



Mitigating Salinity Stress in Tomato (*Solanum lycopersicum* L.) through Foliar Application of Melatonin and Boron

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Abstract

Growing tomatoes is affected by a wide range of environmental conditions, including salt stress. A factorial experiment was conducted to evaluate the efficacy of melatonin and boron in mitigating the adverse effects caused by salinity stress on tomato (*Solanum lycopersicum* L.) physiology and fruit quality. The experiment consisted of applying three levels of melatonin (0, 200, and 400 mg L⁻¹) and three levels of boron (0, 5, and 10 mg L⁻¹) to tomato plants. The results demonstrated that melatonin and boron mitigated several damaging effects of salt stress, including a reduction in chlorophyll content, antioxidant activity, lycopene concentration, and reduced sugar levels. Exogenous application of melatonin and boron improved all traits negatively impacted by salt stress. Melatonin at 400 mg L⁻¹ markedly enhanced chlorophyll synthesis, antioxidant enzyme activity, and elevated lycopene and sugar contents. Similarly, boron at 5 mg L⁻¹ has been shown to promote chlorophyll stability, sugar transport, and enhanced nutrient uptake. Across all salinity levels, the combined application of melatonin and boron produced superior responses compared with individual treatments. The optimal treatment was S0M2B1 (no salt stress, melatonin 400 mg L⁻¹, boron 5 mg L⁻¹), which showed a significant improvement in the overall fruit quality and physiological performance of the tomato plants over all other treatments. The results of this study indicate that through the coordinated use of melatonin and boron, it is possible to effectively mitigate the adverse effects of environmental salinity on tomato plant growth, yield, and quality.

Keywords: *Solanum lycopersicum*, Salinity stress, Melatonin, Boron, Antioxidant defense system, Lycopene.

1. Introduction

Soil salinization occurs due to the accumulation of salts as soils lose their water-holding capacity¹. This degradation leads to millions of acres of agricultural land becoming unusable for crops, with estimates that over 1.5 billion acres (over 10% of the Earth's surface) are already salt-influenced, and an additional 1 billion acres vulnerable to future salt formation from climate change and poor agricultural management practices. Na⁺ and Cl⁻ accumulate in plant root zones, causing osmotic stress, ion toxicity, and oxidative damage that impair water and nutrient uptake and hinder proper development. Damage to plant growth leads to yellow leaves, reduced growth rates, and reduced yields and quality of harvested produce^{2,3}.

Hormones (plant hormones) are important parts of the regulation of the growth and developmental processes of plants and their response to environmental stress⁴. One way to alleviate the negative impact of high salinity is to use plant growth regulators to enhance tomato plant resistance to combined salt stress. Absciscic acid, brassinolide, methyl jasmonate, and salicylic acid are all plant hormones and have been demonstrated to play an important part in the salt stress adaptation of plants⁵. Recently, melatonin has been identified as an important

indoleamine found throughout the plant continuum, which is directly related to important plant physiological processes, such as the germination of seeds, photosynthesis, root and leaf development, and the plant's ability to respond to environmental stress⁶. Because melatonin has a very high lipophilicity, it is able to pass easily through all biological membranes, including those of the chloroplasts, mitochondria, and cell membranes. Once it passes through the membranes, it will be able to reach sites where there is an increased presence of reactive oxygen species (ROS) very quickly, where it will act as an effective antioxidant⁷. Exogenous melatonin application to potato plants increased photosynthetic activity and antioxidant capacity under salt stress, thereby enhancing salt stress resistance compared with untreated plants⁸. All plants require boron as an essential micronutrient, and boron serves as an important structural component of plant cell walls and membranes, as well as supporting the metabolic processes necessary for plant growth and development⁹. Recent research has highlighted the impact of boron on the mitigation of abiotic stressors through boron's regulation of physiological processes, stabilization of cellular structure, and enhancement of cellular antioxidant defense systems¹⁰. Research has also suggested that the physiological responses associated with boron amelioration of drought stress may be linked to decreased cellular membrane damage, increased accumulation of glycine betaine (GB), and improved moisture status in plants grown in drought conditions. Additionally, when applied as a foliar spray, boron increases the concentration of soluble glucose sugars, which improves the plant's osmotic adjustment capabilities.^{10, 11}. The tomato, belonging to the Solanaceae family, is considered one of the most economically important horticultural crops. Tomato fruits are rich in biologically active compounds, particularly carotenoids such as lycopene and β -carotene¹². Tomatoes (*Solanum lycopersicum* L.) are widely consumed worldwide as fresh or processed food products. Salinity is one of the factors significantly affecting tomato growth in all stages of plant development¹³, and the tomato is considered moderately sensitive to salt. A recent review of studies examining the effects of abiotic stress on the primary and secondary metabolism of tomato emphasized that understanding the plant's responses to salt stress (including metabolite accumulation) is essential for optimizing both yield and fruit quality¹⁴. However, the combined effects of melatonin and boron under salinity stress in tomato remain poorly understood. This study is designed to improve salinity tolerance in tomato (*Solanum lycopersicum* L.) through foliar application of melatonin and boron, allowing tomato plants to sustain acceptable growth and productivity under salinity conditions.

