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Alleviation of High Temperature Stress in Bell Pepper through Foliar Application of Melatonin and Sodium Nitroprusside

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ABSTRACT

The rise in global temperatures, resulting from global warming, imposes severe stress on plants, hindering their growth and development. This study aimed to investigate the effects of melatonin and sodium nitroprusside (SNP) on the growth of California Wonder green bell pepper under heat stress conditions. A factorial experiment using a completely randomized design with three replications was conducted. Plants were exposed to temperatures of 25°C (control), 35°C, and 40°C for 24 hours following foliar application of 0 µM, 50 µM, or 100 µM melatonin and SNP. Results showed that 100 µM melatonin increased shoot dry weight by 13.26% compared to the control. Under heat stress, leaf nitrogen content increased by 32.73% and 37.24% with 50 µM and 100 µM SNP, and by 9.61% and 23.72% with 50 µM and 100 µM melatonin, respectively. At 40°C, leaf potassium levels rose significantly—up to 72% with 100 µM SNP. Additionally, 100 µM SNP increased copper and iron levels by 17.96% and 202.98%, respectively. Foliar spraying with 100 µM melatonin improved photosynthetic traits (carotenoid and carbohydrate contents) and reduced malondialdehyde levels, enhancing stress tolerance. Hydrogen peroxide content decreased by 15.16% and 20.99% with 50 µM and 100 µM SNP, respectively, at 40°C. Both melatonin and SNP significantly enhanced the activity of antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase) under heat stress. Overall, 100 µM melatonin was most effective in mitigating heat-induced damage and improving the physiological and biochemical performance of green bell pepper seedlings..

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1. Introduction

As a result of global warming and temperature fluctuations, agricultural crops are increasingly exposed to heat stress due to temperatures exceeding their tolerance thresholds (Lavania *et al*., 2015; Janni *et al*., 2024; Saleem *et al*., 2024). High temperature is one of the most critical abiotic stressors affecting agricultural production (Parankusam *et al*., 2017; Balfagón *et al*., 2020). Pepper (Capsicum annuum), the most widely cultivated vegetable crop globally, accounts for approximately 36 million tons grown on 2 million hectares (FAO, 2021). However, its production is significantly constrained by heat sensitivity. Elevated day and night temperatures adversely affect pepper yield by disrupting carbohydrate metabolism and proline translocation, ultimately reducing productivity (Sangu *et al*., 2015). Temperatures above the optimal range impair plant growth and development (Wahid & Close, 2007; Walne and Reddy, 2022).

High temperature stress causes oxidative damage, lipid peroxidation, pigment degradation, protein breakdown, enzyme inactivation, and damage to macromolecules (Awasthi *et al*., 2015; Prasad *et al*., 2016; Santisree *et al*., 2018; Hong *et al*., 2023; Mondal, *et al*., 2023). It also accelerates the production of reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2), leading to cellular structural disruption (Khan *et al*., 2013a; Afzal *et al*., 2023). These free radicals damage membranes and macromolecules, impairing plant metabolism and yield. Hence, the rapid scavenging of free radicals by antioxidant enzymes is essential for plant survival under heat stress (Parankusam *et al*., 2017; Soufi *et al*., 2023; Rao *et al*., 2025).

Foliar application of osmoprotectants—including plant hormones, signaling molecules, and mineral elements—can enhance plant tolerance to heat stress due to their antioxidant and growth-promoting effects (Song *et al*., 2006; Khan *et al*., 2013b; Mostofa *et al*., 2013; Kazemi *et al*., 2023; Kazemi *et al*., 2024). Melatonin, a naturally occurring molecule with an indole ring structure and low molecular weight, functions as an antioxidant and plays a key role in plant growth and stress response. External application of melatonin has been shown to increase pepper tolerance to stress (Kaya and Doganlar, 2019; Korkmaz *et al*., 2021; Altaf *et al*., 2022; Khosravi *et al*., 2023). For example, foliar melatonin application in tall fescue (Festuca arundinacea) (Kostopoulou *et al*., 2015; Campos *et al*., 2019), Wheat (Buttar *et al*., 2020), Tomato (Khan *et al*., 2024; Khan *et al*., 2024), Mung bean (Kuppusamy *et al*., 2023) and sugar beet (Irfan *et al*., 2025) under heat stress reduced leaf malondialdehyde and ion leakage while increasing chlorophyll, protein content, and antioxidant enzyme activity. Melatonin also upregulates genes associated with antioxidant enzyme production.

