., 2019), chlorophyll a, b and total chlorophyll (Lichtenthaler & Buschmann, 2001).

Hydrogen peroxide (H₂O₂) content and malondialdehyde (MDA) in fresh leaf samples were determined according to Velikova et al. (2000) and Sahin et al. (2018). Proline content was measured spectrophotometrically at 520 nm (Bates et al., 1973). Sucrose content was analyzed using high-performance

liquid chromatography (HPLC) based on the methods of Chopra et al. (2000).

Extraction of fresh leaf samples for antioxidant enzyme (POD, CAT, and SOD) analysis was performed according to the method described by Angelini and Federico (1989) and Angelini et al. (1990). Catalase (CAT) activity was measured at 240 nm, POD activity was measured by absorbance increase at 470 nm, and SOD activity was measured at 560 nm (Agarwal & Pandey, 2004; Gong et al., 2000; Yee et al., 2003; Yordanova et al., 2004).

The study was designed according to a randomized plot experimental design, variance analysis of the obtained data was performed using the SPSS 20 program, and the differences between the applications were determined by the Duncan multiple comparison test.

3. Results

In the experiment, salinity caused decreases in plant growth and development in rocket and the results are given below. In the study, plant height decreased with salinity, plant height which was 15.00 cm in control became 10.63 cm with salinity.

However, iodine applications increased plant height in rocket both in normal and salt conditions. While plant height was the highest (15.83 cm) with T2 in normal conditions, plant height was 11.67 cm in T1 application and 11.71 cm in T2 application under salt stress, which was higher than control plants in the same conditions (Figure 1). There was a decrease in stem diameter with salinity, it was 2.07 mm in the control plant and was determined as 1.51 mm under the effect of NaCl. Under normal conditions, the highest stem diameter was determined as 2.20 mm in the T1 application. Under saline conditions, the highest stem diameter was 2.00 mm in the T2 application (Figure 1). In the study, it was determined that the number of leaves decreased with salinity and the lowest leaf number was 4.25 in the control plants in saline conditions. There was an increase in the number of leaves in both T1 and T2 applications in saline conditions (Figure 1). Iodine applications caused an increase in leaf area both in normal and salinity conditions. In normal conditions, the lowest leaf area was in the control plant with 36.97 cm². The highest leaf area was in the T1 application with 49.81 cm². In salinity, the lowest leaf area was in the control plant with 16.32 cm² and the highest leaf area was in the T2 application with 25.94 cm² (Figure 1).