2. Materials and Methods

The experiment was carried out during the period from September 2024 to April 2025 in the botanical garden of the Biology Department, College of Science for Women, University of Baghdad. A factorial experiment with three factors was implemented in a randomized complete block design (RCBD) with three replications. The treatments included melatonin at three levels (M0, M1, M2: 0, 2, and 3 mg L⁻¹), boron at three levels (B0, B1, B2: 0, 5, and 10 mg L⁻¹), and sodium chloride at three concentrations (S0, S1, S2: 0, 2000, and 4000 mg L⁻¹), along with their interactions. Soil samples were randomly collected from pots after planting at a depth of 15 cm. Plastic pots measuring 30 cm in height and 40 cm in diameter were used in the experiment. They were filled with a growing medium composed of clay soil and peat moss in a 3:1 ratio, thoroughly mixed to form a composite sample, and analyzed in the postgraduate laboratory of the Department of Biology, College of Science, University of Baghdad, for various physical and chemical properties as presented in **Table 1**. During the growth period, seedlings were initially irrigated regularly with non-saline water. At 30 days after planting, they were subjected to three salinity treatments. Salinity was applied several times, coinciding with irrigation needs, and each saltwater irrigation was followed by irrigation with fresh water throughout the plant's life cycle. Weed control was performed manually. Seeds of the salt-sensitive tomato variety *Solanum lycopersicum* var. Seyran F1 (produced by BASF Nunhems Global, Netherlands, and imported by Ard Unifert) was sterilized and sown in specialized germination trays on September 1, 2024.

On October 7, 2024, we used 27 treatments with three replications, totaling 81 experimental units.

Table 1. Several chemical and physical properties of the soil

Physical properties			
Property	Value		
Salt stress levels (S)	S0	S1	S2
Soil texture	Sandy	Sandy	Sandy
Sand %	74.3	66.6	90
Silt %	15.2	21.4	5.3
Clay %	10.5	12	4.7
Bulk density (mg kg ⁻¹)	1	0.86	0.8
Chemical properties			
Property	Value		
Salt stress levels (S)	S0	S1	S2
pH	6.9	6.7	6.8
Electrical Conductivity (dsm.m ⁻¹)	13.5	43.2	41.9
N (mg kg ⁻¹)	75	155	232
P (mg kg ⁻¹)	28.9	31.9	45.9
K (mg kg ⁻¹)	32.6	39.5	81.4

2.1. The Studied Indicators

2.1.1. Proline Content of Leaves

Proline was extracted from 0.5 g of fresh leaf tissue using 10 ml of 3% aqueous sulfosalicylic acid, following the method of ¹⁵. After grinding and filtration, 2 ml of the filtrate was reacted with ninhydrin reagent and glacial acetic acid, heated in a water bath for 1 hour, cooled, and extracted with toluene. The absorbance of the toluene phase was measured at 520 nm using a UV/Visible spectrophotometer (LKB-Biochrom 4050), and proline concentration was calculated from a standard curve.

1.1.2. The Percentage of Nitrogen in the Leaves

Nitrogen percentage was determined using the semi-micro Kjeldahl method¹⁶. A 0.2 g leaf sample was digested with 1 g CuSO₄ and 5 ml concentrated H₂SO₄ (98%) until clear, then cooled. After adding 25 ml distilled water and 10 ml NaOH, the mixture was distilled, and released NH₃ was trapped in 25 ml of 2% boric acid. The distillate was titrated with 0.01 N HCl, and the nitrogen percentage was calculated as in **Equation 1**.

$$\text{Nitrogen \%} = (\text{Volume of HCl titer} \times 0.01 \times 0.014 \times 100) / \text{Sample weight (g)} \quad (1)$$

2.1.3. The Percentage of Phosphorus in the Leaves

Phosphorus percentage was determined according to¹⁶ using 10 ml of the Kjeldahl-digested sample. The sample was mixed with 0.1 g ascorbic acid and 4 ml ammonium molybdate solution, heated for 1 min until blue, cooled, and diluted to 100 ml with distilled water. Absorbance was measured at 620 nm using a spectrophotometer and compared to a standard curve. Phosphorus percentage was calculated as in **Equation 2**.

$$\text{Phosphorus \%} = (\text{Final phos. conc.} \times 50 \times 100 \times 100) / (\text{Sample W. (g)} \times 10 \times 1,000,000) \quad (2)$$

2.1.4. The Percentage of Potassium in the Leaves

Total potassium content was determined from 1 g leaf samples digested using the semi-micro Kjeldahl method with 1 g CuSO₄ and 5 ml concentrated H₂SO₄ (98%). After digestion and cooling, the solution was diluted to 100 ml with distilled water. Potassium concentration was measured using a 2000 Automatic Flame Photometer and compared with a standard potassium curve. Final values were obtained by multiplying by the dilution factor and dividing by sample dry weight. The same procedure was used for boron determination.

2.1.5. Peroxidase Enzyme POD

A weighed leaf sample was homogenized with phosphate-buffered saline (PBS) at 4–6°C, filtered, and centrifuged at 9000 rpm for 15 min to obtain the crude enzyme extract. Peroxidase activity was assayed colorimetrically using guaiacol as the substrate, with a reaction mixture

containing 18 mM guaiacol, 1 mM H₂O₂ in PBS (pH 5), and 0.05 mL of enzyme extract. Absorbance at 470 nm was recorded at 30°C every 10 s, and an absorbance change of 0.01 min⁻¹ was defined as one unit of enzyme activity¹⁷.

