Effects of foliar spraying with melatonin and chitosan Nano-encapsulated melatonin on tomato (*Lycopersicon esculentum* L. cv. Falcato) plants under salinity stress

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Abstract

Melatonin has been found to be crucial in the growth and development of plants under stress conditions. In this study, the effects of melatonin and nano melatonin regarding the growth and development of tomato plants, along with their photosynthetic pigment, phenol, and antioxidant activity, were investigated under saline conditions. The study was conducted using a completely randomized design with three replications, and the applied treatments were salt stress and foliar spraying of melatonin at a concentration of 0 (control), melatonin (Mel), and nano capsule-melatonin (Nano-Mel) at 500 µM. Salinity treatments included application of sodium chloride with two concentration of 0 mM NaCl (S1) and 50 mM NaCl (S2). Under saline conditions, Mel and Nano-Mel increased both shoot and root fresh and dry weights, improved relative water content (RWC), and enhanced antioxidant activity and phenolic content. Salinity elevated leaf ABA content, unaffected by Mel or Nano-Mel. Chlorophyll fluorescence and SPAD values demonstrated resilience to salinity with Mel and Nano-Mel applications. Nano-Mel notably mitigated Na^+ accumulation in leaves under salinity, helping maintain K^+ homeostasis. Proline levels rise due to salinity but decreased with Mel and Nano-Mel treatments. Electrolyte leakage (EL) increased under salinity but is significantly reduced by Mel, indicating enhanced membrane stability. The findings reveal that salinity stress significantly reduced plasma membrane intrinsic protein (PIP) expression in roots and leaves, whereas Mel and Nano-Mel treatments enhance PIP expression, particularly in roots. The study concludes that Mel and Nano-Mel effectively alleviate salinity-induced stress, promoting growth and maintaining physiological homeostasis in tomato plants.

Keywords Abiotic stresses, Antioxidant activity, Aquaporins, Water potential

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Introduction

The decrease in crops yield in semiarid regions is primarily attributed to salt level in the soil or water. However, this phenomenon is influenced by various factors, including species, salt types, phenological stage, crop management, and irrigation [1]. Plants are harmed by salinity present in two ways: osmotic stress and ionic toxicity [2]. High salt concentrations in the culture fluid cause osmotic stress, which restricts plant water uptake. After a 5 h of salt exposure, plants may experience the effects of osmotic stress, which can disrupt the photosynthetic process, decrease carbon uptake, and significantly impede leaf elongation. Some plants have a higher capacity for osmotic adaptation than others, they can take in an adequate amount of water even in a salty environment [3]. As a result, some crops are sensitive to salinity, even at minimal levels, whereas others produce significant yields under high salinity conditions. Ionic effects are caused by the overconsumption of ions, particularly sodium (Na^+) and chloride (Cl^-), which disrupt cellular homeostasis at elevated concentrations [1, 4]. The Na^+ content in old leaves would rise against hazardous threshold with a longer exposure period, hastening leaf senescence and further impeding plant development [1]. Aquaporins (AQPs) are crucial for plant stress response mechanisms, acting as key components of the plasma membrane and performing vital functions in plant cells. The PIP (Plasma membrane Intrinsic Protein) subfamily of AQPs is particularly important for maintaining water homeostasis and enabling plants to adapt to environmental stresses [5]. These PIPs are located in the plasma membrane, functioning as water channels and also facilitating the transport of other neutral molecules. Measuring PIP expression is essential for understanding plant responses to stress conditions, as AQPs play a vital role in regulating water uptake and mitigating the effects of environmental stresses [5].

It has been observed that exposure to high salinity level can lead to a reduction in both growth and biomass, as well as chlorophyll degradation, alterations in water status, disruptions in stomata function, changes in transpiration and respiration, alongside imbalances in ion ratios [6, 7]. Salinization can result in the overproduction of reactive oxygen species (ROS) and maintenance of ROS homeostasis is primarily helped by a cohort of antioxidant enzymes and compounds that are subject to stringent regulation and exhibit interdependent coordination.

Melatonin has been identified as a potent antioxidant, particularly in vertebrate species, according to recent research [8, 9]. The identification of melatonin in plants occurred during the latter part of 1990. However, it was not until 2009 that the investigation of melatonin in plants began to gain prominence [10]. Melatonin is a novel plant hormone which directly regulates many physiological processes during the different stages of plant growth and development is currently receiving more and more attention [11, 12]. Due to its hydrophilic and lipophilic characteristics as well as its capacity to pass through morpho-physiological barriers, melatonin penetrates plant cells easily [13]. According to [14], melatonin affects plant cells in a variety of physiological and biochemical ways, these processes include but are not limited to seed germination, blooming, branching of roots, photosynthesis, delaying the senescence of leaves, and development or maturity of fruit.

Beside its role as a circadian rhythm regulator, melatonin also acts as a strong antioxidant with broad-spectrum action against biotic and abiotic stresses in plants [15, 16]. Exogenously delivered melatonin is very effective in minimizing oxidative damage caused by various of detrimental environmental factors, such as salt, drought, high temperature, and heavy metal stress [12, 17]. As a result, melatonin helps the body get rid of various ROS and free radicals [18]. Furthermore, it has been suggested that plants might reduce the negative impact of oxidative damage on proteins, lipids, and nucleic acids [19] and may experience an increase in endogenous melatonin levels when subjected to stress factors [10, 12]. The impact of melatonin on several plants under salt stress is displayed in Table 1.

Fluctuating of environmental conditions impact the endogenous levels of melatonin in plants. According to Tan et al. [25], the endogenous level of melatonin increases under adverse environmental conditions, leading to an enhancement in stress resistance. Several studies have been conducted to increase endogenous melatonin levels in plants through exogenous melatonin application, following the discovery that plants with high melatonin content exhibit greater stress resistance. These studies include those conducted by Korkmaz et al. [26] and Arnao and Hernández-Ruiz [27]. The efficacy of melatonin in enhancing salt stress tolerance can be summarized as follows: elevation of antioxidant enzyme activities, maintenance of the photosynthetic system, and reduction of Na^+ uptake through inhibition of bypass flow [28–30]. Additionally, transcriptomes have shown that plant hormones like gibberellin, auxin, and abscisic acid (ABA) are involved in the signaling cascades of melatonin-regulating salt stress responses. Polyamine metabolism has been implicated in the melatonin-induced reduction of salt stress modulating ion absorption and leaf abscission [31].

One of the most significant and well-known vegetable crops globally, the tomato (*Solanum lycopersicum* L.) is rich in antioxidant chemicals and mineral nutrients [32]. Melatonin is in tomato seeds at concentrations ranging from 5 to 114,500 pg g^{-1} on a fresh weight basis. Tomato is influenced by several biotic and abiotic stressors,

Table 1 Roles of melatonin in salinity stress tolerance

| Melatonin treatment | | | | | | |
Common name	Scientific name	NaCl (concentration)	Concentration	Application form	Effects	Reference
Broccoli	*Brassica oleracea*	0, 40, 80, 120 mM	50 µM	Foliar spraying	H_2O_2 and MDA ▼ Total soluble solids (TSS), Acidity, Vitamin C, β-carotene, Total phenolic and Activity of antioxidant enzymes ▲	[2]
Basil	*Ocimum basilicum* L	100 mM	1 and 10 µM	Foliar spraying	2,2-diphenyl-1-picrylhydrazyl (DPPH) ▼ Total phenolic and flavonoid ▲	[20]
Common bean	*Phaseolus vulgaris*	100, 200 mM	100, 200 µM	Foliar spraying	H_2O_2 and MDA ▼ Dry weight, Photosynthetic pigments, Transpiration rate, leaf stomatal conductance, K^+, Na^+ and Ca^{+2} content and Antioxidant activity ▲	[21]
Eggplants	*Solanum melongena*	100 mM	200 mM	Seed priming	Photosynthetic pigments, Total soluble sugars, Total soluble proteins and Total free amino acids ▲	[22]
Green bean	*Snap Bean*	50 mM	20 µM	Foliar spraying	Methylglyoxal, H_2O_2, MDA, Diamine oxidase and Polyamine oxidase ▼ Chl a, Carotenoids, TSS, Proline, K^+, Ca^{+2} and EL ▲	[23]
Tomato	*Lycoprsicon esculentum*	150 mM	150 µM	Foliar spraying	H_2O_2, MDA and EL ▼ Height, Biomass, Chlorophyll *a* and *b*, and Proline metabolisms ▲	[24]

▲ or ▼, enhanced or decreased compared to control

including salinity, and its output is insufficient to fulfill demand [33].

Today the utilization of chitosan nanoparticles (ChNPs) in agriculture is a viable and economical substitute for the application of hazardous chemicals. Therefore, the production of green-synthesized ChNPs could benefit the agricultural industry [34]. ChNPs and their Nano formulations have been the subject of extensive research as plant growth enhancers in recent years. The cationic chitosan that is protonated exhibits a heightened attraction to the cellular membranes, resulting in enhanced reactivity within the plant system. Furthermore, it should be noted that chitosan possesses a nitrogen content of approximately 9–10%, which functions as a macronutrient for the plant [34]. Several techniques are employed for the implementation of ChNPs in crops and fields. ChNPs can be utilized in agricultural settings through various modes of application, including foliar sprays, soil application, and seed priming. ChNPs have been investigated in expanding agriculture and its sustainable practices. ChNPs possess biocatalytic activity and may serve as a viable alternative to non-degradable and hazardous compounds. ChNPs have been found to possess the capability of encapsulating diverse supplements, making them suitable for the regulated plant growth promoters and fertilizers access, in the field of agriculture [34].

