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Original Article

Melatonin mitigates salt stress effects on the growth and production aspects of radish[1]

Melatonina mitiga o estresse salino no crescimento e nos aspectos produtivos do rabanete

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HIGHLIGHTS:
Application of melatonin mitigates the deleterious effects of salt stress on the growth and production of radish plants.
Melatonin is a potential attenuator of moderate salt stress (2.75 dS m^{-1}) in radish plants.
Melatonin can be used to optimize growth and protect radish plants under saline conditions.

ABSTRACT: High concentration of salts in the soil impacts the availability of water and nutrients essential for plant growth and physiology. Therefore, management strategies that can mitigate the negative effects of salt stress are necessary, such as the use of plant hormones. Thus, this study aimed to evaluate the effect of salt stress and the application of melatonin on the growth and production aspects of radish plants. The experiment was carried out in a randomized block design, in a 3 × 3 factorial scheme, with four replications, considering one plant per experimental plot. The treatments consisted of three electrical conductivities of irrigation water - ECw (0.5, 2.75, and 5.0 dS m^{-1}) and three concentrations of melatonin (0, 0.5, and 1.0 mM). Growth variables were evaluated, namely plant height, stem diameter, number of leaves, leaf area, dry mass of leaves, petioles and roots, specific leaf area, leaf area ratio, and leaf weight ratio. In addition, production aspects were analyzed, namely bulb length, bulb diameter, and bulb fresh weight. Exogenous application of melatonin reduces the deleterious effects of salt stress on the growth and production aspects of radish plants. Application of melatonin at a dose of 0.5 mM is the most recommended to mitigate the effects of salt stress up to the level of 2.75 dS m^{-1}. The use of melatonin opens new perspectives for the development of management strategies aimed at optimizing growth and protecting the radish crop under saline conditions.

Key words: *Raphanus sativus* L., Brassicaceae, phytohormones, abiotic stresses, salinity

RESUMO: A alta concentração de sais no solo provoca impacto na disponibilidade de água e nutrientes essenciais para o crescimento e fisiologia das plantas. Assim, são necessárias estratégias de manejo que possam mitigar os efeitos negativos do estresse salino, como por exemplo o uso de hormônios vegetais. Assim, este estudo teve como objetivo avaliar o efeito do estresse salino e aplicação de melatonina sobre crescimento e aspectos produtivos de plantas de rabanete. O experimento foi realizado em delineamento em blocos casualizados, em esquema fatorial 3 × 3, com quatro repetições, sendo considerada uma planta por parcela experimental. Os tratamentos foram compostos por três condutividades elétricas da água de irrigação - CEa (0,5; 2,75 e 5,0 dS m^{-1}) e três concentrações de melatonina (0; 0,5 e 1,0 mM). Foram avaliadas a altura de plantas, diâmetro do caule, número de folhas, área foliar, massa seca de folhas, pecíolos e raízes, área foliar específica, razão de área foliar e razão de peso foliar. Além disso, foram analisados aspectos produtivios, como comprimento, diâmetro e peso fresco do bulbo. A aplicação exógena de melatonina reduz os efeitos deletérios do estresse salino no crescimento e aspectos produtivos em plantas de rabanete. A aplicação de melatonina na dose de 0,5 mM é a mais indicada para mitigar os efeitos do estresse salino até o nível de 2,5 dS m^{-1}. O uso da melatonina abre novas perspectivas para o desenvolvimento de estratégias de manejo destinadas a otimizar o crescimento e proteger a cultura do rabanete em condições de salinidade.

Palavras-chave: *Raphanus sativus* L., Brassicaceae, fitormônios, estresses abióticos, salinidade

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Introduction

Climate change represents one of the most concerning phenomena within the environmental context, due to its influence on agricultural systems (Ahmad et al., 2021a). The rise in the global average temperature and variations in precipitation patterns are correlated with an increase in soil salinity (Eswar et al., 2021). The high concentration of salts in water sources intended for irrigation represents a significant challenge for crops, negatively affecting plant growth, soil fertility, and environmental sustainability (Phogat et al., 2020). Therefore, it becomes imperative to develop salinity management strategies that can enhance plant tolerance and ensure food security in affected regions (Suhani et al., 2020).