2.1.6. Catalase Enzyme CAT

Two grams of fresh leaf tissue were homogenized in 0.1 M potassium phosphate buffer (pH 7.8) at 1:2 (w/v), filtered, and centrifuged at 10,000 g for 30 min. For catalase activity, 0.2 ml supernatant was incubated with 1 ml of 65 mM H₂O₂ in 60 mM phosphate buffer (pH 7.4) at 25°C for 4 min, then stopped with 1 ml of 32.4 mM ammonium molybdate. Absorbance at 405 nm was measured, and activity was calculated using the specified **Equation 3**¹⁷.

$$\text{Catalase activity} = (\text{Sample} - \text{Blank 1}) / (\text{Blank 2} - \text{Blank 3}) \times 271 \quad (3)$$

Blank 1: H₂O₂-buffer + molybdate + sample

Blank 2: H₂O₂-buffer + molybdate + buffer

Blank 3: buffer + molybdate + buffer

2.1.7. Lycopene content in fruit

Lycopene was extracted from 10 g dried tomato powder¹⁸ using hexane:acetone:ethanol (2:1:1, v/v/v) under light-protected conditions (aluminum foil). Samples were left 30 min, vortexed 2 min (Vortex-2 Genie, G-5-60E, USA), and separated with 10 ml water into polar/non-polar layers. The hexane layer containing lycopene was collected into 50 ml tubes, re-extracted, treated with 1.5 g KOH (in 100 ml water) to remove by-products, vacuum-filtered, washed with acetone, and dried over anhydrous CaCl₂. The extract was stored in light-proof containers at 25 °C. Lycopene content was measured using a spectrophotometer at 503 nm (hexane blank) and calculated using the extinction coefficient E% = 3150¹⁹.

2.1.8. Ascorbic acid content (Vitamin C)

According to the method adopted by²⁰, 10 g of fresh tomato fruit were homogenized with 20 mL of phosphoric acid. The homogenate was filtered, and the final volume was adjusted to 100 mL using the same solvent. A 2 mL aliquot of the extract was mixed with 5 mL phosphoric acid and titrated with indophenol dye until a stable pink color appeared. For calculation, the dye factor (F) was defined as 7.5 mg ascorbic acid per 1 mL of dye. Vitamin C content was calculated as follows, as in **Equation 4**.

$$\text{Vitamin C} = \text{Volume of dye used for the sample} - \text{Volume of dye for the blank} \times \text{Number of milliequivalents of dye (7.5)} \times 100/2, \text{ all divided by } 10\text{g} \times 100 \quad (4)$$

2.1.9. Reducing sugar content

Reducing sugars were determined according to²¹. A 0.2 g sample was extracted with 8.8 ml of 80% ethanol and incubated in a water bath at 60 °C for 30 min, then centrifuged at 3000 rpm for 15 min. The residue was re-extracted twice with 10 ml of 80% ethanol each time, followed by centrifugation. All filtrates were combined, and the final volume was adjusted to 50 ml with 80% ethanol. From this extract, 1 ml was transferred to a test tube, mixed with 1 ml of 5% phenol, cooled, and then 5 ml of concentrated H₂SO₄ was added. After mixing, absorbance was measured at 490 nm using 80% ethanol as a blank. Standard glucose solutions (0.05–0.20 g/100 ml) were prepared and treated with phenol and H₂SO₄ as above, and their absorbance values were used to construct a standard calibration curve. Statistical analyses were conducted using IBM SPSS Statistics, version 26 (2019)²². Differences between treatment means were evaluated using the least significant difference (LSD) test.

3. Results

3.1. Proline Content of Leaves (μmol/g FW)

Table 2 shows that proline concentration increased with salinity, peaking at S2 (99.259) and lowest at S0 (64.000). Melatonin gave the highest value at M0 (83.740) and lowest at M2 (77.703), while boron peaked at B2 (82.703) and was lowest at B1 (78.777). The M × B interaction was highest at M0B2 (85.333) and lowest at M2B1 (76.000). For B × S, the maximum was at S2B2 (101.33) and the minimum at S0B0 (61.778). The M × S interaction

peaked at S2M0 (101.88) and was lowest at S0M2 (61.222). The three-way interaction recorded the highest at S2M0B0 (105.00) and the lowest at S0M2B0 (59.333).

Table 2. Effect of melatonin, boron, and their interaction on proline ($\mu\text{mol/g FW}$) content of *Solanum lycopersicum* L. leaves under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	63.667 M	66.667 L	69.000 L	66.444 F	64.000 C
	M1	62.333 MN	63.667 M	67.000 L	64.333 F	
	M2	59.333 O	60.667 NO	63.667 M	61.222 G	
S1	M0	83.667 G	79.333 HI	85.667 G	82.889 C	79.259 B
	M1	80.333 H	77.333 IJ	80.667 H	79.444 D	
	M2	77.333 IJ	74.667 JK	74.333 K	75.444 E	
S2	M0	105.00 A	99.333 CD	101.33 CB	101.88 A	99.259 A
	M1	100.33 C	94.667 EF	103.33 AB	99.444 A	
	M2	97.333 DE	92.667 F	99.333 CD	96.444 B	
L.S.D.		LSD: SMB=2.6917			LSD: SM=2.6865	LSD: S=0.8972
S x B		B0	B1	B2		
S0		61.778 F	63.667 EF	66.556 E	LSD: SB=2.6865	
S1		80.444 C	77.111 D	80.222 C		
S2		100.88 A	95.556 B	101.33 A		
M x B		B0	B1	B2	Mean: M	
M0		84.111 A	81.778 A	85.333 A	83.740 A	
M1		81.000 A	78.556 B	83.667 A	81.074 B	
M2		78.000 B	76.000 B	79.111 B	77.703 C	
L.S.D.		LSD: MB=2.6865			LSD: M=0.8972	
Mean: B		81.037 B	78.777 C	82.703 A	LSD: B=0.8972	

3.2. The Percentage of Nitrogen in the Leaves

The experimental treatments significantly affected the percentage of nitrogen in the leaves, as shown in **Table 3**. There was a significant decrease in the amount of nitrogen in the tissues of all treatments, mainly due to salinity, where the highest nitrogen content was in S0 (control) at 1.8333% and the lowest at S2 at 1.6622%. Both melatonin and boron treatments greatly increased nitrogen levels, with the highest nitrogen content for each of these being reached in treatment M2 (1.7900%) and treatment B2 (1.7581%), respectively. The interaction between melatonin and boron was notable, with M2B2 producing 1.8033%. Similarly, the melatonin $\times$ salinity interaction peaked at S0M2 (1.8588%), and the boron $\times$ salinity interaction reached its highest value at S0B2 (1.8511%). The combined three-factor interaction of salinity, melatonin, and boron showed the greatest enhancement, with S0M2B2 achieving the maximum nitrogen percentage of 1.8766%.