Many studies have been conducted on the effect of melatonin on tomatoes under various abiotic stresses, such as salt stress, but this study will look into the impact of melatonin in the form of Nano encapsulated chitosan coating on tomato plant tolerance to salt stress. The theory is that applying melatonin in Nano encapsulated form with chitosan coating can improve tomato plant

resistance to salt stress. In other words, the purpose of this study is to see if using melatonin Nano encapsulated with chitosan coating can improve and strengthen tomato plant tolerance to salt stress. The findings of this study could be used to improve tomato crop output and reduce damage caused by environmental stressors.

Materials and methods
Melatonin production in Nano-capsules and their physicochemical properties
Low molecular weight chitosan (85% deacetylation, 100 ± 5 kD, 98 wt% purity) was obtained from Sabz Gostaresh Azin Turkan Co. (Maragheh, Iran). Hydroxypropyl methylcellulose (90 kDa) was sourced from Merck Co., Germany, and melatonin, acetic acid, and sodium tripolyphosphate from Sigma-Aldrich. To prepare chitosan-melatonin nanoparticles (CTS-HPMC-Mel NPs), 0.5 g of chitosan was dissolved in 25 mL of 1% acetic acid at 26 ℃. A melatonin solution (10 mL, 100 mg L^{-1}) was added and stirred for 3 h. Separately, 0.2 g of HPMC was dissolved in 25 mL of water and added to the chitosan-melatonin solution. This mixture was treated with 0.2 g of TPP in 5 mL of water using ionic gelation, forming flocculated particles. After stirring overnight, the mixture was washed and freeze-dried. The surface morphology of the nanoparticles was examined with SEM (VEGAII, XMU, Czech Republic), and their particle size and zeta potential were measured using a Zetasizer Nano ZS90 (Malvern Instruments) [35].

Experimental design, cultivation, and treatment application

The study was conducted in the IUT greenhouse in Iran. To evaluate the effects of melatonin (Mel) and melatonin in Nano encapsulated form with chitosan coating (Nano-Mel) foliar spraying on tomato plants under salinity stress. This factorial experiment was conducted using a complete randomized design (CRD) with three replications. The treatments comprised foliar spraying with melatonin at 0 (control), 500 μM Mel [36], which was purchased from Merck Germany, and 500 μM Nano-Mel concentrations. The experimental salinity treatments comprised two levels: the control group with 0 mM NaCl (S1) and the treatment group with 50 mM NaCl (S2).

The germination of tomato (*Lycopersicum esculentum* cv. Falcato) seeds, which was provided by Pakanbazar seed company, Isfahan, Iran, took place in a mixture of cocopeat and perlite at a 50:50 volume ratio in the relatively controlled conditions of the greenhouse.

The average daily temperature was maintained at 25 ± 2 °C, while the night temperature was 17 ± 2 °C. The length of the light-dark period was set to 14 h of light and 10 h of darkness. The photosynthetic photon flux density (PPFD) was 270 μmol m^{-2} s^{-1}, and the relative humidity was kept at 70%.

Salinity stress was applied approximately 35 days after seed germination, when the plants were at the 2–4 leaf stage. The treatments included NaCl with 0 concentrations as a control and 50 mM by adding NaCl to a standard nutrient solution and continued during the whole growth period [37]. Seedlings were subjected to a half-nutrient solution concentration during the first week of the experiment, and then to a complete nutrient solution. The nutrient solutions were prepared based on Jones' [38] formula. To mitigate the occurrence of osmotic shock, a salinity stress experiment was conducted in a two-step process. The initial step involved rinsing with regular water, followed by irrigating with saline water. The Mel and Nano-Mel treatments were applied weekly at 0 concentrations as a control and 500 μM as a solution spray at a time interval after ten days of the first apply salinity in the amount of 500 mL for each plant in such a way that it drips from the leaves for a duration of two months. One week after the final treatment application, the following variables were evaluated.

Measured parameters
Characteristics of plant growth
With the help of a steel blade, the roots and shoot of tomato plants were separated, and a scale with an exact accuracy of 0.01 was used to determine the fresh weight. In order to achieve a constant weight, the shoots and roots were dried for two days at 70 °C.

Determination of leaf and root water potential
Two to four mature leaf and root samples from each treatment were sealed within a foil laminate bag for at least 10 min before being removed. In this study, the leaf water potential was determined at midday (11:30–12:30 h) using a scholander pressure chamber (SKPM1405, GBR) [39]. In the same way, shoots are separated from the root, and the root is placed in the Scholander pressure chamber to determine the first drop exerted from the top of the root and considered as a root water potential.

Relative water content
Mature leaves (Fw: 0.2 g) were carefully removed from the base of the shoot. Subsequently, the leaves were placed on the surface of distilled water and allowed to float for 12 h at room temperature. Leaves with excessive moisture were dried using a paper towel to eliminate surface moisture, and the weight was measured as the turgid weight (TW). Subsequently, the dry weight (Dw) was acquired by subjecting the saturated leaves to oven drying 60 °C. In order to determine the RWC, the following formula was used [40].

$$RWC = \frac{(Fw - Dw)}{(Tw - Dw)} \times 100$$

SPAD value
The leaf chlorophyll was measured with a Minolta SPAD-502 (SPAD502 plus, Japan) leaf chlorophyll meter, which enabled non-destructive data collection. Each plant was subjected to three readings, with each reading taken from a separate leaf. The mean of these readings was then calculated [41].

Chlorophyll fluorescence
Chlorophyll fluorescence was measured using a chlorophyll fluorometer (RS232, Handy PEA, UK) between 8:00 and 9:00 AM. Following a 3 min dark acclimation period, the maximum photochemical efficiency of photosystem II (Fv/Fm) was recorded, as described by Maxwell and Johnson [42].

Chlorophyll and carotenoid contents
Fresh leaf tissue (0.2 g) was combined with acetone 80%, filtered, and balanced to a volume of 10 mL (UV 160 A-Shimadzu Corp., Kyoto, Japan), and absorbance was measured at 476, 646, and 663 nm [43]. To assess the levels of chlorophyll *a*, chlorophyll *b* and carotenoid, the formula from Pérez-Grajales et al. [44] was used.

Antioxidant activity
From the method Koleva et al. [45] was used to assess the antioxidant activity of tomato leaves. In a test tube

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or sampling tube, 200 μL of an analytical sample solution and 800 μL of a Tris-HCl buffer (pH 7.4) were first added. Then, 1 mL of the DPPH solution was introduced. A test tube mixer was used to stir the mixture for 10 s immediately. The test tube was then placed at room temperature, protected from direct light, to allow the reaction to proceed. Exactly 30 min after adding the DPPH solution, the solution's absorbance at a wavelength of 517 nm was measured using a spectrophotometer (UV 160 A- Shimadzu Corp., Kyoto, Japan). The blank was a mixture made up of 800 μL of Tris-HCl buffer and 1.2 mL of ethanol. The absorbance measured after the introduction of the analytical sample was designated as As, while the absorbance measured after introducing ethanol in its place was designated as Ac. The inhibition ratio (%) was calculated using the following equation:

$$Inhibition\ ratio\ (\%) = \frac{(Ac - As)}{Ac} \times 100$$

Total phenolic
A solution containing 1 mL of extract (concentration ranging from 100 to 500 μg mL^{-1}) was combined with 2.5 mL of Folin–Ciocalteu reagent (concentration of 10% w/v). Following a 5 min interval, a solution consisting of 2.0 mL of Na_2CO_3 (7.5% concentration; 1 mL) and 1 mL of distilled water was introduced into the mixture. The resulting mixture was then incubated at 50 °C for 10 min, with intermittent agitation. Subsequently, the specimen was subjected to a cooling process, following which the absorbance was quantified using a UV Spectrophotometer (UV 160 A- Shimadzu Corp., Kyoto, Japan) at a wavelength of 730 nm, with a blank sample lacking any extract serving as the reference. The results were quantified in μmg GAE g^{-1} of fresh extract [46].

Concentrations of proline
Proline concentrations can be characterized using the ninhydrin test, as described by Bates et al. [47]. Leaf samples (0.2 g) were homogenized at 4 °C using a 10 mL of 3% solution of sulfosalicylic acid. Subsequently, the resultant solution was incubated and centrifuged at a speed of 5000 revolutions per min for 20 min. The supernatant was mixed with a solution containing 2 mL of ninhydrin (2.5% concentration), 2 mL of phosphoric acid (60% concentration, v/v), and 1 mL of glacial acetic acid (100% concentration). The measurement of absorbance was performed at a wavelength of 518 nm.

Total soluble protein
The determination of the total soluble protein content was conducted using the Bradford [48] method. The protein standard used in the study was bovine serum albumin. A sample of fresh texture weighing 1 g was combined with 4 mL of Na-phosphate buffer at pH 7.2. The resulting mixture was subsequently centrifuged at 4 °C. A volume of 5 mL of dye reagent was added to 100 μL of the extract, and was thoroughly mixed. Simultaneously, a series of standards were prepared by aliquoting 5, 10, 20, 30, 40, 50, and 100 μL of Bovine Serum Albumin (BSA) at a concentration of 2 mg mL^{-1} in extraction buffer into individual tubes. A volume of 100 μL was achieved in each tube by adding an extraction buffer. In addition, 5 mL of dye reagent was introduced into these tubes and thoroughly mixed through excessive agitation. After 5 min, the absorbance should be measured at a wavelength of 595 nm for a reagent blank consisting of 100 μL of extraction buffer combined with 5 mL of dye reagent.

