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## Impact of foliar applied rutin on some physiological and yield parameters of lettuce under different saline irrigation water stress

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### Abstract

Salinity is one of the greatest threats to global food security and is recognized as the largest source of abiotic stress in agricultural production. Rutin is a natural flavonoid found in advanced plants and has a stimulatory effect on the defense mechanism against abiotic and biotic stresses. This study conducted a pot experiment in a climate chamber to determine the effect of rutin flavonoid in lettuce plants under salinity stress. In the study, 4 saline water doses (0, 1.2, 2.4, and 3.6 dS m<sup>-1</sup>) and control (Non-rutin) and 1 mmol L<sup>-1</sup> rutin treatment (Rutin) were applied. Leaf dry matter content, electrolyte leakage, and relative water content were analyzed. As a result of the study, it was determined that increasing saline irrigation stress significantly decreased dry matter and leaf relative water content and increased leaf electrolyte leakage. On the contrary, rutin application alleviated the stress by significantly reducing electrolyte leakage. Based on the study's results, it is concluded that rutin application can be used as a stress mitigant for the sustainable commercial cultivation of lettuce.

**Key Words:** *Electrolyte leakage, Relative water content, Rutin, Salinity stress*

### Introduction

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed leafy vegetables and has significant economic value, especially in the restaurant and food processing sectors (Kim et al., 2016). It is becoming increasingly popular, especially among younger generations worldwide, where it is consumed as a significant ingredient in ready-to-eat meals. However, like other vegetable crops, lettuce production faces significant challenges due to salinity stress (Cakmakci et al., 2022; Gruda et al., 2024). Increasing salinity levels in soil and irrigation water worldwide significantly threaten agricultural production (Sahin et al., 2016). Increasing soil salinity, global warming, erosion of minerals, rising sea levels, and over-fertilization in agriculture contribute to the salinity problem. Also, decreasing arable land and agricultural productivity due to climate change threatens millions of people with poverty and under-nourishment (Pradhan et al., 2017).

In recent years, elicitors against stress factors have been widely recognized as a tested technological tool to improve quality and yield in agricultural production (Kazemi, 2014; Javanmardi and Akbari, 2016). Elicitors or biostimulants can be defined as substances that stimulate the defense mechanism in any plant, triggering or enhancing the biosynthesis of secondary compounds of nutraceutical value (Baenas et al., 2014; Gorni et al., 2021). Furthermore, biostimulants can improve plant growth by stimulating different physiological processes (Gorni et al., 2021). This comprehensive definition of stimulants includes both pathogen-derived substances (exogenous stimulants) and compounds released from plants by the action of the pathogen (endogenous stimulants) (Angelova et al., 2006). Rutin is an allelochemical of the flavonoid family, which is considered to be a plant-produced signaling molecule naturally found in developed plants, possessing antioxidant properties and stimulating the defense mechanism against biotic and abiotic stresses (Merewitz and Liu, 2019; Gorni et al., 2022). Rutin application promoted plant growth by reducing oxidative damage in quinoa and broad bean plants (Ismail et al., 2015). Rutin promoted plant growth by reducing oxidative damage in quinoa and broad bean plants (Ismail et al., 2015). Another study reported that rutin synthesis in grass promoted plant growth under heat stress and protected against stress (Merewitz and Liu, 2019). In addition, rutin is also known to play an important role in various physiological processes such as photosynthetic activity, energy transfer, UV protection, antibacterial activity, growth, and development in plants (Singh et al., 2017; Merewitz and Liu, 2019).

Based on the literature, this study hypothesized that rutin-treated plants under salinity stress conditions would increase plant growth and development and alleviate salinity stress damage. To test these hypotheses, the study aimed to determine the dry matter content, membrane damage index, and leaf proportional water content of rutin application under salinity stress.

### Materials and Methods

#### Material

The experiment was carried out in the climate chamber of the Department of Horticulture, Faculty of Agriculture, Van Yüzüncü Yıl University. The experiment used standard lettuce (*Lactuca sativa*) as plant material. The temperature and relative humidity inside the climate chamber were monitored using an automatic weather station



(HOBO, Campbell Scientific INC., USA). The average daily temperature was  $22\pm 2$  °C, and the relative humidity was  $65\pm 5$  %. Lettuce seeds were sown in a randomised experimental design with three replications, producing 24 pots with four plants in each replicate.

#### *Rutin and salinity treatments*

In the study, 2 rutin (R) applications (R0: control; R1: 1 mmol L<sup>-1</sup>) were applied. Rutin in powder formulation was applied by foliar spray for three consecutive days (20 days after seed planting). For Rutin solution, Rutin was weighed on a precision balance, dissolved in methanol (10 mL), and finally added to 1000 mL of 0.02% Tween solution prepared with distilled water. Leaves of control plants were sprayed with water only.