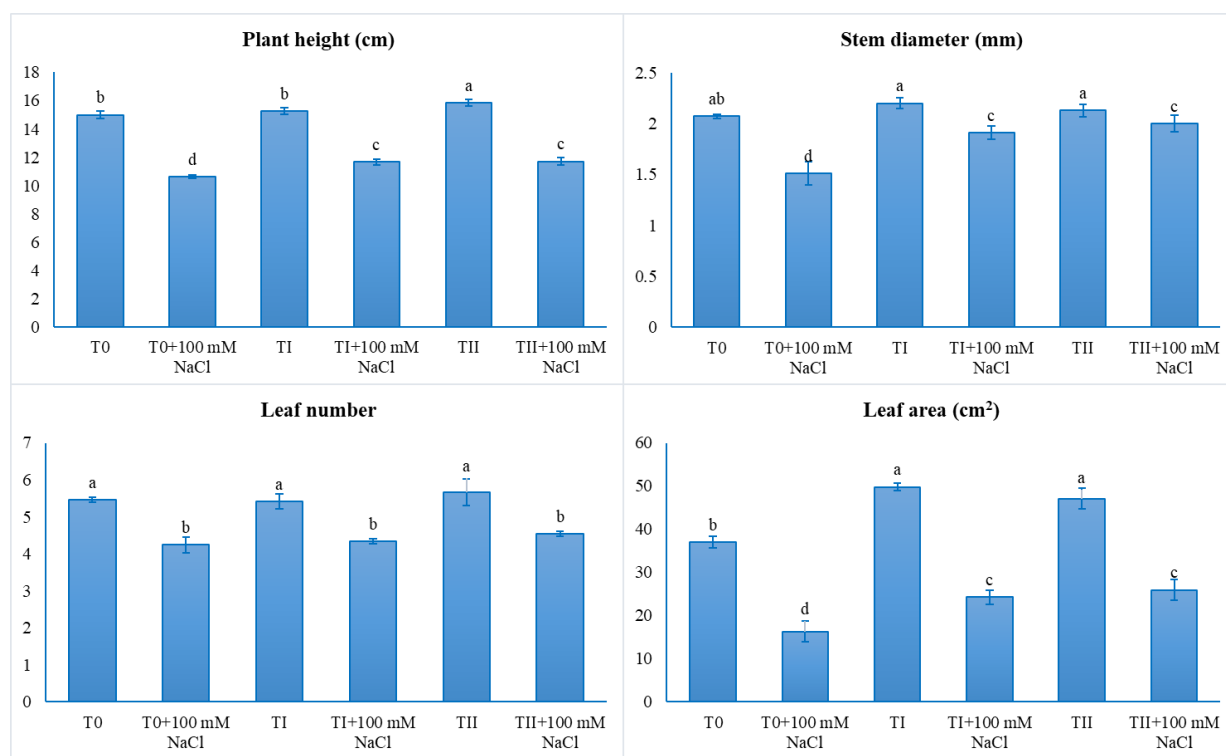


Figure 1. The effect of application on plant height, stem diameter, leaf number and leaf area of plant under salinity. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

EC value increased with salinity. In saline conditions, EC value in treatment plants decreased compared to EC value in control plants (Figure 2). The highest LRWC was 78.67 in normal conditions in the T1 application. The lowest LRWC was 56.67 in the control plant in saline conditions. The iodine

applications increased the LRWC value both in normal conditions and in salinity. While the highest LRWC value was 78.67 in normal conditions in the T1 application, the highest LRWC value in salinity was 59.71 in T2 (Figure 2).

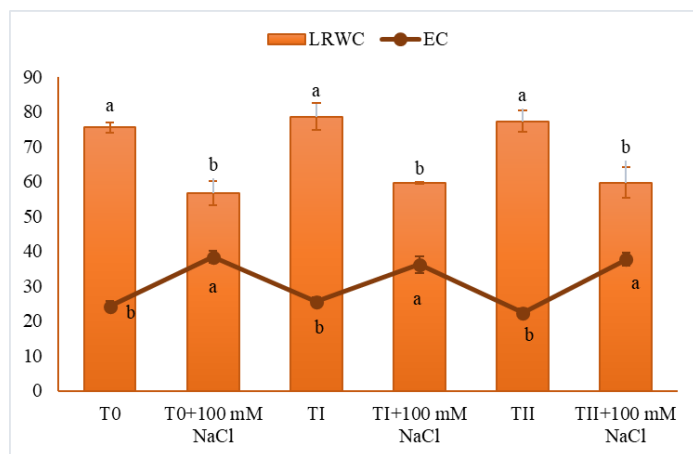


Figure 2. The effect of application on LRWC and EC content of plant under salinity. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

Plant fresh weight decreased in saline conditions. Iodine applications increased plant fresh weight in both normal and

saline conditions. The highest plant fresh weight was in T2 application with 2.17 g in normal conditions. The lowest plant fresh weight was seen in the control plant in saline conditions. T1 and T2 applications increased plant fresh weight in saline conditions (Figure 3). Under salt stress, there was a decrease in the root fresh weight of the plants. On the other hand, both salinity and iodine application under normal conditions increased the root fresh weight. The highest root fresh weight was 0.123 g in T2 application under normal conditions, while the lowest was in the control plant under salinity (Figure 3). Plant dry weight decreased under salt stress. The highest plant dry weight was 0.19 g in T2 treatment under normal conditions. The lowest plant dry weight was 0.08 g in control plant under saline conditions (Figure 3). The lowest root dry weight was 0.008 g in the control plant under saline conditions. The highest root dry weight was in the T2 application under normal conditions. Similarly, the highest root dry weight was in the T2 application under saline conditions (Figure 3).