Determination of electrolyte leakage (EL)
The leaf segments (0.2 g) were immersed in 6 mL of deionized water after being washed three times with deionized water. When the rehydration phase began, an initial evaluation of electrical conductivity (Eci) was done. The segments-containing tubes were then repositioned in a dark room with a 25 °C temperature. After that, measurements (Ecf) were taken at different points during the rehydration process (0.5, 1.5, 3.5, 7.5, and 22.5 h). The samples were autoclaved after the aforementioned measurements were made, and they were then cooled to a temperature of 25 °C. The samples' overall electrical conductivity (Ect) was then determined using a formula presented by Bajji et al. [49].

K + and na + concentration
For each treatment, four uniform leaves were gathered. These leaves had a thorough tap water wash before receiving a deionized water rinse. Once the leaves were dried to a consistent weight at 65 °C. The leaves were then dried for 48 h at 65 °C until they reached a consistent weight. The dried leaves were then crushed (0.5 g) to determine their mineral contents. Following a 5 h dry ashing at a temperature of 550 °C, the plant tissue material was extracted using 10 mL of 2 N hydrochloric acid (HCl). After the digestive process, the amounts of potassium (K $^+$) and sodium (Na $^+$) were assessed using an flame photometer [50].

PIP1 expression
Samples of roots and leaves, were collected at 10:00 AM and promptly frozen in liquid nitrogen. RNA samples were extracted using the Iraizol Kit, a product manufactured by RNA Biotech Co in Iran. To ensure the purity of the RNA samples, DNase treatment was performed to remove any potential DNA contamination. The isolation of total RNA involved mechanical disruption of the

tissue in a mixture consisting of 1 mL of extraction buffer (composed of 0.1 M TRIS, 50 mM EDTA, 1% (w/v) SDS, 0.1 M NaCl, 1% tri-iso-propanylnaphthalene sulfonic acid sodium salt, 50 mM β-mercaptoethanol, pH 8.0) and 1 mL of phenol. Following the phenol: chloroform extraction and ethanol precipitation steps, RNA was acquired from the pellet obtained through ethanol precipitation using LiCl. The assessment of RNA integrity was conducted using denaturing agarose gel electrophoresis.

The Picodrop P200 device, which measured absorbance values at wavelengths of 260 nm and a ratio of 260/280 nm, was used to measure the quantity and quality of RNA. Revert Aid M-MuLV reverse transcriptase was used to transcribe 1 μg of DNase I-treated RNA and oligo into first-strand cDNA. The aquaporin (PIP1) gene's presence in the cDNA sample was evaluated using real-time PCR. The primer pairs utilized for the internal control gene, Actin 1, and the PIP1 amplification are shown in Table 1 supplemental. The ABI StepOne Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) and SYBR Green qPCR Master Mix were used for all qPCR reactions, which were run in triplicate. All reactions were subjected to the same thermal cycling conditions, which included an initial denaturation phase at 95 °C for 10 min, 40 cycles of denaturation at 94 °C for 15 s, annealing for 30 s at the primer pairs' ideal temperatures, and extension for 30 s at 72 °C. The amplified product's specificity for every pair of primers was confirmed using gel electrophoresis and melt curve analysis. The expression of PIP1 was determined using the $2^{-\Delta\Delta Ct}$ method [51].

ABA content quantification of leaves

For 30 min at 4 °C, 1 g of freshly chopped leaves was pulverized combined with 10 mL methanol (80%) and polyvinyl pyrrolidone (0.1 g). Whatman filter paper No. 1 was used to filter the mixture in a different conical flask. A lyophilizer (Model Christ −2–4, Germany) was used to vacuum-evaporate the filtrate. The vacuum-dried residue was then thoroughly mixed with 10 mL of 0.5 M phosphate buffer (pH 8), and the mixture was then redissolved. The material was extracted with ethyl acetate after having its pH brought down to 2.8 using HCl. The extract was lyophilized and then redissolved by phosphate buffer (pH 8, 5 mL of 0.5 M). To inject ABA into an HPLC-DAD (high-performance liquid chromatography-diode array detector), a reverse-phase column (Diamondsic, C18; 5 μm; 25 cm 4.6 mm) was utilized with a 0.45 mm filter. An acetic acid gradient solvent system with methanol in water at a flow rate of 4 mL min^{-1}. A 99.97% pure ABA standard (Sigma Aldrich) was utilized to calibrate the output peak in order to determine the degree of sample extraction. Utilizing the area under the curve and its success in the output peak, the extracted sample value was determined. A method developed for ABA detection

made use of an that was quickly linked to a photodiode array (PDA) device. With 10 L of the extract, a reverse-phase column (C18) (Zorbax SB-C18 100 A; 3.5 μm; 150 mm 2.1 mm) was loaded. 25 °C was chosen as the column temperature. The following operating parameters for the HPLC column were set up: a linear gradient to methanol-water (formic acid) 30:70 was utilized for 5 min after using methanol-water (formic acid) and water (10:90) for 5 min. After maintaining this state for 10 min, a linear gradient system was introduced. A methanol-water (formic acid) 45:55 solution was added and the column was maintained for 15 min in the ensuing 35 min. Within 45 min, all compounds had been successfully separated, and the column had been washed twice with a 95:5 methanol-water (formic acid) solution. Following the aforementioned procedure, the column underwent a 30 min re-equilibration with methanol-water (formic acid) at a 10:90 ratio. Li et al. [52] and Kishwar et al. [53] used the standard curve's peak area as a criteria to calculate sample concentrations.

Statistical analysis

A factorial experiment with three replications and a completely randomized design was used to perform the study. The Tallahassee, Florida, USA-based Statistix 8 program was used to analyze the data. A two-way analysis of variance (ANOVA) was performed on the data, and the means were tested for significance at a level of significance of $P \leq 0.05$ using the least significant difference (LSD) test. Principal component analysis (PCA) was performed using Statgraphics Centurion, Version XVI. A heatmap graph was generated through the website https://discover.nci.nih.gov/cimminer/oneMatrix.do.

Results

The interaction effect of salinity stress and melatonin levels on growth characteristics of the tomato

The variance analysis table of the effect of treatments on tomato growth characteristics is presented in Table 2. The highest shoot fresh and dry weight was observed in the Nano-Mel treatment under non-salinity conditions. Salinity decreased shoot fresh and dry weight compared to the control, while Mel and Nano-Mel treatments increased these weights under salinity stress (Fig. 1A, B). The application of Mel and Nano-Mel significantly increased both fresh and dry weights of roots compared to the control treatment under non-saline conditions. Under saline conditions, the fresh weight of roots treated with Mel and Nano-Mel was also significantly higher than that of the control treatment (Fig. 1C, D).

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Table 2 Analysis of variance of melatonin and salinity on growth characteristic of the shoot and root, water potential and RWC of tomato

Source of variation	df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Leaf water potential	Root water potential	RWC
Melatonin	2	14.169 **	0.337 **	13.115 **	0.041 **	604.390 **	51.690 **	669.632 **
Salinity	1	21.863 **	0.360 **	6.707 *	0.046 *	15.436 n.s	85.755 **	5.741 n.s
Melatonin × Salinity	2	4.389 *	0.095 *	1.675 *	0.009 *	1.514 *	38.041 **	207.864 *
Error	12	1.316	0.017	0.894	0.005	5.460	0.090	52.712
CV		26.36	27.77	39.96	45.76	24.77	4.14	10.19