Nitric oxide (NO), commonly applied via sodium nitroprusside (SNP), is a crucial signaling molecule involved in numerous physiological processes including metabolism, senescence, photosynthesis, and stress responses. NO plays an important role under environmental stress, including heat stress (Santisree *et al*., 2018, Rai *et al*., 2020; Iqbal *et al*., 2021). It reduces ROS accumulation by activating antioxidant enzymes like superoxide dismutase, catalase, and ascorbate peroxidase (Astier *et al*., 2017; Naaz *et al*., 2025). External NO application has been shown to enhance photosynthesis, cell water potential, and overall stress tolerance, thereby increasing yield (Zangani *et al*., 2023; Zhang *et al*., 2023). For example, 100 µM SNP mitigated the negative effects of high temperatures in wheat grown at 40°C (Iqbal *et al*., 2022). NO not only enhances antioxidant activity but also interacts with

other plant signaling molecules, modulating gene expression and osmolyte accumulation under heat stress. It helps maintain cellular structure and increases carotenoid levels, which are key for protection against photo-oxidative damage. For instance, SNP foliar application increased carotenoid content in Chrysanthemum morifolium under high temperature stress (Yang *et al*., 2011b).

Finding effective strategies to maintain crop production under high temperature stress is a major goal in agriculture (Sehar *et al*., 2023). Plants respond to heat stress through changes in vegetative growth, biochemical attributes, and nutrient composition. Thus, foliar application of compounds like melatonin and sodium nitroprusside may improve photosynthetic pigments, antioxidant systems, and mineral content, enhancing heat tolerance in Capsicum annuum. This study aimed to investigate the effects of melatonin and sodium nitroprusside under heat stress on the vegetative and biochemical characteristics of California Wonder green bell pepper seedlings. The goal was to establish an experimental basis for their foliar application under different temperature regimes.

2. Materials and Methods

2.1. Experiment condition

This study was conducted using a 3×5 factorial design within a completely randomized framework, comprising three replications. The first factor consisted of three temperature treatments (25°C, 35°C, and 40°C), while the second factor included five different foliar spray treatments: distilled water (control), melatonin at concentrations of 50 µM and 100 µM, and sodium nitroprusside at concentrations of 50 µM and 100 µM. A total of 45 pots were used in the experiment, with each replication including observations from three plants. California Wonder green bell pepper seedlings were grown in pots measuring 16 cm in height and 17 cm in diameter, filled with a uniform mixture of perlite, cocopeat, and soil. All plants were kept under uniform conditions in the greenhouse of the Faculty of Agriculture, Zabol University. Standard cultivation practices were applied equally across all treatments. When the plants developed five true leaves, foliar spraying was initiated using the designated concentrations (50 µM and 100 µM). Each treatment was applied three times at 24-hour intervals. The spraying was continued for each plant until runoff, and 50 ml of the solution was applied per plant. Control plants received 50 ml of distilled water. Foliar spraying was done using a 2-liter hand sprayer with a valve (Kalacarwash. CO). To minimize the impact of high light intensity during spraying and to prevent direct sunlight exposure, a 50% green shade net was used as a canopy. To apply heat stress, plants were placed in a growth chamber (EYELA LTI-1000SD) set to 65% relative humidity, with a photoperiod of 16 hours of light and 8 hours of darkness, and a light intensity of 270 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The temperature in the growth chamber was gradually increased from 25°C by 5°C every 24 hours until it reached 35°C and then 40°C. Plants were exposed to each temperature level for 24 hours. After the temperature treatments, the plants were returned to the greenhouse under the same growing conditions. One day after the heat stress treatments, various morphological traits were assessed. Additionally, samples were collected from fully developed leaves and immediately preserved in liquid nitrogen at -80°C for subsequent physiological and biochemical analyses.

2.2. Morphological Traits

Plant height, shoot fresh weight, shoot dry weight and number of leaf per plant were measured.

2.3. Physiological and biochemical traits

2.3.1. Leaf relative water Content (RWC)

First, samples (leaves) were placed in distilled water and kept at a temperature of 4°C for 24 hours. After 24 hours, leaf saturated weight was measured and leaves were placed in an oven at 70°C for 24 hours and subsequently dry weight of each treatments was measured. By putting obtained numbers in the following formula, RWC was determined and expressed as a percentage (Ritchie *et al*. 1990): RWC: Fw – Dw / Sw – Dw ×100%, Fw: leaf wet weight, Dw: leaf dry weight after being placed in an oven: Sw: leaf saturated weight after exposure to distilled water.

2.3.2. Leaf Membrane Stability Index

Leaf samples were placed in distilled water with a volume of 20mL and kept at room temperature for 24 hours. Then, electrical conductivity of distilled water with sample was measured as initial leakage. Secondary leakage was measured by measuring electrical conductivity of samples after heating them for one hour at 100°C. Membrane Stability Index was calculated through following equation (Shiferaw and Baker 1996)**.** Membrane Stability Index: (1-(initial leakage/ secondary leakage)× 100)

2.3.3. Leaf Chlorophyll, Carotenoid and SPAD

Leaf samples weighing 0.2 g were immersed in 8 mL of an ethanol-acetone solution (mixed in a 1:1 volume ratio) and kept at room temperature for 24 hours in the dark until the tissues turned pale. After incubation, the absorbance of the resulting extract was measured using a spectrophotometer at wavelengths of 645 nm (A_{645}), 663 nm (A_{663}), and 440 nm (A_{440}). The concentrations of chlorophyll and carotenoids were determined using specific formulas and reported as milligrams per gram of fresh leaf weight, based on the method described by L. Gratani (1992). To measure the SPAD, a hand-held chlorophyll meter model SPAD-502 Minolta, Japan was used.