Plant hormones have emerged as a strategy employed as mitigating agents for abiotic stresses (Bai et al., 2020). Melatonin (MT, N-acetyl-5-methoxytryptamine) is a multifunctional plant hormone that exerts influence on critical processes such as seed germination, growth and development, response to abiotic stress, and plant-microorganism interactions (Ahmad et al., 2021b). Studies indicate that the exogenous application of melatonin enhances salt stress tolerance in wheat (*Triticum aestivum* L.) (Talaat, 2021).

Radish (*Raphanus sativus* L. - Brassicaceae) is a widely cultivated vegetable and known for its high nutritional and antioxidant value (Tuver et al., 2022). It is cultivated in several countries around the world. Brazil produces approximately 9 thousand tons of radish yearly (IBGE, 2017). It is considered a moderately salt-sensitive crop, as exposure to saline conditions results in a reduction in photosynthetic capacity, growth, and yield (Garcia-Ibañez et al., 2022). Thus, this study aimed to evaluate the effect of salt stress and application of melatonin on the growth and production aspects of radish plants.

Material and Methods

The experiment was conducted from June to August 2023 in a greenhouse located at the Department of Agronomic and Forestry Sciences of the Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte, Brazil (5° 12′ 28″ S, 37° 19′ 04″ W, altitude 24 m). The region has a climate classified as BSh, characterized as dry and very hot, with a dry season and summer rainfall (Alvares et al., 2013). Daily mean data for temperature and relative humidity were collected at the experimental site using a digital thermohygrometer, and the data are presented in Figure 1.

The experiment was carried out using a randomized complete block design in a 3 × 3 factorial scheme with four replications, considering one plant per experimental plot. The treatments consisted of three levels of electrical conductivity of irrigation water (ECw: 0.5 – control, 2.75, and 5.0 dS m^{-1}) representing levels of salt stress (no stress, moderate stress and severe stress), prepared by adding sodium chloride (NaCl) in the local supply water, and three concentrations of melatonin (0, 0.5, and 1.0 mM).

The 'Crimson Gigante' radish variety (Topseed Garden®) was used. This variety is characterized by being early in

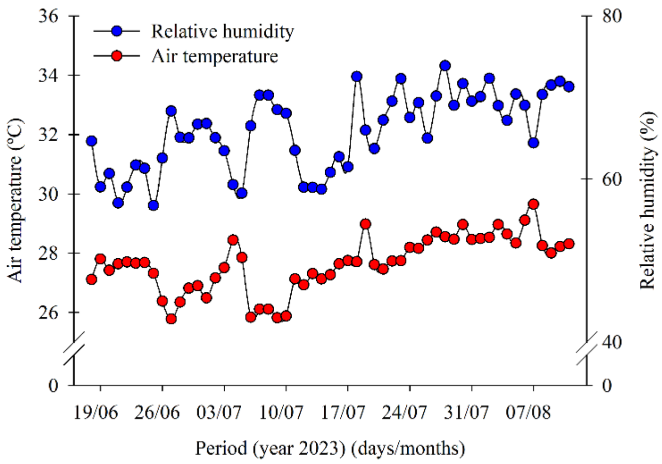


Figure 1. Mean air temperature and relative humidity of air during the experimental period (June to August 2023)

its cycle and having vigorous, medium-sized foliage, large roots, bright red external color, and white and firm pulp. It can be cultivated at temperatures ranging between 20 and 30 °C. Seeds of the 'Crimson Gigante' radish variety were initially sown in polyethylene trays containing 162 cells, which were filled with a commercial substrate (with an electrical conductivity of 0.50 ± 0.30, pH 6.00 ± 0.50, maximum moisture content of 58%, and density of 310 kg m^{-3}). Subsequently, the seedlings were transplanted at 6 days after planting (DAP) into polyethylene pots with a volumetric capacity of 5.0 dm^3. These pots were filled with a substrate mixture consisting of 3 kg of soil and 1 kg of cattle manure. The physicochemical characteristics of the substrate are detailed in Table 1.

Daily irrigation with water of varying electrical conductivities was initiated after the transplanting and establishment of seedlings in the pots, 8 days after planting. Saline waters were stored in 60 dm^3 plastic containers, with electrical conductivity monitored every 3 days to check each salt concentration. Water collection for analysis was performed on the day of preparation of saline solutions for irrigation. Chemical analysis was performed for each saline water solution (Table 2).