ns: not significant, * significant at $P < 0.05$ and ** significant at $P < 0.01$ probability level, df: degree of freedom
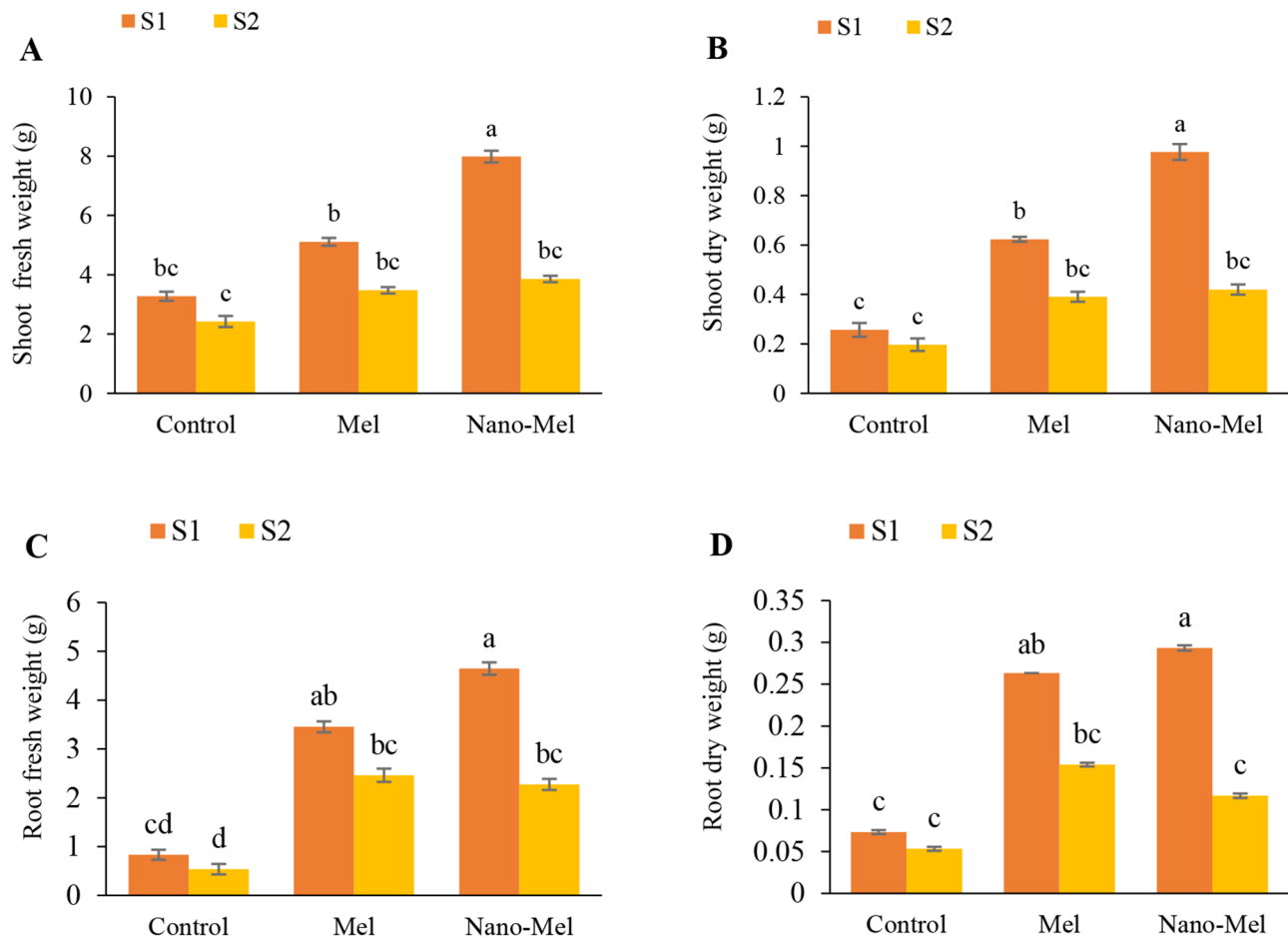

Fig. 1 Effect of melatonin and Nano-melatonin on shoot fresh weight (**A**), shoot dry weight (**B**), root fresh weight (**C**) and root dry weight (**D**) of tomato under salinity stress. Treatments include melatonin (Mel), Nano capsule-melatonin (Nano-Mel) and Control, and salinity treatment include 0 mM NaCl (S1) and 50 mM NaCl (S2). According to the LSD test, various letters indicate significant differences between treatments at ($P ≤ 0.05$). Error bars based on the standard error of the mean do reflect the uncertainty of the mean

The interaction effect of salinity stress and melatonin level on water potential in leaf and root and relative water content of the tomato

The variance analysis table of the effect of treatments on tomato water potential in leaf and root and RWC content is presented in Table 2. Application of Mel significantly reduced leaf water potential at both 0 and 50 mM NaCl concentrations, with the lowest value observed under salt stress when Nano-Me is employed (Fig. 2A). Although the highest root water potential was observed under salinity when Mel was employed, this increase was not statistically significant (Fig. 2B). As shown in Fig. 2C, the RWC decline under salinity stress and exogenous application of Mel significantly promote RWC of stress plants in comaraison with non-treated plants.
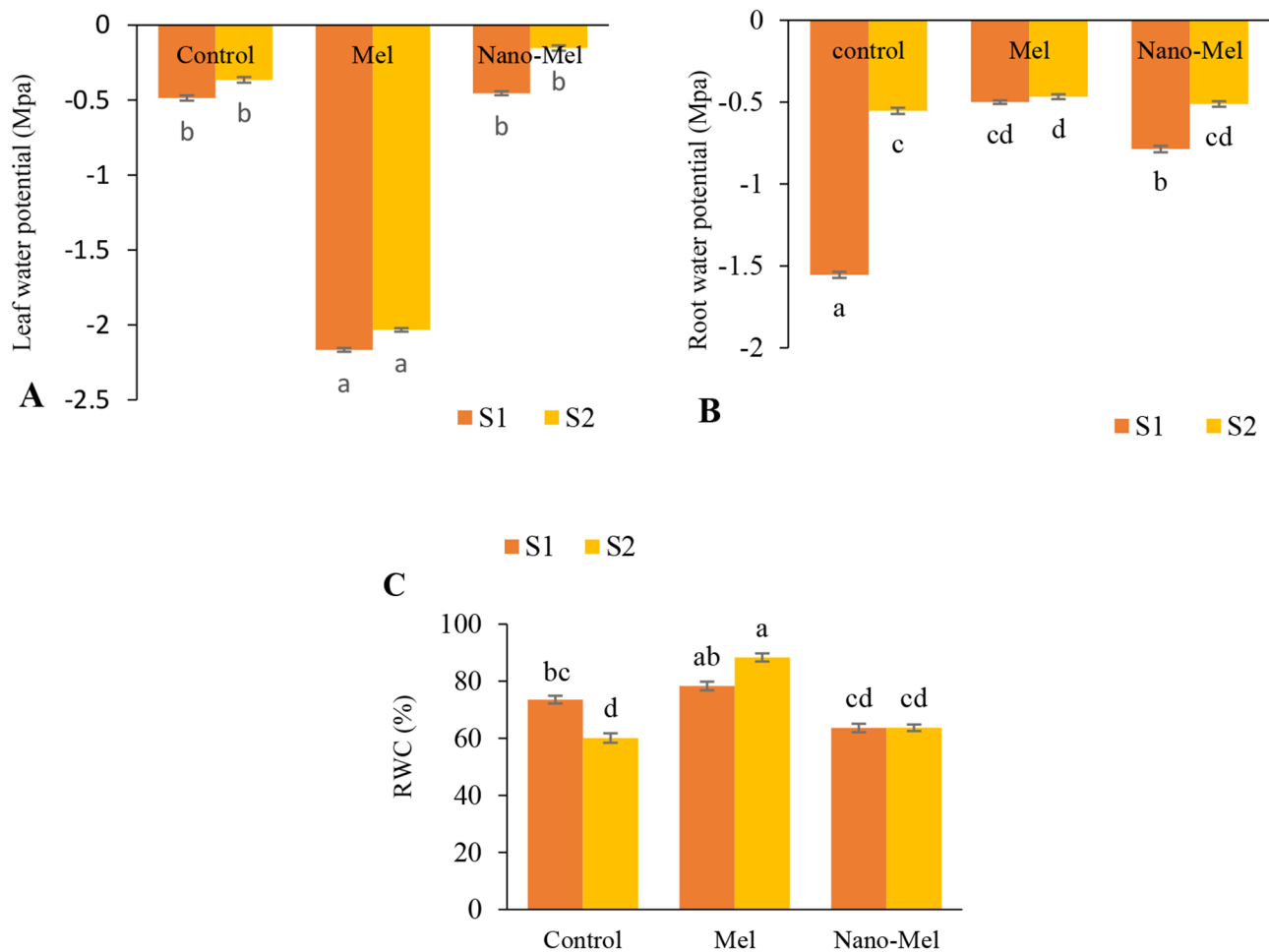
Fig. 2 Effect of melatonin and Nano-melatonin on leaf water potential (**A**) and root water potential (**B**) and RWC (**C**) of tomato under salinity stress. Treatments include melatonin (Mel), Nano capsule-melatonin (Nano-Mel) and Control, and salinity treatment include 0 mM NaCl (S1) and 50 mM NaCl (S2). According to the LSD test, various letters indicate significant differences between treatments at ($P \leq 0.05$). Error bars based on the standard error of the mean do reflect the uncertainty of the mean

Table 3 Analysis of variance of melatonin and salinity on chlorophyll content, chlorophyll fluorescence and chlorophyll a, b and carotenoid of tomato

Source of variation	df	Chlorophyll content	Chlorophyll fluorescence	Chlorophyll a	Chlorophyll b	Carotenoid
Melatonin	2	88.193 [n.s]	2.72E-05 [n.s]	15.938 [n.s]	0.618 [n.s]	46.349 [n.s]
Salinity	1	6.480 [n.s]	7.35E-04 [n.s]	343.640 [**]	10.849 [**]	265.240 [n.s]
Melatonin × Salinity	2	8.435 [n.s]	4.18E-04 [*]	114.727 [**]	3.088 [*]	889.917 [**]
Error	12	38.260	7.04E-03	12.047	0.559	100.651
CV		12.12	2.45E+00	34.33	34.56	49.77

[n.s]: not significant, [*] significant at $P < 0.05$ and [**] significant at $P < 0.01$ probability level, df: degree of freedom

The interaction effect of salinity stress and melatonin level on pigment characteristics of the tomato

The variance analysis table of the effect of treatments on tomato pigment characteristics is presented in Table 3. SPAD value did not reveal significant change under the influence of treatments (Fig. 3A). Chlorophyll fluorescence decreased with salinity when Mel and Nano-Mel were not used (Fig. 3B).