Table 3. Effect of melatonin, boron, and their interaction on nitrogen content (%) content of *Solanum lycopersicum* L. leaves under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	1.7866 G	1.8066 F	1.8233 DEF	1.8055 C	1.8333 A
	M1	1.8266 ED	1.8266 ED	1.8533 BC	1.8355 B	
	M2	1.8366 CD	1.8633 AB	1.8766 A	1.8588 A	
S1	M0	1.7066 KLM	1.7333 J	1.7033 LM	1.7144 E	1.7581 B
	M1	1.7533 I	1.7600 I	1.7633 HI	1.7588 D	
	M2	1.7800 GH	1.8133 EF	1.8100 EF	1.8011 C	
S2	M0	1.6000 P	1.6333 O	1.5966 P	1.61 G	1.6622 C
	M1	1.6566 N	1.6733 N	1.6700 N	1.6666 F	
	M2	1.6933 M	1.7133 KL	1.7233 JK	1.7100 E	
L.S.D.		LSD: SMB=0.0192			LSD: SM=0.0163	LSD: S=0.0064
S x B		B0	B1	B2		
S0		1.8166 A	1.8322 A	1.8511 A	LSD: SB=0.0163	
S1		1.7466 B	1.7688 B	1.7588 B		
S2		1.6500 C	1.6733 C	1.6633 C		
M x B		B0	B1	B2	Mean: M	
M0		1.6977 C	1.7244 CB	1.7077 BC	1.7100 C	
M1		1.7455 ABC	1.7533 ABC	1.7622 ABC	1.7537 B	
M2		1.7700 AB	1.7966 A	1.8033 A	1.7900 A	
L.S.D.		LSD: MB=0.0163			LSD: M=0.0064	
Mean: B		1.7377 B	1.7581 A	1.7577 A	LSD: B=0.0064	

3.3. The Percentage of Phosphorus in the Leaves

In **Table 4**, the effects of the three factors and their interactions are shown. Salt reduces the percentage of phosphorus in the leaves, reaching its highest value at S0 0.5325% and the lowest value at S2 0.4714%. In contrast, the melatonin spraying treatment had the highest percentage at M2 amounting to 0.5229%. There were also significant differences in the rates at which the boron was sprayed—the highest at B2 0.5111 %. Also significant differences were observed in the interaction between M and B treatments; the highest phosphate percentage, 0.532%, was observed at the M2B1 treatment. In contrast, S and B showed differences, with the highest value in treatment S0B2 being 0.5411%. Additionally, the percentage P in leaves exhibiting the interaction between S and M was highest at S₀M₂ (0.5444%). The experimental factors showed highly significant variations, with S0M2B2 0.5533% showing the highest percentage.

Table 4. Effect of melatonin, boron, and their interaction on phosphorus content (%) of *Solanum lycopersicum* L. leaves under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	0.5066 GIH	0.5166 EFGH	0.5266 CDEF	0.5166 B	0.5325 A
	M1	0.5300 BCDEF	0.5366 ABCD	0.5433 ABC	0.5366 A	
	M2	0.5333 BCDE	0.5466 AB	0.5533 A	0.5444 A	
S1	M0	0.4866 JKLM	0.4966 IJKL	0.5000 HIJK	0.4944 C	0.5140 B
	M1	0.5066 GHI	0.5133 FGHI	0.5166 EFGH	0.5122 B	
	M2	0.5200 DEFG	0.5466 AB	0.5400 ABC	0.5355 A	
S2	M0	0.4433 Q	0.4533 PQ	0.4633 OP	0.4533 E	0.4714 C
	M1	0.4666 NOP	0.4733 MNO	0.4766 MNO	0.4722 D	
	M2	0.4833 KLMN	0.5033 GHIJ	0.4800 LMNO	0.4888 C	
L.S.D.		LSD: SMB=0.0179			LSD: SM=0.0118	LSD: S=0.006
S x B		B0	B1	B2		
S0		0.5233 B	0.5333 AB	0.5411 A	LSD: SB=0.0118	
S1		0.5044 C	0.5188 CB	0.5188 CB		
S2		0.4644 D	0.4766 D	0.4733 D		
M x B		B0	B1	B2	Mean: M	
M0		0.4788 D	0.4888 CD	0.4966 CD	0.4881 C	
M1		0.5011 BCD	0.5077 ABC	0.5122 ABC	0.5070 B	
M2		0.5122 ABC	0.532 A	0.5244 AB	0.5229 A	
L.S.D.		LSD: MB=0.0118			LSD: M=0.006	
Mean: B		0.4974 B	0.5096 A	0.5111 A	LSD: B=0.006	

3.4. The Percentage of Potassium in the Leaves

Table 5 presents the effects of the treatments on the K percentage, where significant differences were observed. It appears that as salinity levels increase, the percentage of potassium decreases; the highest average was at S0 1.7459% and the lowest average was at S2 1.6811%. The melatonin treatment showed significant differences; the highest potassium percentage, 1.7466%, was recorded at the M2. Similarly, the boron treatment also showed significant differences, with the highest value being B2 at 1.7266%. The interaction of M and S was clear, as the value was high at S0M2 1.7800 %. Significant differences were observed in the interaction between M and B treatments, with the highest value reaching 1.7544% in M2B2. Significant differences were also observed in the interaction between S and B, with the highest k value, 1.7633%, recorded at the S0B2 treatment. As for the interaction among S, B, and M, there were significant differences, as the highest percentage was in treatment S0M2B2 (1.7966%).