For salinity stress, NaCl was applied as a saline water source. Threshold values and yield loss levels of lettuce plants were taken into consideration while determining salt doses (Ayers and Westcot, 1976; 1985; 1989). In the study, water with 4 different irrigation water salinities (Sw-0: 0.60 dS m<sup>-1</sup>, control; Sw-1.2: 1.2 dS m<sup>-1</sup>; Sw-2.4: 2.4 dS m<sup>-1</sup>; Sw-3.6: 3.6 dS m<sup>-1</sup>) were used. The salinity of the irrigation water was prepared and applied before each irrigation. Salinity applications started when the plants reached 4-5 true leaf stage.

#### *Dry matter ratio (DM)*

Lettuce leaves were separated from the stems, weighed to obtain fresh weight, and recorded. Subsequently, the same leaf samples were dried in an oven at 65°C for 48 h, weighed again, and obtained. The dry weight ratio to fresh weight was calculated to determine the plants' dry matter content (DM).

#### *Electrolyte leakage (EL)*

Electrolyte leakage was calculated by measuring the outflow of electrolyte from leaf cells (Shi et al., 2006). Samples taken as discs (10 mm diameter) from each plant leaf before harvesting were placed in 50 ml tubes containing 30 ml de-ionised water for 24 hours at room temperature, and then the electrical conductivity of the water in the tube was measured, and the EC1 value was obtained. Then, the tubes containing the sample were kept in a water bath at 95 °C for 20 minutes, cooled to room temperature, and the electrical conductivity of the water in the tube was measured again, and the EC2 value was obtained. Using the EC1 and EC2 values found, the electrolyte leakage (MZI) will be determined with the help of the following Equation.

$$EL = (EC1 / EC2) \times 100$$

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

To determine leaf relative water content (LRWC), fresh weights (LFW) of leaf samples taken 10 mm in diameter before harvesting were weighed on a precision balance and recorded as LFW. Leaf turgor weights (LTW) were recorded by soaking the same samples in distilled water for 4 hours. Leaf dry weights (LDW) were determined by drying and weighing the samples in an oven at 65°C for about 48 hours. LRWC was calculated from the obtained weights using the following equation (Smart and Barss 1973, Ors et al., 2021):

$$LRWC (\%) = 100 \times (LFW - LDW) / (LTW - LDW)$$

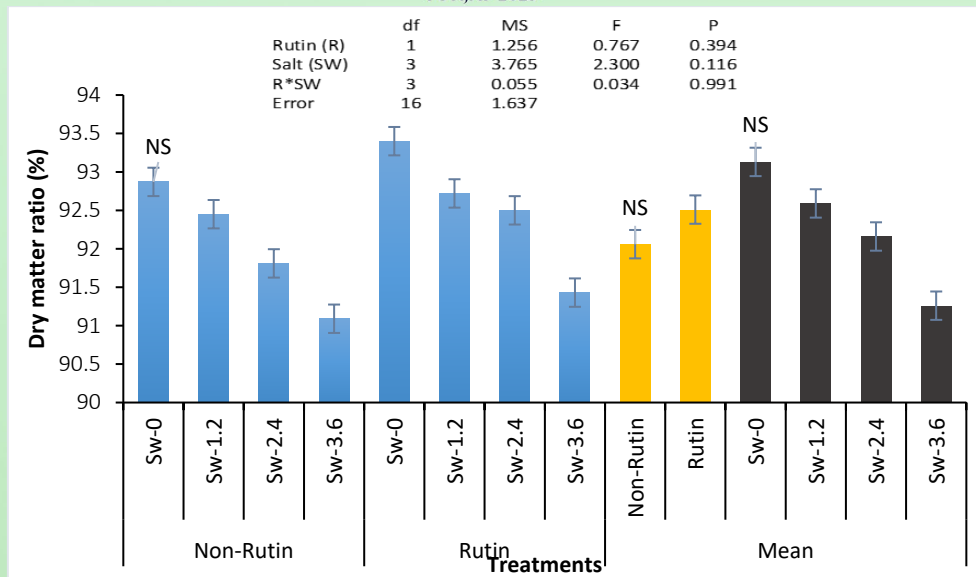
#### *Statistical Analysis*

All multivariate analyses were performed with IBM SPSS Statistics version 21.0. One-way analysis of variance (One-way ANOVA) was performed for data comparison, and Duncan's multiple comparison test (at 0.05 % probability) was used to determine the differences between groups. All values were presented as mean  $\pm$  standard deviation (SD).