The highest content of chlorophyll *a* and *b* and carotenoid was observed at 0 mM NaCl when Nano-Mel was used, (Fig. 3C, D, E).

The interaction effect of salinity stress and melatonin level on the physiological characteristics of the tomato

The variance analysis table of the effect of treatments on tomato physiological characteristics is presented in Table 4. Antioxidant activity increased with
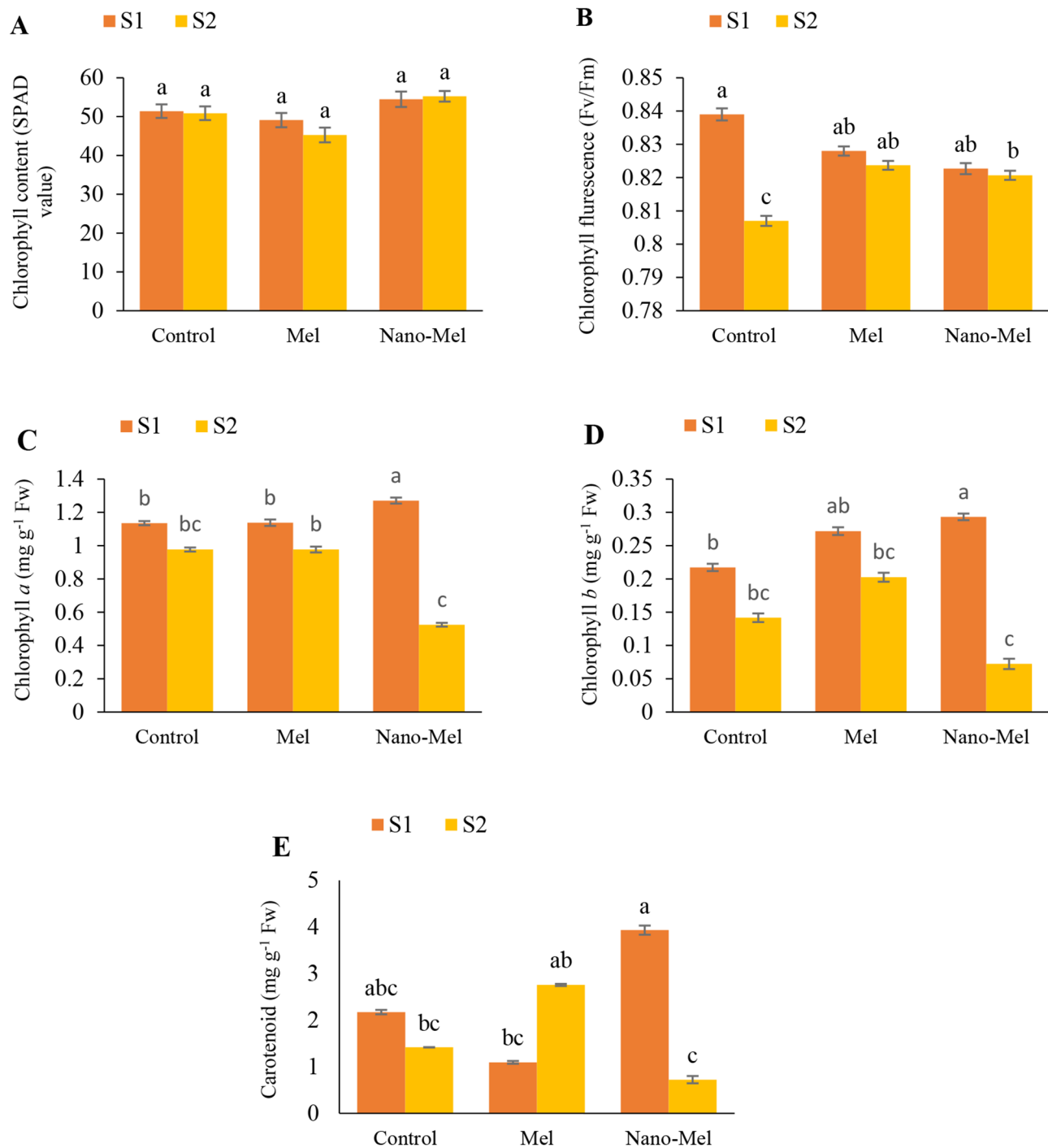
Fig. 3 Effect of melatonin and Nano-melatonin on chlorophyll content (**A**), chlorophyll flurescence (**B**), chlorophyll *a* (**C**), chlorophyll *b* (**D**) and carotenoid levels (**E**) of tomato under salinity stress. Treatments include melatonin (Mel), Nano capsule-melatonin (Nano-Mel) and Control, and salinity treatment include 0 mM NaCl (S1) and 50 mM NaCl (S2). According to the LSD test, various letters indicate significant differences between treatments at ($P \leq 0.05$). Error bars based on the standard error of the mean do reflect the uncertainty of the mean

the application of both Mel and Nano-Mel under both stress and non-stress conditions, with the highest values observed under saline conditions. The highest phenol content was observed with the Nano-Mel supplementation under saline conditions (Fig. 4A, B). Proline content

significantly increased in response to salinity stress conditions. However, in tomato plants treated with Mel and Nano-Mel, proline levels were decreased compared to those subjected to salinity alone (Fig. 4C). Protein soluble content was significantly affected by both salinity

Table 4 Analysis of variance of melatonin and salinity on physiological characteristics and na $^+$ and K $^+$ concentration of tomato

Source of variation	df	Antioxidant activity	Phenol Conc.	Prolin	Soluble protein	EL	Na $^+$ Conc.	K $^+$ Conc.
Melatonin	2	26.791 [n.s]	1937.160 [**]	188.547 [n.s]	0.069 [**]	193.760 [n.s]	45.906 [n.s]	28.184 [n.s]
Salinity	1	68.608 [n.s]	191.050 [n.s]	39.768 [n.s]	0.004 [n.s]	91.643 [n.s]	25.730 [n.s]	124.272 [n.s]
Melatonin × Salinity	2	545.843 [*]	130.860 [*]	39.515 [*]	0.010 [*]	62.278 [*]	1.324 [*]	30.230 [*]
Error	12	158.940	239.370	173.013	0.001	117.497	31.257	56.221
CV		21.76	77.57	81.13	10.01	29.53	43.77	27.06

[n.s]: not significant, [*] significant at $P<0.05$ and [**] significant at $P<0.01$ probability level, df: degree of freedom

and melatonin treatments. Under non-saline conditions, the application of Mel and Nano-Mel resulted in higher protein soluble content compared to the control. Under saline conditions, protein soluble content was significantly reduced in the control treatment. However, the application of Mel and Nano-Mel mitigated this reduction (Fig. 4D).

EL, an indicator of membrane stability and oxidative stress, was significantly influenced by salinity and melatonin treatments. Under saline conditions (50 mM NaCl), EL was significantly increased in the control treatment, reflecting enhanced membrane damage due to salt stress. However, the application of Mel significantly reduced EL under these conditions, indicating improved membrane stability (Fig. 4E).

The interaction effect of salinity stress and melatonin level on Na$^+$, and K$^+$concentration of the tomato

The variance analysis table of the effect of treatments on tomato Na $^+$, and K $^+$ concentration is presented in Table 4. The Na $^+$ concentration in leaves increased in response to salinity in both the control and Mel-treated plants compared to those treated with Nano-Mel (Fig. 5A). However, the application of Nano-Mel resulted in a significant reduction in Na$^+$concentration under saline conditions compared to both the control and Mel treatments. This indicates that Nano-Mel effectively mitigates the accumulation of Na $^+$ in leaves under salinity stress, enhancing the plant's tolerance to high salinity. The concentration of K $^+$ in leaves slightly decreased in response to salinity (Fig. 5B). However, there was no significant negative effect on K $^+$ concentration in plants treated with Mel and Nano-Mel compared to the control under saline conditions (Fig. 5B). This indicates that both Mel and Nano-Mel treatments help maintain K $^+$ homeostasis in leaves despite salinity stress.

The interaction effect of salinity stress and melatonin level on PIP expression and ABA contet of the tomato

According to the findings, PIP expression in roots and leaves decreased by salinity. PIP expression in root increased by Nano-Mel then Mel as compared with control. PIP expression in leaves increased with both Mel and Nano-Mel. Salinity increased the ABA content of leaves and Mel and Nano-Mel did not affect it (Table 5).

The correlation between various characteristics

EL, Na $^+$, K $^+$ Phenolic compound, and DPPH were the parameters changed greater by Mel and Nano-Mel under salinity (Red circle). Photosynthesis pigments that including chlorophyll *a*, chlorophyll *b* and carotenoid and SPAD were the parameters changed greater by Mel under optimum conditions (blue circle). Proline was the main parameter change with salinity and Mel and Nano-Mel had a lower effect on them as compared with other parameters (green circle) (Fig. 6A).

The parameters that are changed with Mel and Nano-Mel in tomatoes are RWC, DPPH, EL, and K $^+$ concentration. These parameter changes help the tomato reduce the stress condition and keep growth in the tomato (Fig. 6B).