Table 5. Effect of melatonin, boron, and their interaction on potassium content (%) of *Solanum lycopersicum* L. leaves under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	1.6933 MNO	1.7133 IJKL	1.7400 DEFG	1.7133 C	1.7459 A
	M1	1.7266 FGHI	1.7433 CDE	1.7533 CD	1.7422 B	
	M2	1.7633 CB	1.7800 AB	1.7966 A	1.7800 A	
S1	M0	1.6866 NOP	1.6966 LMN	1.7066 JKLM	1.6966 D	1.7222 B
	M1	1.7166 HIJK	1.7366 DEFG	1.7166 HIJK	1.7233 C	
	M2	1.7333 EFGH	1.7433 DEF	1.7633 CB	1.7466 B	
S2	M0	1.6366 S	1.6633 QR	1.6700 PQR	1.6566 F	1.6811 C
	M1	1.6533 RS	1.6766 OPQ	1.6900 MNO	1.6733 E	
	M2	1.7133 IJKL	1.7233 GHIJ	1.70333 KLMN	1.7133 C	
L.S.D.		LSD: SMB=0.0196			LSD: SM=0.0161	LSD: S=0.0065
S x B		B0	B1	B2		
S0		1.7277 CB	1.7466 AB	1.7633 A	LSD: SB=0.0161	
S1		1.7122 CD	1.7255 CB	1.7288 CB		
S2		1.6677 E	1.6877 ED	1.6877 ED		
M x B		B0	B1	B2	Mean: M	
M0		1.6722 D	1.6988 CD	1.7055 C	1.6896 C	
M1		1.6911 CD	1.7200 BC	1.7200 BC	1.7129 B	
M2		1.7366 AB	1.7488 AB	1.7544 A	1.7466 A	
L.S.D.		LSD: MB=0.0161			LSD: M=0.0065	
Mean: B		1.7025 C	1.7200 B	1.7266 A	LSD: B=0.0065	

3.5. Peroxidase Enzyme POD (unit /g)

The effect of salinity on peroxidase enzyme activity is presented in **Table 6**, which indicates that the activity is concentration-dependent. The result in the S0 treatment showed that the highest rate was 0.2198, compared to S2 with the lowest rate (0.1928), whereas the highest rate was reached at M2 (0.2117) in the melatonin treatment. The results also revealed a significant effect due to the boron treatment, where the highest value was at B2 (0.2113). The interaction of M with B, the highest value, was recorded at the M2B2 (0.2177). Similarly, the interaction of B and S gave the highest value at S0B2 (0.2241). In the interaction between M and S, the highest value was observed at S0M2 (0.2266). As for the combined interaction of M, B, and S, the highest value for enzyme activity was S0M2B2 (0.2340).

Table 6. Effect of melatonin, boron, and their interaction on peroxidase enzyme activity (unit /g) activity in *Solanum lycopersicum* L. leaves under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	0.2113 HI	0.2150 FG	0.2136 GH	0.2133 C	0.2198 A
	M1	0.2126 GHI	0.2216 D	0.2246 C	0.2196 B	
	M2	0.2166 EF	0.2293 B	0.2340 A	0.2266 A	
S1	M0	0.1990 LM	0.2046 J	0.2026 JK	0.2021 D	0.2080 B
	M1	0.2013 KL	0.2106 I	0.2166 EF	0.2095 C	
	M2	0.2053 J	0.2133 GHI	0.2190 DE	0.2125 C	
S2	M0	0.1813 Q	0.1883 P	0.1906 OP	0.1867 F	0.1928 C
	M1	0.1916 O	0.1956 N	0.2003 KL	0.1958 E	
	M2	0.1910 OP	0.1966 MN	0.2003 KL	0.1960 E	
L.S.D.		LSD: SMB=0.0058			LSD: SM=0.0049	LSD: S=0.0009
S x B		B0	B1	B2	LSD: SB=0.0049	
S0		0.2135 B	0.2220 A	0.2241 A		
S1		0.2018 C	0.2095 B	0.2127 B		
S2		0.1880 E	0.1935 D	0.1971 CD		
M x B		B0	B1	B2	Mean: M	
M0		0.1972 D	0.2026 BCD	0.2023 CD	0.2007 C	
M1		0.2018 CD	0.2093 ABC	0.2138 AB	0.2083 B	
M2		0.2043 BCD	0.2131 ABC	0.2177 A	0.2117 A	
L.S.D.		LSD: MB=0.0049			LSD: M=0.0009	
Mean: B		0.2011 C	0.2083 B	0.2113 A	LSD: B=0.0009	

3.6. Catalase Enzyme POD (unit/g)

According to **Table 7**, an increase in salinity levels decreased catalase enzyme activity in leaves; the maximum value recorded was 169.03 at S0 and 138.62 at S2. Application of melatonin positively influenced catalase activity in plants, where the highest value recorded with melatonin was at M2 (165.14), and that recorded with boron was at B2 (159.20). Based upon the interaction between melatonin and boron, catalase activity significantly increased to 168.55% more than that of the individual treatments alone. For the interaction of boron × salinity, the maximum was registered at S0B2 (173.00), while for melatonin × salinity, a maximum of S0M2 (175.55) was observed. The interaction of the three factors produced the highest recorded catalase activity at S0M2B2 (177.66).