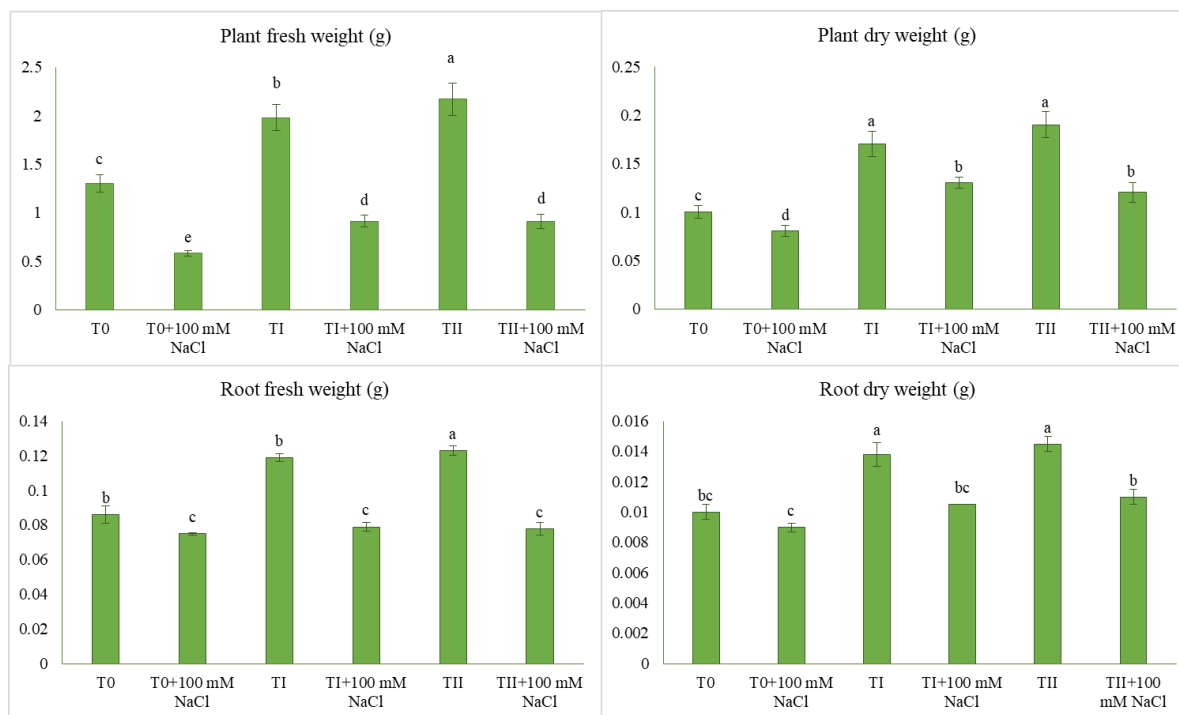


Figure 3. The effect of application on plant and root fresh and dry weight of plant under salinity. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

The highest chlorophyll a value was 5.57 mg/g in T1 application under normal conditions. The lowest was 4.90 mg/g in control plant under normal conditions. It was observed that applications caused an increase in chlorophyll a under both normal and saline conditions (Figure 4). The lowest chlorophyll b was 2.65 mg/g in the control plant under normal conditions. The highest was 4.30 mg/g in the T1 application under saline

conditions. Iodine applications provided an increase compared to control plants (Figure 4). The lowest total chlorophyll value was 7.57 mg/g in the control plant under normal conditions, while the highest total chlorophyll value was 9.78 mg/g in the T1 application under saline conditions. Applications had a positive effect on chlorophyll in both conditions and increased it (Figure 4).

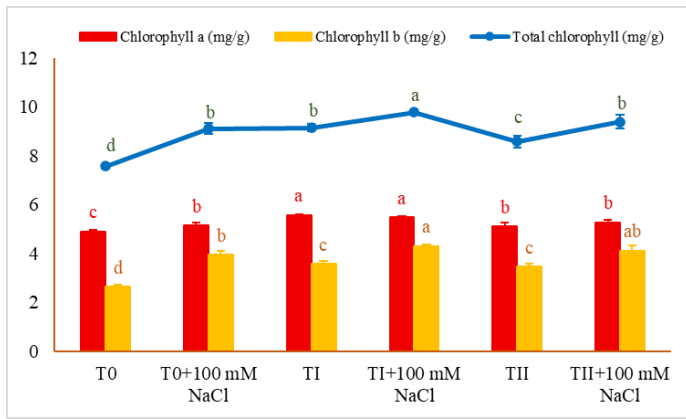


Figure 4. The effect of application on chlorophyll content of plant under salinity. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

An increase in H_2O_2 was observed under salinity stress compared to normal conditions. The highest value was 95.52 mmol/kg in T2 application under saline conditions. The lowest value was 8.28 mmol/kg in the control plant under normal conditions. According to MDA values, there was a significant increase in salinity compared to normal conditions. The highest MDA value under salt stress was 92.11 in the T2 application (Figure 5). Proline increased in saline conditions, the highest proline value was in the T2 application (0.70 mmol/kg) in saline conditions. The lowest proline value was determined as 0.06 mmol/kg in the control plant under normal conditions. Iodine applications increased the proline value in all conditions. A significant increase in sucrose value was observed in salty conditions compared to normal conditions. The highest sucrose value was 16.44% in the T2 application in salty conditions. The other value following this value was 6.18% in the T1 application in salty conditions (Figure 5).