### **Results and Discussion**

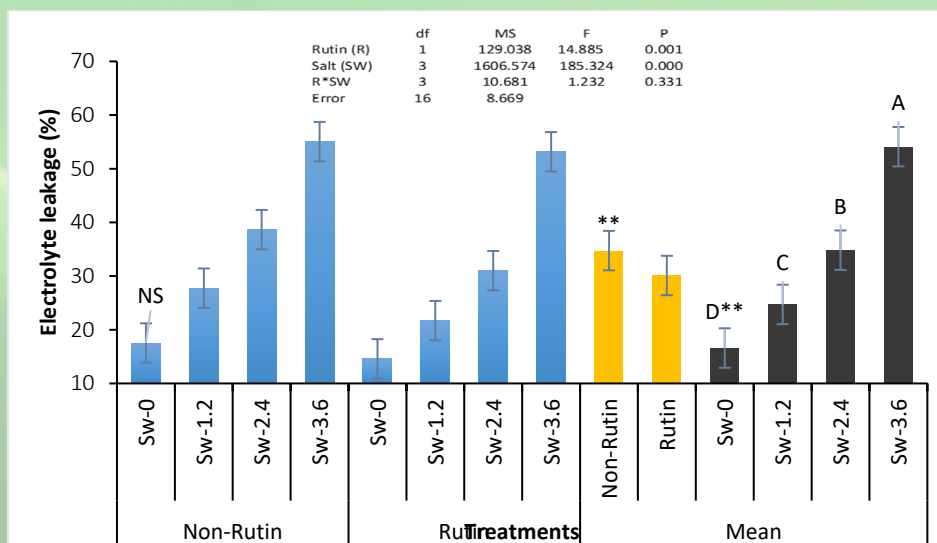
Rutin treatments caused an increase in lettuce leaf dry matter ratio, but this effect was not found to be significant statistically (Fig. 1). The rutin increased dry matter ratio by 0.5% compared to control plants. On the other hand, the salt stress affected DM negatively, but these differences were not statistically significant. Also, 3.6 dS m<sup>-1</sup> of saline water application decreased the dry matter ratio by 2%. No significant interaction was found between rutin and salinity treatments. The highest DM (93.4%) was found in control SW-0 and rutin-treated plants. Biostimulants play important roles in plant growth and development, and these properties can be associated with improving photosynthesis and carbohydrate accumulation in plants (Siddiqui et al., 2020; Gorni et al., 2020). An increase in leaf dry matter content was observed in lettuce plants treated with a 1 mmol L<sup>-1</sup> dose of rutin. In a previous study, rutin application increased growth and development parameters such as leaf number, leaf area, fruit, and shoot weight in tomato plants (Gorni et al., 2022). Another study reported that exogenous application of rutin decreased total reactive oxygen species and improved the growth of rice plants (Singh et al., 2017). These responses may be related to increased photosynthetic activity (Hussain and Reigosa, 2016; Singh et al., 2017) and interaction with hormones (Hussain and Reigosa, 2014) and may be associated with increased dry matter in lettuce plants.





**Figure 1.** Effect of rutin treatment to dry matter ratio on lettuce plant under salinity stress. Sw-0: 0.60 dS m<sup>-1</sup>, control; Sw-1.2: 1.2 dS m<sup>-1</sup>; Sw-2.4: 2.4 dS m<sup>-1</sup>; Sw-3.6: 3.6 dS m<sup>-1</sup>. R: Rutin Sw: Saline water. Different letters indicate significant differences between treatments at P<0.05 according to Duncan’s multiple range test. “NS” indicates not significant.