Table 1. Physical and chemical analysis of the substrate used in the experiment

Physical	Value	Fertility	Value
Coarse sand (g kg^{-1})	480	pH in water (1: 2.5)	7.11
Fine sand (g kg^{-1})	330	EC (dS m^{-1})	0.06
Silt (kg kg^{-1})	140	P (mg dm^{-3})	104.7
Clay (g kg^{-1})	050	K^+ (mg dm^{-3})	177.4
		Na^+ (mg dm^{-3})	16.4
Textural class	Sandy loam	Ca^{2+} ($cmol_c$ dm^{-3})	3.20
		Mg^{2+} ($cmol_c$ dm^{-3})	1.10
		Al^{3+} ($cmol_c$ dm^{-3})	0.00
		$H^+ + Al^{3+}$ ($cmol_c$ dm^{-3})	0.00
		SB ($cmol_c$ dm^{-3})	4.83
		t ($cmol_c$ dm^{-3})	4.83
		CEC ($cmol_c$ dm^{-3})	4.83
		V (%)	100
		m (%)	0
		ESP (%)	1

EC - Electrical conductivity of the soil:water extract, in the ratio 1:2.5; SB - Sum of exchangeable bases; t - Effective cation exchange capacity; CEC - Cation exchange capacity; V - Base saturation; m - Aluminum saturation; ESP - Exchangeable sodium percentage

Table 2. Chemical attributes of the saline solutions employed in the experiment

pH water	EC (dS m^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl	CO_3^{2-}	HCO_3^-	SARse (mmol L^{-1})$^{0.5}$	Hardness (mg L^{-1})	Cations	Anions
		(mmol$_c$ L^{-1})									(mmol$_c$ L^{-1})	
8.8	0.50	0.25	4.23	0.70	1.90	3.00	0.60	2.80	3.7	130	7.08	6.40
8.9	2.75	0.24	31.41	0.80	1.3	25.60	0.80	2.90	30.7	105	33.75	29.30
8.7	5.00	0.24	51.49	0.90	1.3	48.60	0.40	2.70	49.1	110	53.93	51.70

SARse - Sodium adsorption ratio

Irrigation for the experiment was conducted daily using the weighing lysimetry method, and the volume of each type of water evaporated or transpired over a 24-hour period was measured to maintain soil moisture at 80% of field capacity, as described by Girardi et al. (2016). During the experiment, manual weeding was carried out to control weeds.

To prepare the melatonin concentrations, distilled water was used along with the adhesive spreader Tween 80 (0.05%) to enhance plant absorption. The control (0 mM) was prepared using distilled water with the same concentration of Tween 80 (0.05%). Applications were carried out with manual sprayers every seven days over a span of 28 days (four applications). Melatonin application started on the first day of irrigation with saline waters, at 8 days after planting and 2 days after transplanting.