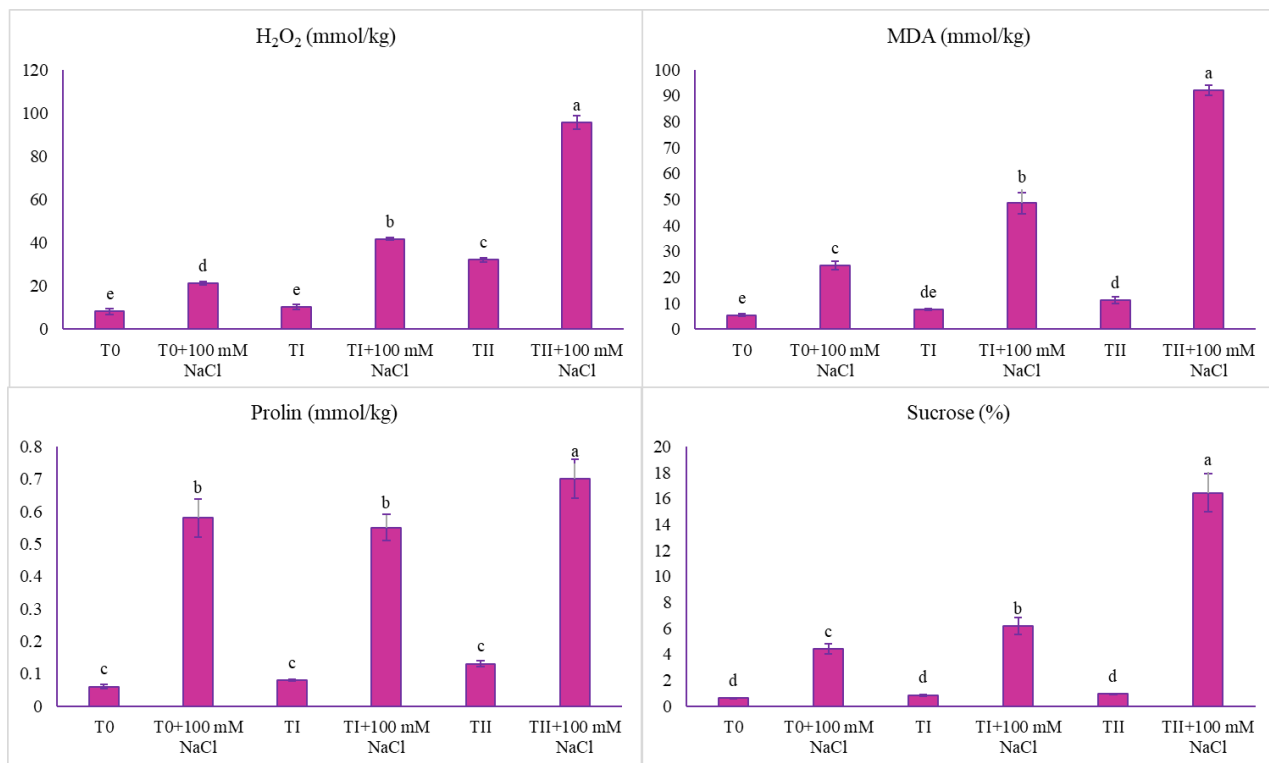


Figure 5. The effect of application on H_2O_2 , MDA, proline and sucrose content of plant under salinity. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

CAT increased under salty conditions compared to normal conditions. The highest CAT value was 541.63 eu/g in the T2 application under salty conditions. In the study, it was observed that the POD value increased under salt stress. Similarly, there were increases in the T1 and T2 applications. The highest POD value was 10538.72 eu/g in the T2 application under salty

conditions. The SOD value, which increased under stress, was also observed to increase as a result of the study. The SOD value, which was 379.74 eu/g in the control plant under normal conditions, was 555.11 eu/g in the T1 application and 1316.39 eu/g in the T2 application (Figure 6).