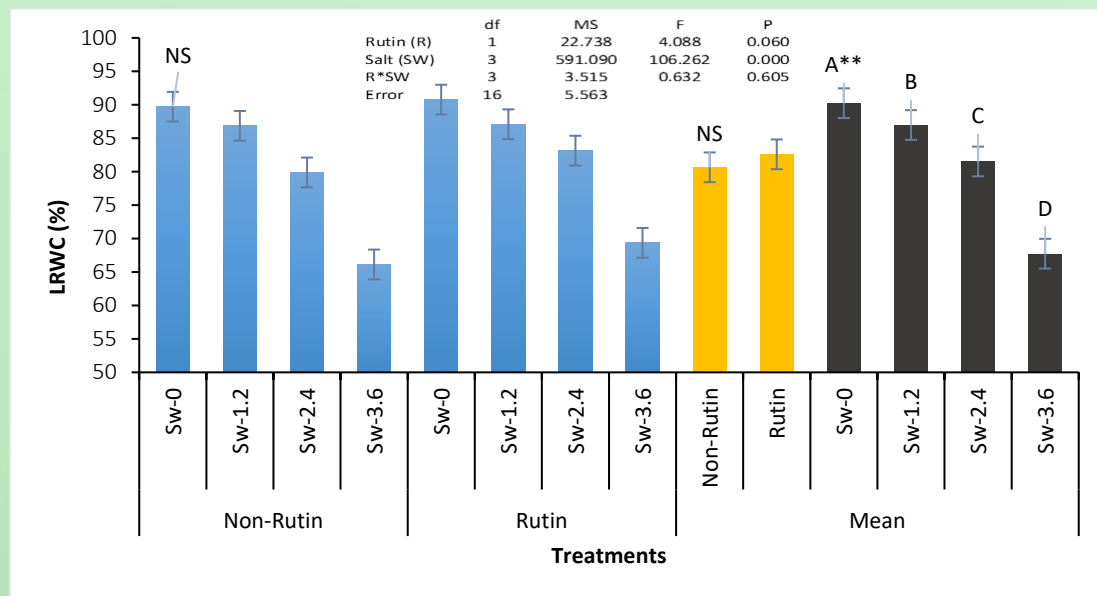
Electrolyte leakage (EL) value caused by oxidative damage caused by salinity stress was significantly decreased by rutin treatments ( $p < 0.01$ ) (Fig 2). Rutin application reduced the EL value by approximately 15% compared to the control. On the contrary, the EL value increased progressively with increasing saline irrigation applications. To clarify, Sw-1.2, Sw-2.4, and Sw-3.6 salinity stress treatments increased the EL value of lettuce leaves by approximately 1.5, 2, and 3-fold, respectively, compared to the control. Also, the interaction between rutin and saline irrigation treatments was not significant. In previous studies, applying rutin to bean leaves improved tissue tolerance, reduced the harmful effects of salinity on leaf photochemistry, and activated the antioxidant defense mechanism (Ismail et al., 2015). Another study demonstrated that exogenous application of rutin in corn seedlings alleviated the harmful effects of salinity stress (Muslu, 2024). These beneficial effects of rutin are attributed to improved potassium uptake and increased Na<sup>+</sup> pumping rate from cells (Ismail et al., 2015). Additionally, rutin, a natural flavonoid, enhances antioxidant resistance by promoting the binding of free radicals. In this study, EL induced by salt stress was significantly reduced with rutin application, suggesting that stress was alleviated in lettuce leaves.



**Figure 2.** Effect of rutin treatment to electrolyte leakage on lettuce plant under salinity stress. Sw-0: 0.60 dS m<sup>-1</sup>, control; Sw-1.2: 1.2 dS m<sup>-1</sup>; Sw-2.4: 2.4 dS m<sup>-1</sup>; Sw-3.6: 3.6 dS m<sup>-1</sup>. R: Rutin Sw: Saline water. Different letters indicate significant differences between treatments at P<0.05 according to Duncan’s multiple range test. “ns” indicates not significant.



Increasing doses of salinity treatments negatively affected leaf relative water content (LRWC) in lettuce leaves (Fig. 3). Increasing doses of salinity treatments negatively affected leaf relative water content (LRWC) in lettuce leaves. Salinity treatments at doses of 1.2 dS m<sup>-1</sup>, 2.4 dS m<sup>-1</sup>, and 3.6 dS m<sup>-1</sup> decreased LRWC by about 4%, 11%, and 30%, respectively, compared to the control. On the other hand, rutin treatment increased LRWC by about 2% compared to the control, but this effect was not statistically significant. Furthermore, no significant interaction was found between rutin and saline irrigation treatments. LRWC is a valuable measure of the physiological water status of plants (Gonzalez and Gonzalez-Vilar, 2001). In this study, salt stress significantly reduced the LRWC of lettuce. Previous studies have demonstrated decreased LRWC values in lettuce under salt stress (Ekinici et al., 2012; Yildirim et al., 2015). As observed in the present study, the increase in LRWC is known as osmotic adjustment. Osmotic adjustment is one of the factors that can increase salt tolerance (Ashraf 2004; Raza et al. 2006).



**Figure 3.** Effect of rutin treatment to leaf relative water content (LRWC) on lettuce plant under salinity stress. Sw-0: 0.60 dS m<sup>-1</sup>, control; Sw-1.2: 1.2 dS m<sup>-1</sup>; Sw-2.4: 2.4 dS m<sup>-1</sup>; Sw-3.6: 3.6 dS m<sup>-1</sup>. R: Rutin Sw: Saline water. Different letters indicate significant differences between treatments at P<0.05 according to Duncan's multiple range test. "ns" indicates not significant.

In summary, the present study concluded that the exogenous application of rutin had beneficial effects on lettuce plants and alleviated the damage caused by salinity stress. The data revealed that rutin promotes plant growth and increases the leaf dry matter content. Based on these results, the application of rutin in lettuce cultivation is recommended. Additionally, it is anticipated that rutin could be used as an alternative to biofertilizers to develop effective plant growth promoters and maximize their use under field conditions.

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