Chlorophyll a = (19.3 ∗ A663 - 0.86 ∗ A645) V/100W
Chlorophyll b = (19.3 ∗ A645 - 3.6 ∗ A663) V/100W
Total Chlorophyll = Chl a + Chl b
Carotenoides = 100(A470) - 3.27(mg chl. a) - 104(mg chl. b)/227

V = The volume of filtered solution (superior solution from centrifugation), A= absorption of light at wavelengths of 663, 645 and 470 nm, W= Fresh weight of the sample in grams

2.3.4. Anthocyanin Determination in Leaves

The anthocyanin content in leaf tissues was quantified using a modified protocol based on Xu *et al*. (2005). Fresh samples were incubated in 20 mL of 60% ethanol solution for two hours in a water bath. After extraction, the solution was filtered through a volumetric flask. The absorbance of the filtrate was measured at 535 nm using a UV-Vis spectrophotometer, and results were expressed as milligrams per gram of fresh leaf mass.

2.3.5. Leaf Proline Quantification

A slightly adapted method from Bates *et al*. (1973) was employed to estimate proline levels. Leaf tissue (500 mg) was homogenized in 5 mL of 10% sulfosalicylic acid. The

homogenate was centrifuged at 15,000 × g for 20 minutes at 4°C. Then, 2 mL of the supernatant was mixed with 2 mL of acidic ninhydrin and 2 mL of glacial acetic acid. The reaction mixture was incubated in a 100°C water bath for one hour. Once cooled, 4 mL of toluene was added, and the mixture was vortexed briefly. The chromophore-containing upper phase was isolated and its absorbance was measured at 520 nm. Proline concentration was determined using a standard curve and reported as millimoles per gram of dry leaf tissue.

2.3.6. Total Soluble Carbohydrates

Soluble sugars were measured by the anthrone method following McCready *et al*. (1950), with some modifications. Anthrone reagent was prepared by dissolving 150 mg of anthrone in 100 mL of diluted sulfuric acid. For this, 76 mL of concentrated sulfuric acid was combined with 38 mL of distilled water. A 100 µL aliquot of the leaf extract was mixed with 3 mL of anthrone reagent and heated at 100°C for 20 minutes. After cooling, the absorbance was recorded at 620 nm, and sugar content was calculated accordingly.

2.3.7. Hydrogen Peroxide Estimation

Hydrogen peroxide (H_2O_2) content in leaves was determined as per Loreto and Velikova (2001). About 100 mg of leaf material was ground in 1 mL of 0.1% trichloroacetic acid. After centrifugation at 12,000 × g for 15 minutes at 4°C, 0.5 mL of the clear supernatant was mixed with 0.5 mL of phosphate buffer (10 mM, pH 7) and 1 mL of 1 M potassium iodide. Absorbance was measured at 390 nm and H_2O_2 levels were expressed in µmol per gram of fresh tissue using a standard curve.

2.3.8. Lipid Peroxidation (Malondialdehyde Content)

Membrane damage was assessed by quantifying malondialdehyde (MDA), an indicator of lipid peroxidation. Leaf samples (0.1 g) were macerated with 1 mL of 0.1% trichloroacetic acid in the presence of liquid nitrogen. The homogenate was centrifuged at 12,000 × g for 20 minutes at 4°C. The resulting supernatant (1 mL) was reacted with 3 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid and heated at 95°C for 30 minutes. After rapid cooling and centrifugation, absorbance was measured at 532 nm and corrected for non-specific turbidity at 600 nm. MDA content was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

2.3.9. Glutathione Content in Leaves

To assess glutathione levels, 0.2 g of fresh tissue was extracted in 2 mL of cold 5% trichloroacetic acid and centrifuged at 10,000 × g for 10 minutes at 4°C. A 0.5 mL portion of the supernatant was combined with 0.5 mL phosphate buffer (100 mM, pH 7.0) containing 0.5 mM EDTA and 50 µL of 3 mM DTNB. Absorbance was read at 512 nm. The glutathione concentration was interpolated from a standard curve and expressed in mg per gram of fresh mass.