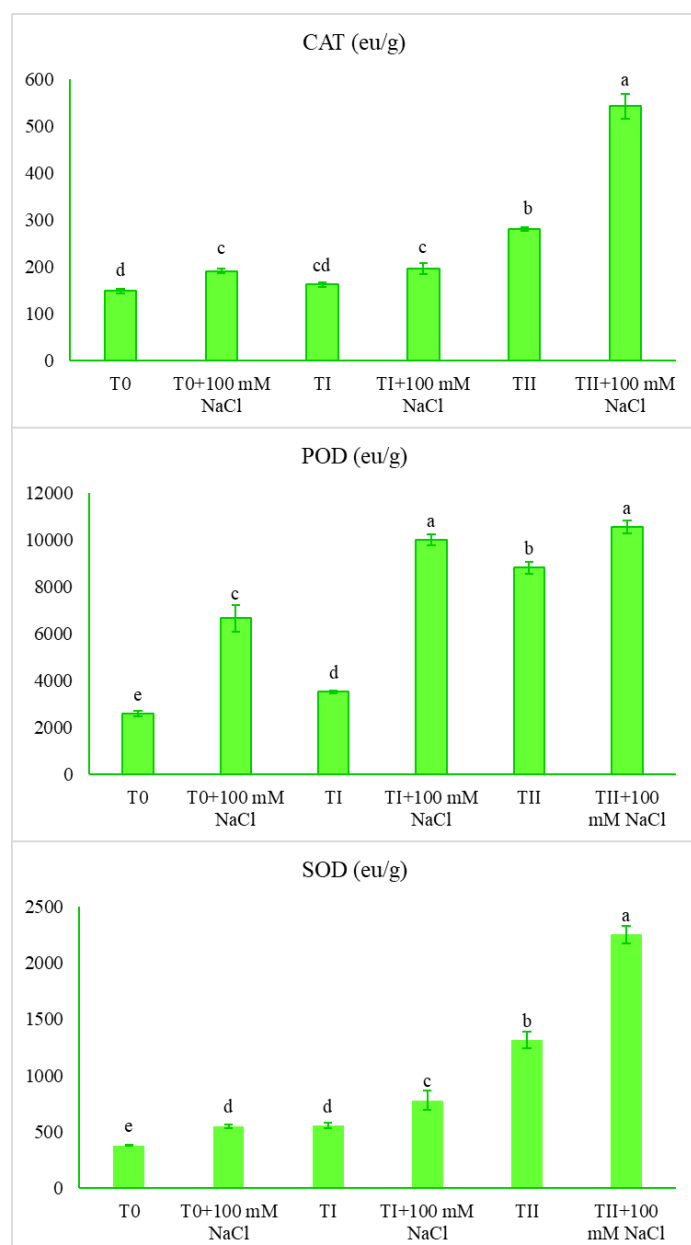


Figure 6. The effect of application on antioxidant enzyme activity of plant under salinity. There is no statistical difference between means indicated with the same letter ($p < 0.001$).

4. Discussion

In the cultivation of agricultural products, adverse environmental conditions significantly reduce production and quality. Salinity is one of them which is a serious problem worldwide and researchers are working on it intensively. Increasing stress tolerance or reducing damage, especially with exogenous applications, is an important issue. Biostimulant application and biofortification is one of them. It can be used both to support plant growth in production and to increase defense against stress. Iodine, which was used in this study, has such a feature. In the study, salt stress significantly reduced plant growth and development in rocket. Iodine application alleviated this damage and supported growth in rocket against salt stress. In rocket, plant height, stem diameter, leaf number

and leaf area decreased by 29%, 27%, 22% and 56%, respectively, under salt stress, while iodine applications reduced this decrease. In a study conducted on beans, it was observed that salinity caused a significant decrease in growth parameters (Çirka et al., 2022). The osmotic effect caused by salt stress prevents the absorption of water by the plant, negatively affecting cell metabolism, biological activities, photosynthesis and other important activities such as respiration. This situation is the most important reason for the decrease in plant development (Hniličková et al., 2017; Munns, 2002; Mushtaq et al., 2020).