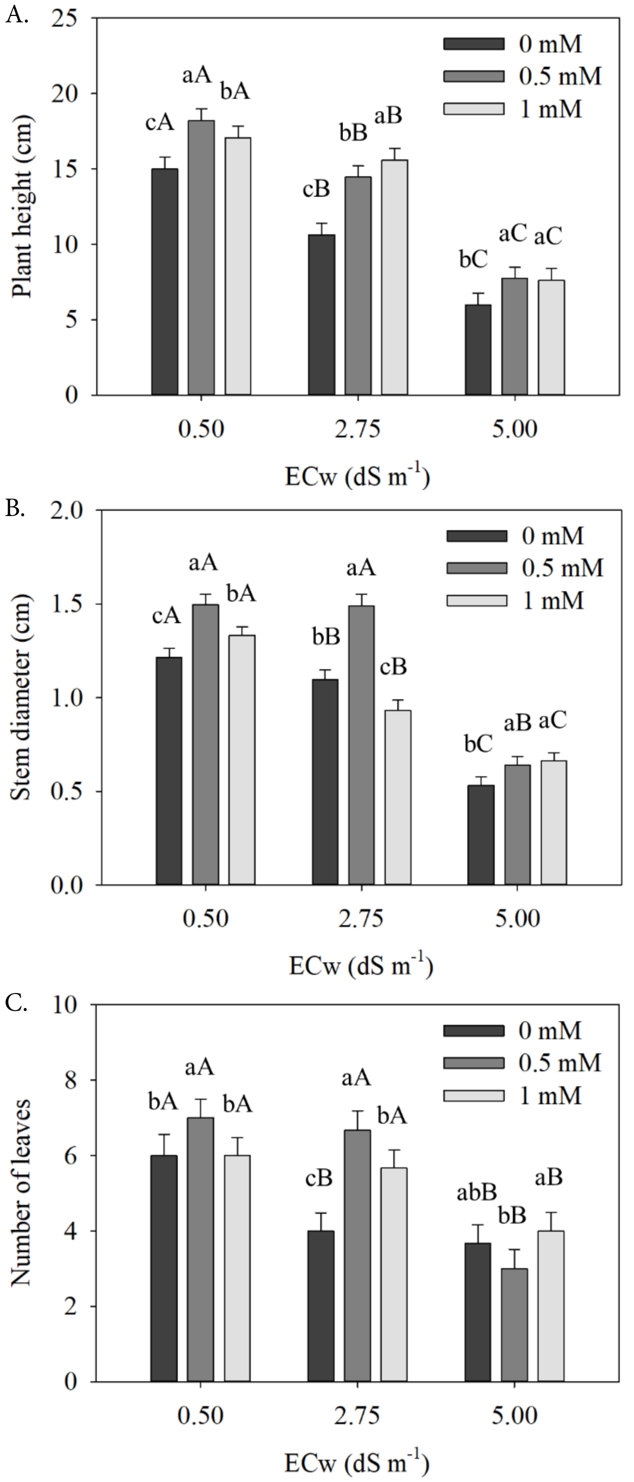
Data collection was conducted at 35 days after planting, which marked the start of treatments involving saline waters and melatonin. Measurements were taken for plant height (cm), stem diameter (cm), and number of leaves. Plant height was assessed using a graduated ruler, while stem diameter was measured three centimeters above the substrate level using a digital caliper. Leaf area (LA, cm^2) was calculated by multiplying the length and width (LW) dimensions of the leaves, following the equation proposed by Aminifard et al. (2019): LA = 0.847 (LW) + 29.39.

Harvested plants were separated into various parts for specific determinations, namely bulb length (cm), bulb diameter (cm), and fresh bulb weight (g), at 35 days after planting. Subsequently, leaves, petioles, and roots (bulbs) were placed on Kraft paper and subjected to drying in a forced-air circulation oven at 65 °C until a constant mass was achieved. Leaf dry mass (LDM), petiole dry mass (PDM), root dry mass (RDM), and total dry mass (TDM) were determined using a semi-analytical balance (0.01 g), with the results expressed as g per plant. Using the dry mass and leaf area data, specific leaf area (cm^2 g^{-1}), leaf area ratio (cm^2 g^{-1}), and leaf mass ratio (g g^{-1}) were calculated (Benincasa, 2003).

The data were subjected to analysis of variance (F-test, $p \leq 0.05$). In cases of significance, means were compared using the Tukey's test ($p \leq 0.05$). These analyses were performed using the statistical software R (R Core Team, 2023).

Results and Discussion

Salt stress had a negative impact on various aspects related to the growth and yield of 'Crimson Gigante' radish variety (Figures 2, 3, 4, and 5). Without melatonin application, there were significant reductions in plant height, stem diameter, and number of leaves as salt stress levels increased (Figure 2). When exposed to a salinity level of 2.75 dS m^{-1}, the plants



Means followed by the same lowercase letters do not differ for melatonin, and means followed by the same uppercase letters do not differ from each other for salt stress according to the Tukey's test ($p \leq 0.05$)

Figure 2. Plant height (A), stem diameter (B), and number of leaves (C) of 'Crimson Gigante' radish variety grown under different levels of electrical conductivity of irrigation water (ECw) and melatonin application (concentrations 0, 0.5, and 1 mM)