2.3.10. Superoxide Dismutase (SOD) Activity

SOD activity was measured following the method of Giannopolitis and Ries (1977). The reaction mixture contained phosphate buffer (50 mM), methionine (13 mM), EDTA (0.1 mM), riboflavin (2 µM), and NBT (75 µM), with 10 µL of enzyme extract. The mixture was

illuminated for 15 minutes and then kept in darkness to stop the reaction. Absorbance was recorded at 560 nm, and SOD activity was expressed in µmol per gram of fresh tissue.

2.3.11. Catalase (CAT) Activity

CAT enzyme activity was determined spectrophotometrically based on the method of Beers and Sizer (1952). The reaction mixture included 5 µL of enzyme extract, 30 µL of potassium phosphate buffer, 665 µL of 0.5 mM ascorbic acid, and 1 µL of 0.1 mM H_2O_2. The decrease in absorbance at 240 nm was monitored, and results were expressed as µmol per gram of fresh weight.

2.3.12. Ascorbate Peroxidase (APX) Activity

APX activity was measured based on the oxidation rate of ascorbate, following the approach of Cakmak and Marschner (1992). The assay medium contained 3 mL of phosphate buffer (50 mM, pH 7.0), 100 µL of 5 mM ascorbate, 51.4 µL of hydrogen peroxide, and 50 µL of enzyme extract. The change in absorbance at 290 nm over one minute was used to calculate APX activity, expressed as µmol per gram fresh weight per minute.

2.3.13. Guaiacol Peroxidase (GPX) Activity

GPX activity was determined using the guaiacol oxidation assay described by Zhou *et al*. (2009). The assay solution included 3.35 µL of guaiacol, 3.8 µL of H_2O_2, 50 µL of enzyme extract, and 3 mL of sodium phosphate buffer. The increase in absorbance at 470 nm was recorded for 120 seconds. One unit of GPX activity was defined as the amount of enzyme required to oxidize 1 µmol of guaiacol per minute.

2.3.14. Mineral Content Analysis in Leaves

Leaf nitrogen content was assessed by combustion using a CHNS-O elemental analyzer (ECS4010, Italy). Phosphorus was quantified by a colorimetric method as outlined by Ryan (2008), following dry ashing at 500°C for 4 hours, acid digestion, and dilution. Absorbance was recorded at 420 nm. Calcium, potassium, magnesium, iron, copper, zinc, and manganese levels were measured using atomic absorption spectroscopy. Dried leaf samples (0.5 g) were digested in 10 mL of concentrated nitric acid at 70°C for 24 hours, diluted with deionized water, and analyzed with an FSAA 240 atomic absorption unit.

2.4. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SAS 9.4 software. Principal component analysis (PCA) and correlation matrices were performed using the FactoMineR and corrplot packages in R. Hierarchical clustering and heat map visualization were employed to explore treatment effects. Mean comparisons were carried out using the LSD test at a significance level of 5%.

3. Results

3.1. Macro- and Micronutrient Content in Leaves

The data in Table 1 indicate that the highest leaf nitrogen concentration (4.70% D.W.) was observed at 25°C, while the lowest value (3.05% D.W.) occurred at 40°C. According to Table 2, the highest nitrogen content (4.57% D.W.) was recorded in plants treated with 100 µM

sodium nitroprusside, whereas the lowest (3.33% D.W.) was found in the control treatment (0 µM). Leaf phosphorus levels, as shown in Table 1, reached a maximum of 0.36% D.W. at 25°C and a minimum of 0.21% D.W. at 40°C. Similarly, Table 2 shows that the highest phosphorus content (0.31% D.W.) was detected in plants treated with 100 µM sodium nitroprusside. However, this value was not significantly different ($P < 0.05$) from the 100 µM melatonin treatment. For calcium, Table 1 reveals that the maximum concentration (2.35% D.W.) was found at 25°C, while the minimum (1.58% D.W.) was recorded at 35°C. Leaf magnesium content also peaked at 25°C (0.42% D.W.) and was lowest at 35°C (0.28% D.W.), as shown in Table 1. In Table 2, the highest magnesium level (0.42% D.W.) was associated with 100 µM melatonin application. There were no significant differences in magnesium content among plants treated with 50 µM melatonin, 50 µM sodium nitroprusside, and 100 µM sodium nitroprusside. Potassium levels in the leaves, presented in Table 3, were highest (3.23% D.W.) in plants grown at 25°C and treated with 100 µM sodium nitroprusside. The lowest potassium concentration (1.25% D.W.) was observed at 40°C in the control group (0 µM). Additionally, no significant differences were found in potassium content among treatments with 0 µM, 50 µM, or 100 µM melatonin, or with 50 µM and 100 µM sodium nitroprusside at 25°C. Table 1 also shows that the highest iron concentration (168.59 mg/kg D.M.) was recorded at 25°C. No significant difference ($P < 0.05$) was observed in iron levels between the 35°C and 40°C temperature treatments. According to Table 2, the highest iron content (165.02 mg/kg D.M.) was measured in plants treated with 100 µM sodium nitroprusside, while the lowest (139.89 mg/kg D.M.) was found in the control. Regarding zinc, the highest concentration (44.49 mg/kg D.M.) was detected in plants treated with 100 µM melatonin, as shown in Table 1. Lastly, the greatest manganese content (87.28 mg/kg D.M.) was recorded in the treatment combining 25°C with 0 µM foliar application, according to Table 3.