According to the heatmap analysis, the experimental treatments had the most significant impact on traits such as RWC, SPAD chlorophyll content, and DPPH radical scavenging activity. These traits were grouped into a distinct cluster, indicating their similar responses to the treatments. Furthermore, the study found that carotenoid content, phenolic compounds, and EL were also influenced by the experimental treatments, while growth-related traits showed the least sensitivity to the treatments. The highest RWC under saline conditions was observed in the Me treatment (red color), while the control and Nano-Mel treatments exhibited the lowest RWC in saline conditions. Similarly, the highest DPPH radical scavenging activity under saline conditions was recorded in the Me and N-Me treatments, with the control treatment showing the lowest DPPH activity in both optimal and saline conditions. Regarding electrolyte leakage, the highest EL under saline conditions was observed in the control treatment, followed by the Nano-Mel treatment, while the lowest EL was found under optimal conditions. Additionally, the lowest leaf water potential was detected in the Mel treatment, irrespective of the optimal or saline conditions.The lowest leaf water potential (blue color) was observed in both optimum and salinity conditions in the Mel treatment (Fig. 6C).

Discussion

Melatonin is increasingly recognized as a crucial phytohormone that enhances plant tolerance to a wide range of biotic and abiotic stresses [54]. Its antioxidant properties
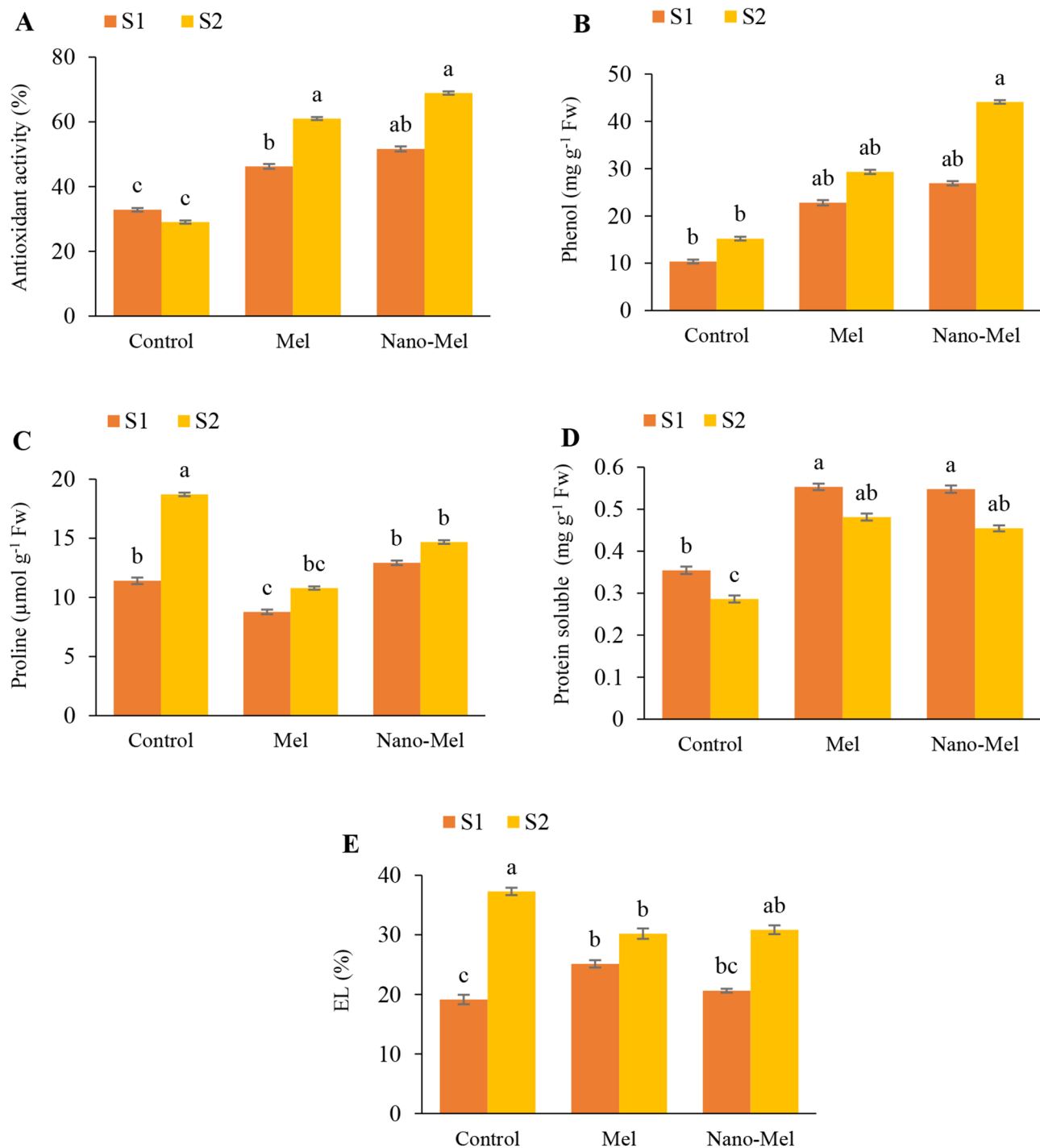
Fig. 4 Effect of melatonin and Nano-melatonin on antioxidant activity (**A**), phenol concentration (**B**), proline levels (**C**), soluble protein content (**D**) and EL (**E**) of tomato under salinity stress. Treatments include melatonin (Mel), Nano capsule-melatonin (Nano-Mel) and Control, and salinity treatment include 0 mM NaCl (S1) and 50 mM NaCl (S2). According to the LSD test, various letters indicate significant differences between treatments at ($P \leq 0.05$). Error bars based on the standard error of the mean do reflect the uncertainty of the mean

are vital in mitigating oxidative stress induced by environmental factors such as salt, drought, and pollutants. Melatonin effectively enhances the activity of key antioxidant enzymes, including ascorbate peroxidase, superoxide dismutase, and catalase, thereby reducing ROS

levels and preventing oxidative damage in plants [55, 56]. Beyond its antioxidant functions, melatonin positively influences plant growth and development under stress conditions. Studies have demonstrated its ability to promote root growth, stimulate lateral root formation, and
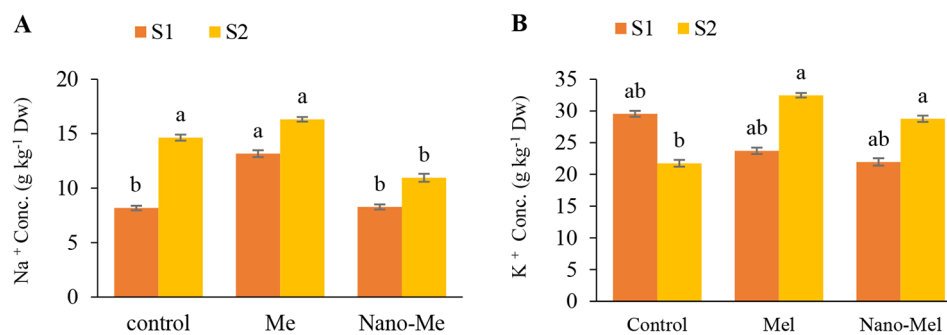
Fig. 5 Effect of melatonin and Nano-melatonin on Na $^+$ (**A**) and K $^+$ (**B**) concentrations of tomato under salinity stress. Treatments include melatonin (Mel), Nano capsule-melatonin (Nano-Mel) and Control, and salinity treatment include 0 mM NaCl (S1) and 50 mM NaCl (S2). According to the LSD test, various letters indicate significant differences between treatments at ($P \leq 0.05$). Error bars based on the standard error of the mean do reflect the uncertainty of the mean

Table 5 Real-time quantitative PCR analysis of PIP1 mRNA levels in leaf and root and amount of ABA after effect of salinity stress and melatonin application

	PIP1 expression in root	PIP1 expression in leaves	ABA in leaves
Non -salinity	0.8104a	2.0162a	14.503b
Salinity	0.5575b	0.9621b	41.783a
Melatonin application			
Control	0.1547c	0.457b	17.847a
Nano- Melatonin	1.0296a	1.2523a	18.612a
Melatonin	0.3383b	1.726a	16.674a

Within a column of stress and melatonin application, the means followed by the same letter are not significantly different at $P \leq 0.05$ according to the least significant difference test. Expression values are normalized by actin expressed transcripts

enhance overall biomass production, thereby improving crop yield in stress-prone environments [57].

There are several adverse abiotic factors that affect plant growth stages, including salinity [58]. In addition to the decrease in water availability and toxicity of sodium chloride, salinity may also cause osmotic stress, which reduces cell division and elongation, resulting in less plant growth [59]. Research by Khosravi et al. [60] showed that the foliar application of melatonin improved certain growth characteristics of the shoot and root in the hot pepper under water stress compated to control. Li et al. [61] found that melatonin improved low light tolerance in pepper seedlings by alleviation root growth limitation induced by low light stress, possibly by upregulating key genes in melatonin biosynthesis in roots. In our study, 50 mM NaCl reduced the fresh and dry weight of the shoots in the control treatment. Application of Nano-Mel in control conditions enhanced the shoot fresh and dry weight as compared to control treatment without Nano-Mel. It was found that the fresh and dry weight of the roots decreased in the presence of 50 mM NaCl compared to non saline conditions. As a result of the application of Nano-Mel under salinity stress conditions, the fresh and dry root weights increased as compared to the control treatment. Altaf et al. [62] found that drought stress negatively affected the root morphological traits in tomato, but melatonin pretreatment enhanced

root characteristics and contributed to better growth under stress. This is likely due to the role of melatonin as growth regulators and its ability to increase tolerance to salinity stress by improving mineral nutrition, water uptake, and scavenging ROS. Melatonin also regulates the biosynthesis of GA4 and ABA [63]. Nano-fertilizers have been shown to enhance physiological processes such as cell division and elongation, photosynthetic activity, and ATP production, contributing to improved nutrient utilization and stress resilience [64, 65].