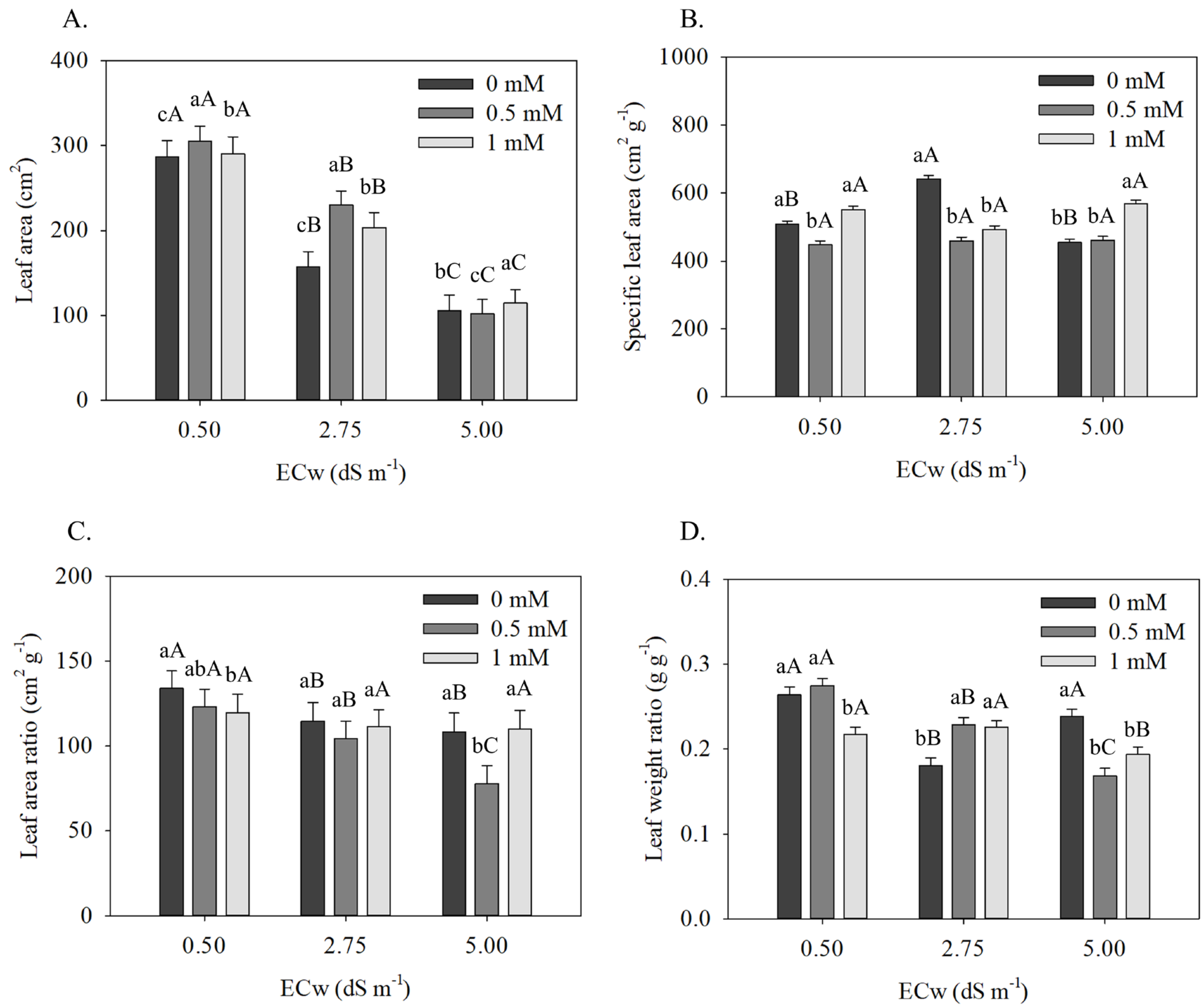
exhibited intermediate growth in plant height; however, the use of 0.5 or 1.0 mM melatonin mitigated the damage caused by salt stress, with increases of 26.5 and 31.9%, respectively (Figure 2A). Furthermore, increasing salinity led to decreases in stem diameter and number of leaves in *R. sativus* plants. The melatonin concentration of 0.5 mM proved to be the most effective in alleviating moderate salt stress (2.75 dS m^{-1}), resulting in similar values to those obtained with the control (0.5 dS m^{-1}) (Figures 2B and C).