Table 7. Effect of melatonin, boron, and their interaction on catalase enzyme activity (unit /g) activity in *Solanum lycopersicum* L. leaves under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	153.00 HI	162.00 F	166.33 E	160.44 C	169.03 A
	M1	166.66 E	171.66 D	175.00 BC	171.11 B	
	M2	172.33 D	176.66 AB	177.66 A	175.55 A	
S1	M0	141.66 M	147.66 L	150.33 JK	146.55 E	157.11 B
	M1	151.00 IJK	157.66 G	162.33 F	157.00 C	
	M2	161.33 F	168.33 E	173.66 CD	167.77 B	
S2	M0	121.33 Q	128.33 P	132.00 O	127.22 G	138.62 C
	M1	130.66 O	137.33 N	141.66 M	136.55 F	
	M2	149.66 KL	152.33 HIJ	154.33 H	152.11 D	
L.S.D.		LSD: SMB=4.902			LSD: SM=4.2371	LSD: S=0.7301
S x B		B0	B1	B2	LSD: SB=4.2371	
S0		164.00 CB	170.11 AB	173.00 A		
S1		151.33 ED	157.88 CD	162.11 CB		
S2		133.88 G	139.33 FG	142.66 EF		
M x B		B0	B1	B2	Mean: M	
M0		138.66 E	146.00 DE	149.55 CDE	144.74 C	
M1		149.44 CDE	155.55 BCD	159.66 ABC	154.88 B	
M2		161.11 ABC	165.77 AB	168.55 A	165.14 A	
L.S.D.		LSD: MB=4.2371			LSD: M=0.7301	
Mean: B		149.74 C	155.77 B	159.25 A	LSD: B=0.7301	

3.7. Lycopene content in tomato fruit (mg / 100 g)

Table 8 showed that the treatments had a clear effect on lycopene content in tomato fruits. The results indicated that increasing salinity levels led to a reduction in lycopene concentration. The highest value was recorded under the S0 treatment (10.141), while the lowest value was observed in the high-salinity control S2 (7.6770). In the presence of melatonin, the highest value was at M2 (10.073). In treatment with boron, the highest value was at B1 (9.0721). Moreover, clear significant differences were noticed in the interaction of M and B, where the highest rate was recorded at M2B1 (10.538). When M interacted with S, a significant difference was found, as shown; the highest value was at M2S0 (11.325). Significant differences were also found between B and S, where the highest value was at S0B2 (10.475), followed by S0B1 (10.184), respectively. At the triple interaction, the significant differences were very clear, and the highest value of lycopene concentration was at S0M2B2 (11.820).

Table 8. Effect of melatonin, boron, and their interaction on lycopene content (mg/ 100 g) in *Solanum lycopersicum* L. fruits under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	8.6343 L	9.4260 I	9.5726 H	9.2110 C	10.141 A
	M1	9.7766 G	9.8553 F	10.033 E	9.8884 B	
	M2	10.884 C	11.273 B	11.820 A	11.325 A	
S1	M0	6.9456 S	7.2243 R	7.6780 Q	7.2827 F	8.5461 B
	M1	7.9873 O	8.4280 M	8.2646 N	8.2267 E	
	M2	9.3646 J	10.893 C	10.129 D	10.129 B	
S2	M0	5.8820 V	6.8846 T	6.0536 U	6.2734 G	7.6770 C
	M1	7.7570 P	8.2163 N	8.0053 O	7.9929 E	
	M2	8.8286 K	9.4476 I	8.0180 O	8.7648 D	
L.S.D.		LSD: SMB=0.4584			LSD: SM=0.3959	LSD: S=0.0195
S x B		B0	B1	B2	LSD: SB=0.3959	
S0		9.7650 AB	10.184 A	10.475 A		
S1		8.0992 CD	8.8487 CB	8.6907 C		
S2		7.4892 D	8.1829 CD	7.3590 D		
M x B		B0	B1	B2	Mean: M	
M0		7.1540 D	7.8450 CD	7.7681 CD	7.5890 C	
M1		8.5070 C	8.8332 BC	8.7678 BC	8.7026 B	
M2		9.6924 AB	10.538 A	9.9891 A	10.073 A	
L.S.D.		LSD: MB=0.3959			LSD: M=0.0195	
Mean: B		8.4511 C	9.0721 A	8.8416 B	LSD: B=0.0195	

3.8. Ascorbic acid content (Vitamin C) (mg/100g)

Table 9 showed the results, indicating that increasing the level of salinity led to an increase in the concentration of ascorbic acid. In S2 treatment, the highest value was 12.848; the lowest value, recorded under the control treatment S0, was 10.903. In the presence of melatonin, the highest value was at M0 (12.214) compared to M2 (11.755). In treatment with boron, the highest value was at B2 (12.340). Moreover, clear significant differences were noticed in the interaction of M and B, where the highest rate was recorded at M0B2 (12.544). When the M was interacted with S, the highest value was recorded at treatment S2M0 (13.177). Significant differences were also found between B and S, where the highest value was at S2B2 (13.211). At the triple interaction the highest value of ascorbic acid concentration was at S2M0B2 (13.466).