EC increased with salinity, while leaf relative water content decreased in rocket. This effect of salt was low in iodine application. Plant fresh weight, root fresh weight, plant dry weight and root dry weight decreased by 55%, 13%, 20% and 10% in the control group with salinity, and iodine application mitigated this decrease. Contrary to the decrease, root fresh and dry weight of plants, especially those under salt stress, increased with iodine application. However, chlorophyll increased with salt stress and iodine application. Reduction of leaf area due to salinity may be an important factor in this increase. Similarly, in another study, it was determined that the fresh and dry weight of leaves and roots decreased with increasing salinity in rocket (Jesus et al., 2015). Shariatnia et al. (2021) found that increasing salinity decreased relative water content, leaf chlorophyll content and leaf area, and increased electrolyte leakage of rocket. The decrease in leaf area due to salt stress is a result of the decrease in leaf turgor pressure, the decrease in water amounts due to osmotic effect, and the inability to transfer nutrients and hormones taken from the roots to other parts of the plant (Acosta-Motos et al., 2017; Hu & Schmidhalter, 2004). While the decreases in cell division and elongation that begin with salinity cause slower leaf formation and size, visual damage begins to the plant due to increased salt stress and plant development is completely affected in the later stages (Läuchli & Grattan, 2007). Shalaby (2025) stated that low iodine levels can increase the plant's resistance to adverse conditions such as salt stress, while high levels can be harmful and even cause reduced growth and yield. Similarly, it was determined that IO_3^- (20-80 μM) application to lettuce plants under salinity stress (100 mM NaCl) increased plant biomass, decreased Na^+ and Cl^- concentrations, and increased the activity of antioxidant enzymes. Due to these effects, the researchers stated that IO_3^- was effective without reducing the harmful effects of salinity stress (Leyva et al., 2011). In one study, it was determined that exogenously applied KIO_3 (0, 50 and 100 μM) improved the development, yield and fruit characteristics of pepper plants exposed to salt. In particular, 100 μM KIO_3 was determined to reduce the electrical conductivity of fruit juice under saline conditions and increase the amount of leaf relative water, thus it may be effective in increasing the stress tolerance of plants (Akinoğlu et al., 2025).

H₂O₂, MDA, proline and sucrose, which increased with salinity, showed a greater increase with iodine. This suggests that the osmotic effect on the plant, which increased with salt stress, was tried to be balanced with iodine application. It is suggested that iodine protects cell membranes under stress conditions by acting as a free radical scavenger to prevent membrane lipid peroxidation (Dey & Mukherjee, 1984). Similarly, CAT, POD and SOD enzyme activities increased with salt stress, and this increase was higher in iodine applications. In this way, it was observed that it had an effect on ROS formation. Shariatnia et al. (2021) determined that CAT, SOD, guaiacole peroxidase and APX activities increased in rocket with 6 or 12 dS/m salinity. Similarly, it was shown that the increase in CAT, ascorbate peroxidase (APX) and polyphenoloxidase (PPO) enzymatic activities in rocket activated the antioxidative defense system in maintaining the growth of rocket genotypes under increasing salinity (Jesus et al., 2015). Similar to our study, Leyva et al. (2011) determined that iodine application in lettuce under salt stress increased soluble sugar and antioxidant enzyme activities. It has been stated that this increase with iodine varies depending on the dose, with an increase at low doses but not at high doses (Shalaby, 2025). The dose used in our study was effective in these. Iodine applied exogenously to the plant is absorbed and accumulated in various plant parts and photosynthesis, reductase activity, antioxidant level, various biochemical and physiological activities are positively affected. In adverse conditions, iodine also balances tolerance by triggering enzymatic and non-enzymatic antioxidants that clean uncontrolled ROS production (Riyazuddin et al., 2023). It is stated that iodine species can control stress and defense-related gene expression by triggering the transcription of various photosynthesis-related genes, genes involved in salicylic acid (SA) metabolism, and several stress and defense-related genes (Kiferle et al., 2021; Riyazuddin et al., 2023). Exogenous iodine application is stated to be a good biostimulant because it increases redox metabolism, improves antioxidant capacity, creates synergy with essential minerals and increases tolerance to adverse factors (Fuentes et al., 2022). According to the studies, it has been stated that commercial use of iodine in agriculture and application of the element may be an alternative approach to reduce damage caused by biotic and abiotic stresses and to promote plant growth (Medrano-Macías et al., 2016).

5. Conclusion

The aim of the study was to reduce the harmful effects of salt stress on rocket with iodine application. Iodine, which is a good biofortification product, supports plant development and is also effective in salt stress tolerance. Previous studies have shown that the effectiveness of iodine application varies and that the dose should be selected well. Depending on the plant species and the severity of stress, the effective dose of iodine will be beneficial in plant production and will also provide

economic benefits. More research needs to be done in this sense.

Compliance with Ethical Standards

This study does not require ethical committee approval.

Acknowledgment

This study includes the results of Hande Dizkara's Master's Thesis.

Conflict of Interest

The authors declare no conflict of interest.

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