Melatonin functions as a potent scavenger of free radicals and an antioxidant, contributing to the enhancement of antioxidant systems under salt stress conditions, thereby reducing the damage incurred in plant growth (Cen et al., 2020), as observed in the current study for plant height, stem diameter, and number of leaves. The exogenous application of melatonin has proven effective in mitigating the detrimental effects of salt stress on plant growth, making it a promising phytohormone for safeguarding the growth of cultivated plants under saline conditions (Bielach et al., 2017; Sarwar et al., 2018).

Leaf area, specific leaf area, leaf area ratio, and leaf mass ratio were adversely affected by increasing salt concentrations in the absence of melatonin application (Figure 3). The application of 0.5 mM melatonin mitigated the effects of salt stress at electrical conductivity of 2.75 dS m^{-1}, resulting in a 46% increase in leaf area compared to the treatment without melatonin application (Figure 3A). Specific leaf area was reduced at salt levels of 0.50 and 2.75 dS m^{-1} for the 0.5 mM concentration (Figure 3B). An increase in specific leaf area was observed only at salt levels of 0.50 dS m^{-1} for the 1 mM concentration, with an 8.5% increment, and at 5.00 dS m^{-1} for the 1 mM concentration, with a 25% increment (Figure 3B).

There was no significant difference in leaf area ratio concerning melatonin concentrations when plants were subjected to the salt level of 2.75 dS m^{-1} (Figure 3C). It was observed that, as salt levels increased, there was no reduction in the proportion of leaf area at a melatonin concentration of 1.0 mM (Figure 3C). At the salt level of 2.75 dS m^{-1}, the leaf mass ratio showed similar means for the treatments that received melatonin concentrations (0.5 and 1.0 mM), being higher (21 and 20%) than those found in the treatment without the application of this mitigating agent (Figure 3D).

The specific leaf area is related to the number and size of leaf mesophyll cells, being the ratio between the morphological



Means followed by the same lowercase letters do not differ for melatonin, and means followed by the same uppercase letters do not differ from each other for salt stress according to the Tukey's test ($p \leq 0.05$)

Figure 3. Leaf area (A), specific leaf area (B), leaf area ratio (C), and leaf weight ratio (D) of 'Crimson Gigante' radish variety grown under different levels of electrical conductivity of irrigation water (ECw) and melatonin application (concentrations 0, 0.5, and 1 mM)

(leaf surface) and anatomical (leaf dry mass) components of the leaves. The reduction in these variables related to leaf area and dry mass is attributed to decreased cellular activities such as photosynthesis and respiration. Consequently, the osmotic effect leads to salt accumulation in the soil, compromising the plant's absorption of water and nutrients, thereby diminishing cell growth and leaf surface (Al-Khafajy et al., 2020). Abeed et al. (2023) confirmed that salt stress reduced leaf area and the dry mass of both shoot and root in radish genotypes, which supports the findings of the current study, as specific leaf area, leaf area ratio, and leaf mass ratio rely on these variables for measurement.

The melatonin concentration of 0.5 mM increased leaf dry mass by 51% at the salt level of 2.75 dS m^{-1}, compared to the treatment without melatonin application (Figure 4A). Melatonin concentration of 0.5 mM increased petiole dry mass at salt level of 2.75 dS m^{-1} by 28% compared to the treatment without melatonin application (Figure 4B).

Melatonin concentrations of 0.5 and 1 mM mitigated salt stress at all salt levels; however, at salt levels of 2.75 and 5.00 dS m^{-1}, the 0.5 mM concentration of the mitigating agent resulted in greater increases in root dry mass, with increments of 55 and 49%, respectively (Figure 4C). The 0.5 mM concentration led to a 16, 63, and 34% increase in total dry mass at the three ECw levels, respectively, compared to the treatment that did not receive melatonin (Figure 4D).

The results of this study demonstrated that the increases in leaf, petiole, root, and total dry mass were a result of melatonin induction, which mitigated the negative effects of salt stress. Li et al. (2019) noted that melatonin treatments lead to a reduction in the effects of salinity on photosynthesis and dry mass, as melatonin plays a significant role in photosynthesis and photoprotection, which could explain the gain in radish dry mass. Melatonin also possesses antioxidant properties, aiding in the removal of reactive oxygen species (ROS), thus reducing oxidative stress (Zhan et al., 2019). This further emphasizes the alleviation of salt stress in the studied crop when melatonin is added.