Abiotic stress typically decrease in leaf water status and an increase in osmotic regulators [66]. Relative water content is commonly used to assess plants salt tolerance [67]. Our results showed that plants not treated with melatonin showed a decrease in relative water content when 50 mM NaCl was present. In contrast, plants treated with melatonin and Nano-Mel showed higher relative water content as compared to the control plants. According to these results, melatonin may contribute to plant stress mitigation. Salinity can disturb water relations by decreasing leaf osmotic potential. Sarwar et al. [68] found that salinity decreased leaf water potential and osmotic potential in cucumber plants, with a positive correlation between leaf proline and glycinbetaine contents and leaf water potential. Korkmaz et al. [69] reported that melatonin increased water potential in tomato plants under chilling stress by improving the root system, enhancing
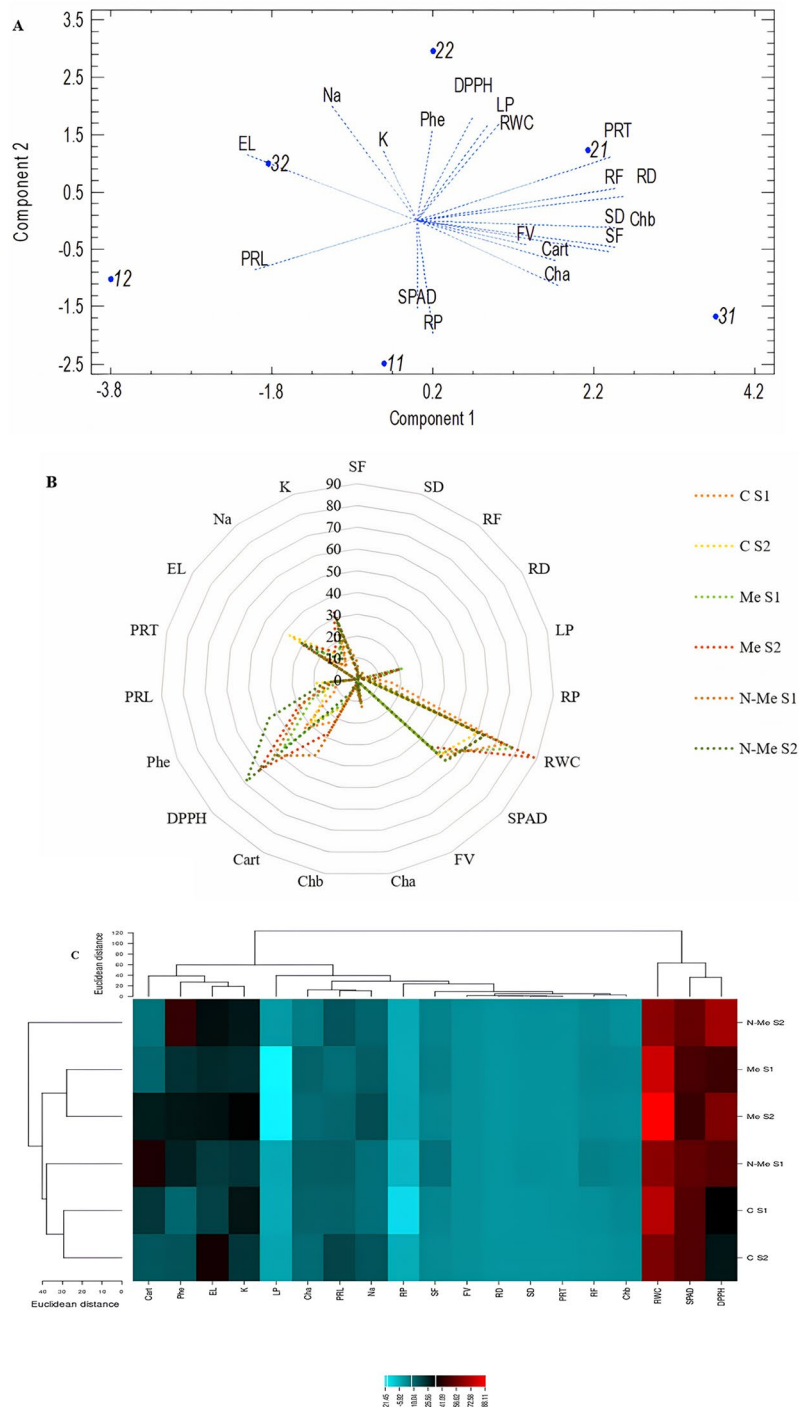
Fig. 6 Biplot analysis (**A**), spider (**B**) and heatmap (**C**) graph of interaction effect of melatonin and salinity stress on the tomato. Shoot fresh (SF), shoot dry weight (SD), root fresh weight (RF), root dry weight (RD), leaf water potential (LP), root water potential (RP), RWC (RWC), SPAD (SPAD), Fv/Fm (FV), chlorophyll a (Cha), chlorophyll b (Chb), carotenoid (Cart), antioxidant (DPPH), phenol (Phe), proline (PRL), protein soluble (PRT), EL (EL), sodium (Na), potassium (K). control, 0 mM NaCl (11), control, 50 mM NaCl (12), Me, 0 mM NaCl (21), Me, 50 mM NaCl (22), Nano-Me, 0 mM NaCl (31), Nano-Me, 50 mM NaCl (32)

water capture, and maintaining balance between absorption and loss.

Melatonin improves the root system by capturing water efficiently, which leads to a better balance between absorbing water and losing it to the atmosphere.

Melatonin reduces osmotic potential and increases leaf water potential when applied foliarly. Therefore, it maintains a higher turgor pressure necessary for maintaining stomatal conductance [70]. Our research showed that

Nano-Mel improved leaf water potential under salt stress as compared to the control treatment.

Chlorophyll fluorescence is negatively affected by salinity stress, which decreases the maximum efficiency of PSII (Fv/Fm) in sweet pepper plants [71]. This effect is due to inhibition of electron transport, damage to reaction centers at PSII sites, and disruption of the oxygen-evolving complex [72]. Salinity also decreases enzyme activity, affecting water-splitting enzyme complexes and electron transport chains, resulting in reduced Fv/Fm [71]. Our research showed that salt stress reduced chlorophyll fluorescence in tomato plants as compared to optimal conditions, but melatonin treatment increased chlorophyll fluorescence under salinity stress. Melatonin may help maintain high photosynthetic efficiency and chlorophyll integrity [73]. Altaf et al. [62] reported that melatonin mitigated cold-induced changes to Fo and Fv/Fm, positively impacting PSII quantum yield and electron donation under cold stress [74].

According to research by Bahcesular et al. [20], steady increases in salinity led to the development of a defense mechanism against salinity, which prevented salt stress from significantly altering photosynthetic pigments. There was no difference between the treatments in our experiment's chlorophyll concentration under salt stress compared to non-stress circumstances. Exogenous melatonin treatments increase the contents of photosynthetic pigments during salt stress by reducing chlorophyll degradation, maintaining chloroplast, and thus protecting the photosystems in response to stress [11, 75]. According to the findings of our study, melatonin application under salinity stress conditions there was a significant difference in the increase of chlorophyll *a* and *b* compared to the witness was absent. It has been demonstrated that melatonin can regulate the activity of 5-aminolevulinate synthase and the biosynthesis of succinyl-CoA, glycine, and porphyrins, which are precursors to chlorophyll [76]. Alternatively, melatonin may be able to inhibit the oxidative degradation of chlorophyll under salinity stress and the production of photosynthetic pigments is altered by salt stress, which is one of its important impacts [77]. Radi et al. [78] claims that the decrease in chlorophyll content under salt stress may be due to the development of proteolytic enzymes such chlorophyllase, which destroys chlorophyll and harms the photosynthetic system. Furthermore, the primary reason for the drop in chlorophyll levels is the reduction in aminolevulinic acid synthase under salt stress [79]. Salt stress is thought to produce oxidative stress, which may be brought on by the breakdown of the chloroplast structure, the photosynthetic apparatus, and the photooxidation of pigments, which results in a reduction in photosynthetic pigments [80]. Additionally, salt stress decreases the activity of the Rubisco enzyme, damages the membrane proteins that make up the photosynthetic apparatus, and makes the photosynthetic pigment complex unstable [81]. The results of our research showed that under salinity stress conditions, the application of melatonin in tomato plants increased the amount of carotenoid as compared to the control treatment. The result of a study by He et al. [82], revealed that endogenous melatonin potentially affects the transcription and translation levels of genes involved in carotenoid synthesis and occurs in increased carotenoid content in the tomato plants.