3.2. Leaf Glutathione, Proline, and Carbohydrates

According to the results presented in Fig. 1a, the maximum glutathione content in leaves (0.77 $mg \cdot g^{-1}$ F.W.) was recorded under the 40°C treatment, while the minimum value (0.40 $mg \cdot g^{-1}$ F.W.) was observed at 25°C. As shown in Fig. 1b, leaf proline concentration peaked at 25.46 $mmol \cdot g^{-1}$ D.W. under the 40°C treatment and reached its lowest level (11.99 $mmol \cdot g^{-1}$ D.W.) at 25°C. Similarly, Fig. 1c illustrates that total carbohydrate content in leaves was highest (124.91 $mg \cdot g^{-1}$ F.W.) at 40°C and lowest (89.25 $mg \cdot g^{-1}$ F.W.) at 25°C. The data in Fig. 2a also show that the application of 100 µM melatonin resulted in the highest carbohydrate accumulation in leaves (116.10 $mg \cdot g^{-1}$ F.W.), whereas the control treatment (0 µM) had the lowest value (99.60 $mg \cdot g^{-1}$ F.W.). Moreover, no significant difference ($P < 0.05$) was found between treatments with 100 µM melatonin and 100 µM sodium nitroprusside, as both belonged to the same statistical group in terms of leaf carbohydrate content.

3.3. Leaf Membrane Lipid Peroxidation, Hydrogen Peroxide, and Antioxidant Enzymes (SOD, CAT, APX, GPX)

As illustrated in Fig. 2b, the highest level of lipid peroxidation (2.34 $\mu mol \cdot g^{-1}$ F.W.) in leaf tissues was recorded in the control treatment (0 µM foliar application), while the lowest value (1.68 $\mu mol \cdot g^{-1}$ F.W.) occurred with the 100 µM sodium nitroprusside treatment. Additionally, no statistically significant difference ($P < 0.05$) was observed in lipid peroxidation levels between the control and the 50 µM melatonin treatment.

Table 1. Effects of temperatures on Leaf Nitrogen, Phosphorus, Calcium, Magnesium and Iron in California Wonder green bell pepper

T (°C)	Nitrogen	Phosphorus	Calcium	Magnesium	Iron (PPM D.W)
		% D.W			
25	4.70a	0.36a	2.35a	0.42a	168.59a
35	4.30b	0.29b	1.58c	0.38b	142.95b
40	3.05c	0.21c	2.08b	0.28c	146.52b
LSD	0.18	0.01	0.12	0.01	7.33

Temperature: T, Differences letters indicate significantly different values at $p < 0.05$

Table 2. Comparison of means effects of foliar application of melatonin and Sodium Nitroprusside on Leaf Nitrogen, Phosphorus, Magnesium, Zinc and Iron in California Wonder green bell pepper

Foliar Application (µM)	Nitrogen (% Dry mass)	Phosphorus (% Dry mass)	Magnesium (% Dry mass)	Zinc (mg/kg Dry mass)	Iron (mg.kg Dry mass)
0	3.33d	0.27c	0.30c	33.69c	139.89c
50 M	3.65c	0.27c	0.36b	41.49b	149.69b
100 M	4.12b	0.30ab	0.42a	44.49a	153.62b
50 SNP	4.42a	0.29bc	0.36b	35.53c	155.22b
100 SNP	4.57a	0.31a	0.36b	35.44c	165.02a
LSD	0.23	0.02	0.02	2.48	9.41

Melatonin (M) and Sodium Nitroprusside (NSP), Differences letters indicate significantly different values at $p< 0.05$. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside

Table 3 Comparison Means Effects of Foliar application (Melatonin and Sodium Nitroprusside) and Temperatures, on Leaf Manganese, Potassium and Copper in California Wonder green bell pepper.