Bulb length, bulb diameter, and bulb fresh weight were negatively affected by increasing salt levels (Figures 5 and 6). At the salt level of 2.75 dS m^{-1}, the application of 0.5 mM melatonin proved to be the most effective, resulting in a 41% increase compared to the treatment without melatonin application (Figure 5A). Bulb diameter decreased with rising salinity, but the application of 0.5 mM melatonin promoted the best results

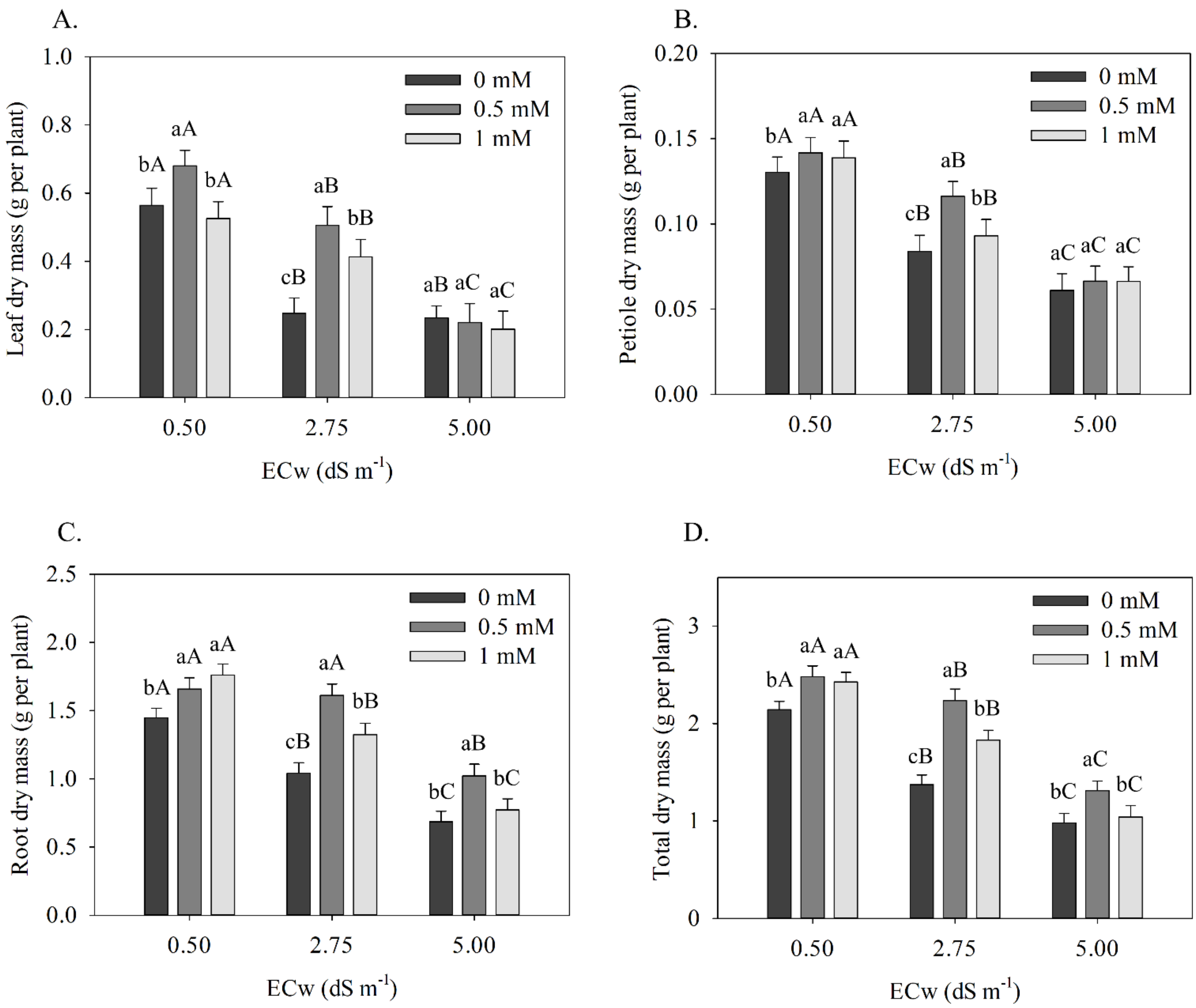


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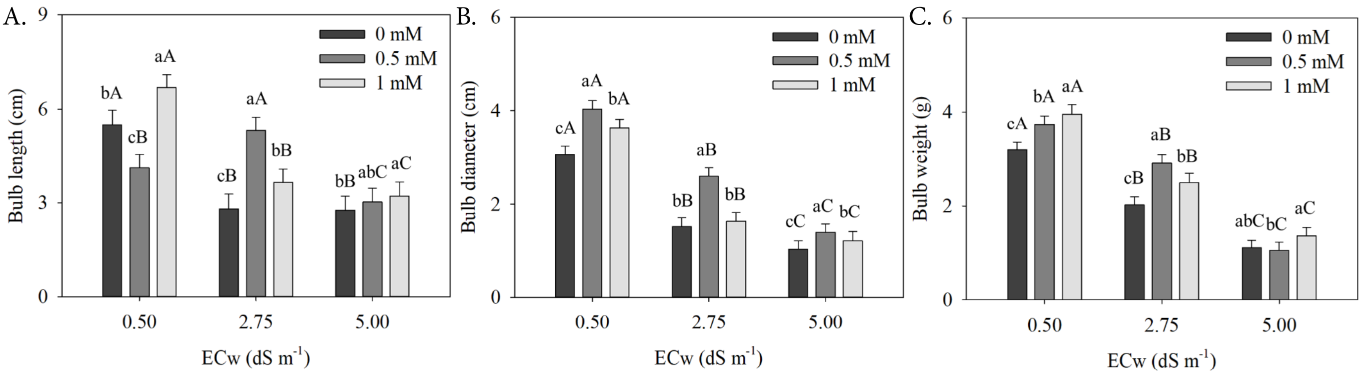
Figure 4. Leaf dry mass (A), petiole dry mass (B), root dry mass (C), and total dry mass (D) of 'Crimson Gigante' radish variety grown under different levels of electrical conductivity of irrigation water (ECw) and melatonin application (concentrations 0, 0.5, and 1 mM)



Means followed by the same lowercase letters do not differ for melatonin, and means followed by the same uppercase letters do not differ from each other for salt stress according to the Tukey's test ($p \leq 0.05$)

Figure 5. Bulb length (A), bulb diameter (B), and fresh bulb weight (C) of 'Crimson Gigante' radish variety grown under different levels of electrical conductivity of irrigation water (ECw) and melatonin application (concentrations 0, 0.5, and 1 mM)

Figure 6. 'Crimson Gigante' radish variety grown under different levels of electrical conductivity of irrigation water (ECw) and melatonin application (concentrations 0, 0.5, and 1 mM)