Table 9. Effect of melatonin, boron, and their interaction on ascorbic acid content (mg/ 100 g) in *Solanum lycopersicum* L. fruits under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	11.200 K	10.566 MN	11.533 J	11.100 F	10.903 C
	M1	10.933 L	10.433 NO	11.366 JK	10.911 FG	
	M2	10.666 M	10.266 O	11.166 K	10.700 G	
S1	M0	12.533 EF	11.933 I	12.633 DE	12.366 CD	12.192 B
	M1	12.266 GH	11.900 I	12.400 FGH	12.188 DE	
	M2	11.966 I	11.766 I	12.333 FGH	12.022 E	
S2	M0	13.266 AB	12.800 CD	13.466 A	13.177 A	12.848 A
	M1	12.933 C	12.333 FGH	13.200 B	12.822 B	
	M2	12.466 EFG	12.200 H	12.966 C	12.544 BC	
L.S.D.		LSD: SMB=0.4228			LSD: SM=0.3418	LSD: S=0.0743
S x B		B0	B1	B2		
S0		10.933 F	10.422 G	11.355 E	LSD: SB=0.3418	
S1		12.255 C	11.866 D	12.455 C		
S2		12.888 B	12.444 C	13.211 A		
M x B		B0	B1	B2	Mean: M	
M0		12.333 AB	11.766 ABC	12.544 A	12.214 A	
M1		12.044 ABC	11.555 BC	12.322 AB	11.974 B	
M2		11.700 BC	11.411 C	12.155 ABC	11.755 C	
L.S.D.		LSD: MB=0.3418			LSD: M=0.0743	
Mean: B		12.025 B	11.577 C	12.340 A	LSD: B=0.0743	

3.9. Reducing sugar content in the fruit ($\mu\text{mol/g FW}$)

Table 10 shows that the results indicate that increasing the level of salinity led to a decrease in the concentration of reducing sugar. In the S0 treatment, the highest value was (3.2681), and the lowest value was (2.8511) in the S2 concentration. In the presence of melatonin, the highest value was at M0 (3.1144), while, in treatment with boron, the highest value was at B2 (3.1188). In the interaction of M and B, the highest rate was recorded at M0B2 (3.1688). When M was interacted with S, the highest value recorded was at treatment S0M0 (3.3166). Significant differences were also found between B and S, where the highest value was at S0B2 (3.3077). At the triple interaction, the significant differences were very clear, and the highest value of reducing sugar content was at S0M0B2 (3.3466).

Table 10. Effect of melatonin, boron, and their interaction on reducing sugar ($\mu\text{mol/g FW}$) of *Solanum lycopersicum* L. fruits under salt stress conditions.

Salt	Melatonin	Boron			S x M	Mean: S
		B0	B1	B2		
S0	M0	3.3166 AB	3.2866 ABC	3.3466 A	3.3166 A	3.2681 A
	M1	3.2766 BC	3.2233 CDE	3.3133 AB	3.2711 A	
	M2	3.1833 EF	3.2033 DEF	3.2633 BCD	3.2166 B	
S1	M0	3.1433 FGH	3.0966 GHIJ	3.1633 EFG	3.1344 C	3.1040 B
	M1	3.1133 GHI	3.0566 IJK	3.1366 FGH	3.1022 CD	
	M2	3.0833 HIJ	3.0366 JK	3.1066 GHI	3.0755 D	
S2	M0	2.8633 LM	2.8166 MN	2.9966 K	2.8922 E	2.8511 C
	M1	2.8533 LM	2.7966 MN	2.8866 L	2.8455 EF	
	M2	2.8300 LM	2.7600 N	2.8566 LM	2.8155 F	
L.S.D.		LSD: SMB=0.0697HG			LSD: SM=0.0532	LSD: S=0.0232
S x B		B0	B1	B2	LSD: SB=0.0532	
S0		3.2588 AB	3.2377 B	3.3077 A		
S1		3.1133 C	3.0633 D	3.1355 C		
S2		2.8488 F	2.7911 G	2.9133 E		
M x B		B0	B1	B2	Mean: M	
M0		3.1077 A	3.0666 A	3.1688 A	3.1144 A	
M1		3.0811 A	3.0255 A	3.1122 A	3.0729 B	
M2		3.0322 A	3.0000 A	3.0755 A	3.0359 C	
L.S.D.		LSD: MB=0.0532			LSD: M=0.0232	
Mean: B		3.0737 B	3.0307 C	3.1188 A	LSD: B=0.0232	

4. Discussion

Salt stress triggers a series of secondary stresses in plants, including osmotic stress, ionic imbalance, and oxidative damage, through its effects on water uptake, stomatal regulation, and ion homeostasis²³. Salinity stress leads to an ionic imbalance, which leads to the accumulation of harmful ions in plants such as Na^+ and Cl^- and hinders the uptake of macro elements such as N, P, and K. It leads to an increase in the production of reactive oxygen species (ROS) as it causes cellular imbalance, which mainly results in cell membrane damage and biomolecule disruption such as lipids, DNA, and proteins²⁴. Exposure to salinity reduces the strength of cell membranes through the production of toxic by-products like MDA, which results in electrolyte leakage and ultimately reduces plant growth and development. To counteract these damaging effects of ROS, plants employ a range of enzymatic and non-enzymatic antioxidant mechanisms that either increase the endogenous levels of antioxidants or scavenge excess amounts of these radicals. Osmotic stress and ionic imbalances also play a crucial role in exacerbating the severity of damage sustained by plants from salinity. The absorption of water by the roots is hindered by high levels of salinity, which causes dehydration of the cellular contents and reduces turgor

pressure, thus decreasing the plant's ability to take up nutrients and water. The accumulation of Na^+ in plant tissues also results in the manifestation of ionic toxicity, which negatively affects metabolic and physiological processes, further limiting plant growth. To alleviate osmotic stress, plants accumulate various compatible solutes, e.g., soluble sugars, soluble proteins, and proline—as osmotic regulators. These compounds function to protect cellular structures from the adverse effects of salt exposure and provide the necessary osmotically active pressure to maintain cellular turgor^{25, 26}.