T (°C)	Foliar Application (µM)	Potassium (% Dry mass)	Manganese (mg.kg Dry mass)	Copper (mg.kg Dry mass)
25	0	2.99a	87.28a	13.69a
	50 M	3.12a	80.56b	12.74a
	100 M	3.20a	77.05b	13.51a
	50 NSP	3.00a	76.08b	13.39a
	100 NSP	3.23a	75.60b	12.49ab
35	0	2.19bcd	68.61cd	6.77g
	50 M	2.32bc	76.68b	9.20ef
	100 M	2.40bc	75.68b	9.42e
	50 NSP	2.20bcd	75.03b	11.18bc
	100 NSP	2.43b	74.68bc	10.94cd
40	0	1.25f	46.28f	3.36h
	50 M	1.94d	45.16f	7.87fg
	100 M	2.15bcd	47.07f	7.75g
	50 NSP	1.57e	58.13e	10.18cde
	100 NSP	2.13cd	67.35d	9.61de
LSD		0.28	6.39	1.41

Temperature:T, Melatonin:M, Sodium nitroprusside:SNP, Differences letter. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside

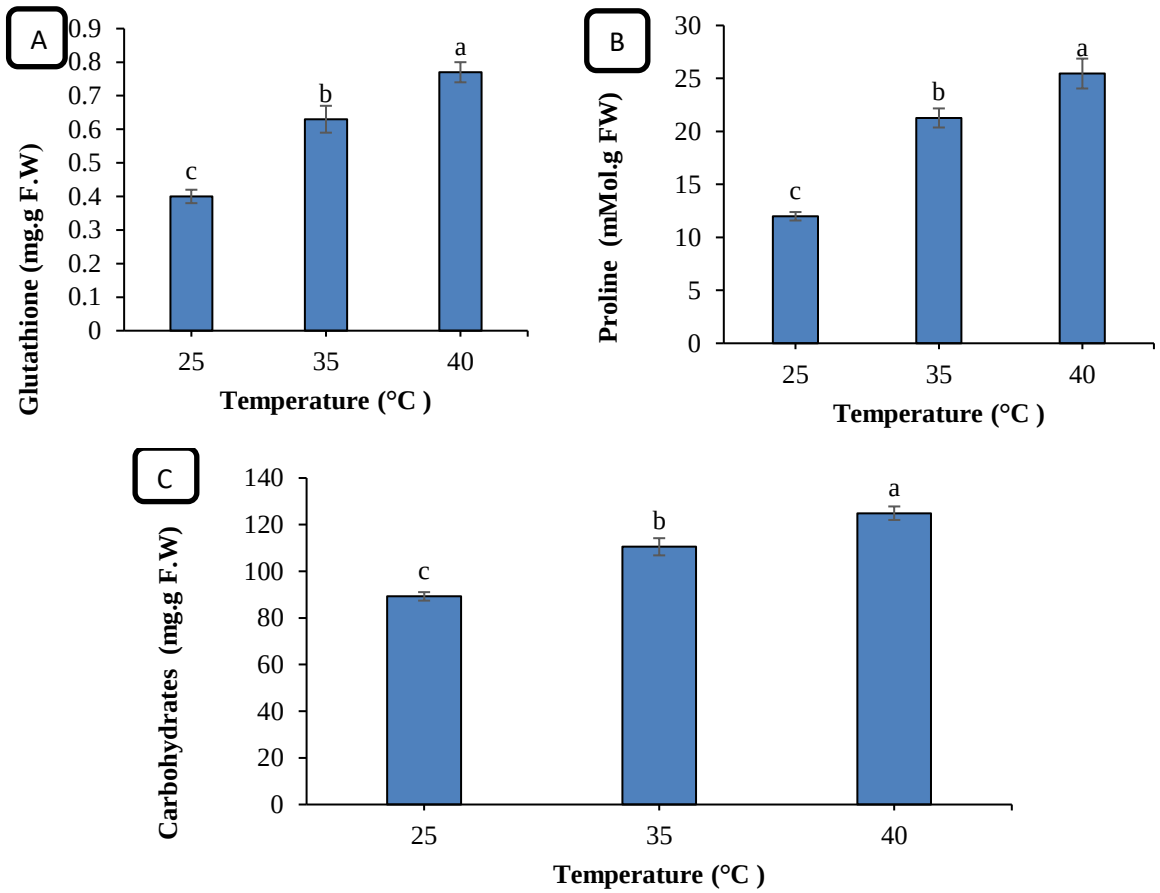

Fig. 1. Comparison of means effects of temperatures on Leaf Glutathione (A), Leaf Proline (B) and Leaf Carbohydrates (C) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05 and 25°C Temperature: Control.rs indicate significantly different values at p < 0.05. Temperature 25 °C was Control treatment.