in mitigating the effects of salt stress, with a 41% increment at salt level of 2.75 dS m^{-1} (Figure 5B). The treatments subjected to melatonin application yielded higher fresh bulb weight, with the 0.5 mM concentration being the most effective in mitigating moderate salt stress (2.75 dS m^{-1}), resulting in a 30.6% increase compared to the treatment without application (Figure 5C).

The high salt concentration in irrigation water induces a series of morphological and physiological changes in plants, impairing root growth and development (Zou et al., 2022). Elevated sodium levels in roots reduce cell division rates and cell elongation, negatively impacting the absorption and transport of essential nutrients such as calcium and magnesium, which are crucial for root development (Abeed et al., 2023). On the other hand, the use of melatonin increases potassium content in leaves, acting as a plant growth regulator, similar to auxins, as they share the same biosynthetic precursor (Jiang et al., 2016). This is essential for mitigating the effects of salt stress on radish bulb development.

Conclusions

1. Exogenous application of melatonin reduces the deleterious effects of salt stress on the growth and production aspects of radish plants.

2. Application of melatonin at a dose of 0.5 mM is the most recommended to mitigate the effects of salt stress up to the level of 2.75 dS m^{-1}.

3. The use of melatonin opens new perspectives for the development of management strategies aimed at optimizing growth and protecting the radish crop under saline conditions.

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