Plants have integrated antioxidant systems that maintain ROS levels and protect against oxidative stress [83]. Antioxidant enzymes detoxify H_2O_2 and defend against stress [84]. Ibrahim et al. [85] reported that increased antioxidant enzyme activities under water deficit reflect the integrated role of these enzymes in ROS detoxification under unfavorable conditions. Our research showed that antioxidant activity increased under salt stress compared to optimal conditions in melatonin treatment. Melatonin affects H_2O_2 burst, increasing antioxidant enzyme activities and enhancing the ascorbate glutathione cycle [86]. Ibrahim et al. [85] also demonstrated that melatonin boosted antioxidant enzyme activities in tomato plants under water deficit stress. Melatonin preserves tissue redox homeostasis by activating the antioxidant defense mechanism, increasing stress tolerance [85]. It acts as an antioxidant, enhancing antioxidant enzyme activities in leaves under salt stress [20]. Ali et al. [87] also reported increased antioxidant enzymes in tomato plants with melatonin under salt stress. Our study found that melatonin and Nano-Mel increased antioxidant activity under salt stress as compared to the absence of melatonin. By controlling the levels of gene transcription, melatonin acts as a free radical scavenger that eliminates ROS [88] and enhances the activity of antioxidant enzymes [55].

In the study by Attia et al. [89], salinity stress increased the content of total phenols in the shoots of tomato plants. Phenolic compounds exhibit potent antioxidant properties by effectively scavenging free radicals. This is achieved by their remarkable reactivity as electron or hydrogen donors, which enables them to stabilize unpaired electrons and perform chain-breaking functions. Additionally, phenolic compounds possess the potential to chelate transition metal ions, further enhancing their antioxidant capabilities [90]. The elevated amounts of phenolic compounds observed in response to stress may be attributed to disruptions in plant cell metabolic processes induced by stress, ultimately resulting in the accumulation of phenolic compounds [91]. Furthermore, the impact of melatonin on phenolic contents is attributed to its ability to stimulate diverse metabolic pathways and help the synthesis of numerous chemicals, particularly in response to stressful conditions [13]. The application of melatonin to various plant

species increased phenolic compounds [92, 93]. The results show that the application of salt stress raises phenol in compared to without salinity conditions. However, it is important to note that this rise was not found to be statistically significant. Specifically, the amount of phenol increased with the use of Mel and Nano-Mel in salinity condition compared to with without salinity. The use of Nano-Mel resulted in a threefold increase in phenol levels under conditions of salt-induced stress, as compared to the control. Hussain et al. [94], reported that Nanomaterials have a cumulative effect on antioxidant metabolites such as phenols, flavonoids and antioxidant enzymes which causes a decrease in the oxidative effects of ROS.

In order to thrive under salt stress, plants accumulate proline content. A significant increase in proline content was observed in salt-stressed tomato plants after exogenous melatonin application [95]. Using Melatonin can increase soluble carbohydrates by improving proline biosynthesis genes and Δ1-pyrroline-5-carboxylate synthetase activity [77]. This resulted in an increase in proline levels in salt-stressed plants with melatonin application, which was linked to a greater leaf osmotic potential that led to an increase in water uptake, boosting photosynthetic processes, and ultimately yields [77]. Unlike Khan et al. [95], our findings revealed that the control treatment had the highest amount of proline, which increased in saline conditions compared to melatonin. 50 mM NaCl increased proline as compared to 0 mM NaCl in our study. According to Ali et al. [87], salinity stress significantly increased tomato plant proline production, but melatonin significantly eliminated this effect.

Soluble protein is a crucial component of plant tolerance against stress and is needed for energy production. In response to environmental changes, stress has been shown to change the concentration of soluble proteins [96]. During stress, plant tolerant species release high levels of osmolytes in their cytosol that increase protein function and tissue water content [61, 97]. Our study's findings demonstrated that, in comparison to the absence of salt, the amounts of protein in the control, Mel, and Nano-Mel treatments fell when salt stress was administered. The protein levels produced during salt stress increased when melatonin was applied compared to the conditions without melatonin. An experiment conducted by Li et al. [61] demonstrated that pepper plant soluble protein levels decreased after short stresses, but increased after 48 and 168 h. A foliar spray of melatonin maintained soluble sugar levels after 48 and 168 h of stress, but kept cell water, and improved plant tolerance to low light by maintaining stable soluble protein contents. Stresses like salt and drought frequently cause the amount of protein to decrease, which in turn causes the synthesis of alternative proteins while suppressing the synthesis of some proteins [98]. High NaCl

concentrations may cause proteins to hydrolyze more quickly or produce fewer proteins overall [99]. Since melatonin increases the resistance and antioxidant capacity of plants exposed to various stresses, it can absorb active oxygen [100]. Excessive salinity triggers the production of ROS, which can cause oxidative damage to various compounds, including membrane proteins [101]. Therefore, melatonin potent involvement in enhancing antioxidant capacity and avoiding oxidative damage to proteins and their degradation may be one of the causes for the increase in protein content following its use in stressful situations.

The EL is one of the markers that may be used to evaluate the membrane's integrity under an abiotic stress situation. The findings of Altaf et al. [102] demonstrated that the increases in EL were compatible with the build-up of H_2O_2 and O^{-2} brought on by salinity stress, suggesting that excess ROS may have caused membrane damage from NaCl. Under stress, the administration of melatonin reduced the build-up of ROS and ROS-induced lipid peroxidation in tomato seedlings [103]. Altaf et al. [104], reported that EL caused by salt stress can be significantly reduced by 1 μM melatonin in tomato plants. Our study's findings also showed that in comparison to 0 mM NaCl, 50 mM NaCl damaged the cell membrane and increased EL in the control treatment. The EL might be reduced when Mel is applied under salinity stress as opposed to the control.

Plant growth may be slowed by salinity by preventing minerals from being absorbed into the plant. For plants to function properly, minerals must be absorbed sufficiently [105]. As reported by Ghorbani et al. [24], NaCl treatment of tomato seedling leaves and roots improved Na^+ concentrations and declined K^+ concentrations. Nevertheless, melatonin significantly reduced Na^+ uptake and transport to leaves under NaCl stress, resulting in K^+ homeostasis and an increase in the K^+/Na^+ ratio. In the control treatment, salt stress increased Na^+ and decreased K^+ in tomato plants. Under salinity, melatonin also decreased Na^+ transport to leaves, possibly due to a decrease in Na^+ loading in the xylem [106]. According to a study [107], melatonin relieves oxidative stress, regulates K^+ transporters, including HAK5, and maintains K^+ homeostasis in plants under salinity. Consequently, melatonin reduces Na^+ uptake by maintaining ionic homeostasis and improving plant growth under salinity stress [24].

Overall, it has been found that increased AQP expression improves cell membrane integrity under salt stress. Moreover, plants can modify the protein composition and membrane lipids in the plasma membrane to cope with abiotic stress, in addition to the possibility that AQPs would affect the cell's transport capacities [108]. In spite of the presence of 14 SlPIP genes in tomato, their

functions remain unclear [109]. According to Jia et al. [110], the expression of each SlPIP gene is different across tomato genotypes, and it may also be influenced by growth conditions. In this study, NaCl treatment reduced tomato SlPIP1;1 and SlPIP2;1 expression by 28.3 and 32.7%, respectively. According to Jia et al. [110], after 1 h of salt stress, root hydraulic conductivity is dramatically reduced, resulting in decreased SlPIP activity. With NaCl added, AQP can sense osmotic shock, thereby decreasing hydraulic conductivity [111]. In leaves, SlPIP1;3 and SlPIP2;8 were respectively decreased by 39.2% and 21.0% after 1 h of salt stress. As a result of salt stress, SlPIP expression is upregulated as an adaptive mechanism [112]. Our study's findings demonstrated that whereas the expression of PIP1 genes in the roots and leaves was decreased by salt stress, it was enhanced in these same tissues when Mel and Nano-Mel were applied in comparison to the control. Melatonin was used to enhance the fresh and dry weights of pepper roots and pepper shoots in the study by Khosravi et al. [55]. Consequently, root and shoot PIP expression increased significantly under stress. Furthermore, melatonin increased PIP expression in leaves under waterlogging, boosting RWC and shoot fresh weight.

Water status is maintained by physiological and biochemical processes induced by increases in ABA levels in response to abiotic stresses [113]. According to previous study, exogenous melatonin affects ABA levels in stressed plants in different ways. Mel seed pre-treatment decreased the quantity of ABA in cucumbers growing in salt by up-regulating two genes involved in ABA catabolism and down-regulating two genes involved in ABA production [114]. ABA synthesis and catabolism may be regulated through repeated exogenous melatonin treatments, which may reduce salt stress [115]. According to Zahedi et al. [116], increases in ABA and Mel concentrations in leaves and fruits would be predicted as a result of the positive effects of exogenous melatonin applications. As a result, the melatonin-induced antioxidant defense may involve both ABA-dependent and ABA-independent signaling pathways. In accordance with the aforementioned results, our investigation also showed that using Nano-Mel reduced decreased the amount of ABA in comparison to Mel.

Conclusion

In optimal conditions, Nano-Mel was more effective in enhancing photosynthetic pigments (chlorophylls and carotenoid) as well as growth parameters. On the other hand, Under salinity stress conditions, both forms of melatonin (Mel and Nano-Mel) were effective in increasing parameters such as K^+, phenolic compounds, and DPPH and decreasing EL. However, the use of Mel at a concentration of 500 µM is more effective than Nano-Mel.

Therefore the Mel is recommended for tomato production under saline conditions.

Data availability

All data are available upon request to the corresponding author, Maryam Mozafarian (Mozafarianmeimandi.Maryam@uni-mate.hu).

Declarations

Ethics approval and consent to participate

We compiled all the relevant institutional, national, and international guidelines and legislation for the cultivation of plants were followed.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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