The data in Table 4 show that the highest hydrogen peroxide content in leaves (3.57 µmol·g⁻¹ F.W.) was observed at 40°C with 100 µM melatonin, while the lowest value (1.09 µmol·g⁻¹ F.W.) occurred at 25°C with the same melatonin concentration. Additionally, there was no significant difference ($P < 0.05$) between the treatments of 40°C with 100 µM melatonin and 40°C with the control (0 µM). In terms of superoxide dismutase (SOD) activity, the highest value (89.84 µmol·g⁻¹ F.W.) was recorded under the 40°C condition with 100 µM melatonin, whereas the lowest activity (50.91 µmol·g⁻¹ F.W.) occurred at 25°C with 50 µM sodium nitroprusside (Table 4). No significant differences were found ($P < 0.05$) among the treatments with 50 µM and 100 µM melatonin or sodium nitroprusside at 40°C, indicating they belong to the same statistical group. Regarding catalase (CAT) activity, the maximum value (3.14 µmol·g⁻¹ F.W.) was detected at 25°C under the control treatment, while the minimum (1.05 µmol·g⁻¹ F.W.) was measured at 40°C, also under the control condition (Table 4). Furthermore, no significant differences ($P < 0.05$) were found among the 25°C treatments with 0 µM, 50 µM, and 100 µM melatonin, as well as with 50 µM and 100 µM sodium nitroprusside, all of which were statistically similar. The results for ascorbate peroxidase (APX) activity indicated the highest value (53.25 µmol·g⁻¹ F.W.) at 25°C with no foliar application, while the lowest (27.32 µmol·g⁻¹ F.W.) was observed at 40°C under control conditions (Table 4). According to Table 4, guaiacol peroxidase (GPX) activity reached its peak (0.60 U·mg⁻¹ protein) at 40°C with 100 µM melatonin. However, no significant differences ($P < 0.05$) were noted among treatments with 0 µM, 50 µM, and 100 µM melatonin, or with 50 µM and 100 µM sodium nitroprusside, as they all fell within the same statistical category.

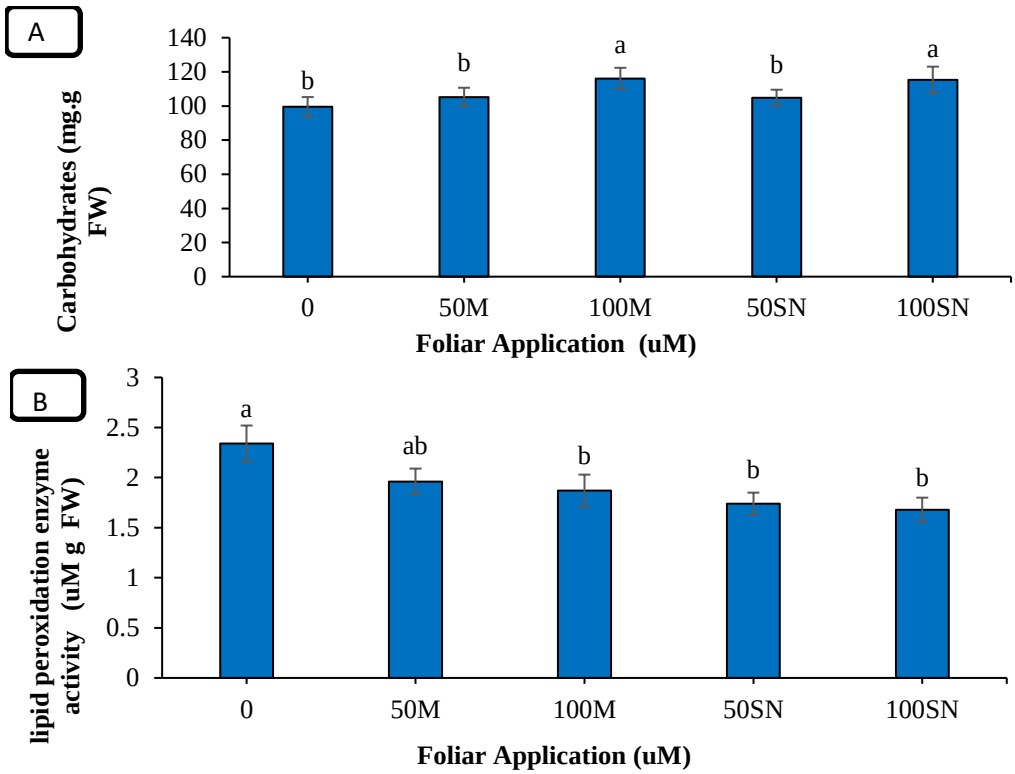

Fig. 2. Comparison of means effect of foliar application with melatonin (M) and sodium nitroprusside (SNP) on Leaf carbohydrates (A) and of leaf membrane lipid peroxidation enzyme activity (LPLM, B) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside.

Table 4. Comparison Means Effects of Foliar Spraying (Melatonin and Sodium Nitroprusside) and Temperatures on LeafHydrogen Peroxide, Superoxide Dismutase, Catalase, Ascorbate Peroxidase and Guayacol Peroxidase in California Wonder green bell pepper