The applied exogenous melatonin had a positive effect on the fruit by increasing glucose, fructose, and sucrose levels, resulting in sweeter/flavorful fruit. Melatonin treatment increased lycopene and carotenoids in the fruit, creating a richer color and better nutritional value. The fruit will also contain more ascorbic acid (vitamin C), total phenolic compounds, and Flavonoids which will contribute to more robust antioxidant capabilities²⁷. Melatonin lowers reactive oxygen species (ROS) and lipid peroxidation while enhancing antioxidant enzymes (SOD, CAT, POD, and APX), preserving fruit quality under salinity²⁸. Melatonin protects chlorophyll, maintains photosystem II functioning, and supports sugar production in fruit under saline conditions. Melatonin-treated plants (with NaCl) retain more lycopene, vitamin C, and phenolics than untreated stressed plants and support K^+/Na^+ ratios, indirectly enhancing physiological and biochemical quality traits^{29, 30}. Concerning photosynthetic efficiency, melatonin improves chlorophyll by inhibiting its breakdown and increasing the greenness of the leaves.²⁷ Proline accumulation is greatly enhanced by exogenous melatonin (M), thereby improving osmotic adjustment and protecting cellular structures from the osmotic stress associated with salt. Osmotic adjustment and salt tolerance lead to improvements in water status. Exogenous M. reduces the negative effects of sodium chloride (NaCl) on the nutrient homeostasis of plants. By inducing a significant improvement in nitrogen (N) and phosphorus (P) uptake, M. enhances the K^+/Na^+ ratio of plants, which is extremely important for enzyme activity and maintaining ion balance under saline conditions²⁸. Exogenous melatonin has been shown to significantly enhance catalase (CAT) activity, which is responsible for converting hydrogen peroxide (H_2O_2) into water and oxygen, thereby reducing the oxidative injury to plant cells caused by salinity and aiding in the maintenance of membrane integrity and chloroplast stability. Exogenous M. also enhances peroxidase (POD) activity, leading to the detoxification of H_2O_2 and other reactive oxygen species. The enhancement of enzymatic activity through the application of M. has reduced lipid peroxidation (indicated by the amount of malondialdehyde (MDA), strengthened cell walls, and increased overall salt tolerance in plants^{28, 29}.

Tomato's enhanced ability to withstand salinity stress may stem from a combination of various physiological and biochemical processes due to boron's positive effects when applied from outside sources. The principal mechanism through which boron increases is only the amount of chlorophyll present in tomato plants under conditions of salinity stress. Salinity stress induces an increase in chlorophyll breakdown because of changes in ion concentrations and increased oxidative damage; however, by providing structural support for the thylakoid membranes of the chloroplast, boron contributes to the maintenance of chloroplast stability, thus allowing chloroplasts to maintain structural integrity while continuing to perform photosynthesis, despite the effects caused by salinity³⁰. Moreover, Boron plays a significant role in osmotic regulation. Boron decreases excessive osmolyte accumulation by stimulating greater uptake of potassium and nitrate, thereby reducing proline levels and signaling improved stress mitigation³¹. Boron also enables the efficient uptake and translocation of macronutrients by stabilizing plasma membranes and forming cross-links between cell-wall pectins and ion selectivity, thus allowing for successful absorption and transportation of N, P, and K in saline environments, thereby decreasing sodium toxicity and maintaining important metabolic and enzymatic activity³². Additionally, in addition to the role it plays in reinforcing the protection against oxidative stress, boron also helps strengthen the plant's immune system against oxidative stress caused by excessive production of reactive oxygen species (ROS), which leads to oxidative damage. Boron

increases the activity of antioxidant enzymes (e.g., catalase (CAT) and peroxidase (POD)), thereby improving the detoxification of hydrogen peroxide and decreasing lipid peroxidation, thus protecting cell membranes and organelles from oxidative stress-induced injury¹⁰. Boron isn't just helping plants have a high tolerance to stress. It has also been shown to be beneficial, positively affecting secondary metabolism and improving the quality of fruit. Tomatoes accumulate lycopene through stabilization of carotenoid biosynthetic enzymes. The accumulation occurs through the efficient conversion of precursors to lycopene even when under oxidative stress.³³ In addition, Boron will help with sugar accumulation. Sugar accumulation will happen because of the improvement in photosynthesis and the transport of assimilates to the fruit. It increases the transport of assimilates by cross-linking with and creating strong rhamnogalacturonan-II structures within the phloem cell wall, which will enhance phloem loading and the movement of sugar over long distances³⁴.

The coordination of mechanisms rather than a specific function of boron is the focus when referring to how boron functions within plants. Through a myriad of ways, including safeguarding photosynthesis pigments, regulating osmotic balance, regulating nutrient absorption, enhancing antioxidant mechanisms, and the formation of secondary metabolites and sugars, boron resources assist in supporting both growth and fruit quality.

5. Conclusion

Salt stress significantly impairs tomato productivity and fruit quality by disrupting key physiological and biochemical processes and inducing the excessive accumulation of Reactive Oxygen Species (ROS). In the present study, salinity reduced chlorophyll content and antioxidant capacity, leading to decreased levels of lycopene and reducing sugars. Consequently, both the nutritional value and sweetness of tomato fruits were adversely affected. The application of melatonin at the second-most effective concentration (M2) markedly improved these parameters by enhancing chlorophyll biosynthesis, stabilizing chloroplast membranes, and stimulating antioxidant enzyme activity. Similarly, boron application at the lowest concentration (B1) contributed to maintaining chlorophyll stability, increasing antioxidant levels, and improving sucrose transport and nutrient uptake, thereby supporting better fruit quality under salinity stress. Therefore, the combined application of M2 and B1 appears to effectively mitigate the salt stress, leading to increased production of high-quality tomatoes.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

This article does not contain any studies performed on animals by any of the authors

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