T (°C)	Foliar Application (µM)	H_2O_2 (µM/g FW)	SOD (µM/g FW)	CAT (µM/g FW)	APX (µM/g FW)	GPX (Unit mg-1 protein)
25	0	1.34i	52.81fg	3.14a	53.25a	0.13f
	50 M	1.14i	51.41g	2.96ab	51.43a	0.13f
	100 M	1.09i	51.46g	3.00ab	52.19a	0.14f
	50 NSP	1.14i	50.91g	2.98ab	51.83a	0.13f
	100 NSP	1.13i	51.06g	3.13a	52.26a	0.13f
35	0	2.42fe	60.48f	2.01fg	39.36b	0.30e
	50 M	2.03gh	72.75de	2.39de	33.56cd	0.41d
	100 M	1.92h	78.67cde	2.76bc	31.31def	0.45c
	50 NSP	2.37fg	79.51bcd	2.39de	31.91de	0.42cd
	100 NSP	2.01h	80.50bcd	2.53cd	29.00ef	0.44c
40	0	3.43ab	70.86e	1.05h	27.32f	0.40d
	50 M	3.16bc	83.20abc	1.88fg	38.43b	0.50b
	100 M	3.57a	89.84a	2.24def	3618bc	0.60a
	50 NSP	2.91cd	83.72abc	1.86g	36.78bc	0.50b
	100 NSP	2.71de	87.48ab	2.13efg	33.87cd	0.50b
LSD		0.34	8.57	0.36	4.21	0.02

Temperature:T, Melatonin:M, Sodium nitro prusside:SNP, Differences letters indicate significantly different values at $p < 0.05$. Hydrogen Peroxide (H2O2), Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) and Guayacol Peroxidase (GPX). Foliar treatment were include: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside.

3.4. Total Leaf Chlorophyll, Carotenoids, Anthocyanin, and SPAD Values

According to Fig. 3A, the highest leaf chlorophyll content (1.95 mg·g⁻¹ F.W.) was recorded at 25°C, while the lowest value (1.31 mg·g⁻¹ F.W.) occurred at 40°C. There was no significant difference ($p < 0.05$) in chlorophyll levels between the 25°C and 35°C treatments. Figure 4a shows that leaves treated with 100 µM sodium nitroprusside had the greatest chlorophyll concentration (1.93 mg·g⁻¹ F.W.), whereas the control group (0 µM) exhibited the lowest (1.46 mg·g⁻¹ F.W.). No significant difference ($p < 0.05$) was found between the 100 µM melatonin and 100 µM sodium nitroprusside treatments; both belonged to the same statistical category for chlorophyll content. In Fig. 3B, leaf carotenoid levels peaked at 25°C with 0.68 mg·g⁻¹ F.W. and were lowest at 40°C with 0.25 mg·g⁻¹ F.W. Similarly, Fig. 4b indicates that the highest carotenoid content (0.67 mg·g⁻¹ F.W.) was found in leaves treated with 100 µM melatonin, while the lowest (0.39 mg·g⁻¹ F.W.) corresponded to the untreated control (0 µM). Table 5 data reveal that the maximum leaf anthocyanin concentration (2.83 mg·g⁻¹ F.W.) was observed at 35°C under the 0 µM foliar application, whereas the minimum (1.90 mg·g⁻¹ F.W.) was recorded at 25°C with 50 µM sodium nitroprusside. Furthermore, SPAD values ranged from a high of 48.83 at 25°C with 100 µM sodium nitroprusside to a low of 27.31 at 40°C without foliar treatment (0 µM), as shown in Table 5.

Table 5 Comparison Means Effects of Foliar Application (Melatonin and Sodium Nitroprusside) and Temperatures on Leaf anthocyanins and Leaf spad in California Wonder green bell pepper

T (°C)	Foliar Application (µM)	Anthocyanins (mg/g F.W)	Spad
25	0	2.16ef	35.33cd
	50 M	2.41bcd	35.80c
	100 M	2.14efg	35.40c
	50 NSP	1.90h	45.31b
	100 NSP	1.99gh	48.83a
35	0	2.83a	35.19cd
	50 M	2.49bc	34.01cde
	100 M	2.27de	33.62cde
	50 NSP	2.34cd	32.71edf
	100 NSP	2.34cd	33.27cdef
40	0	2.06fgh	27.31h
	50 M	2.52b	28.48gh
	100 M	2.16ef	32.15ef
	50 NSP	2.09fg	32.40ef
	100 NSP	2.11efg	30.92fg
LSD		0.17	2.67

Temperature:T, Melatonin:M, Sodium nitroprusside:SNP, Differences letters indicate significantly different values at $p < 0.05$. Foliar treatment were include: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside.

3.5. Relative Water Content (RWC) and Leaf Membrane Stability Index

As shown in Fig. 5a, the highest relative water content (RWC) was observed at 25°C (71.77%), while the lowest value (54.07%) occurred at 40°C. Similarly, Fig. 6a indicates that leaves treated with 100 µM melatonin exhibited the highest RWC (69.94%), whereas the untreated control (0 µM) had the lowest (58.23%). These results also revealed no statistically significant difference ($p < 0.05$) in leaf relative water content between treatments with 100 µM and 50 µM melatonin. Regarding leaf membrane stability, Fig. 5b illustrates that the highest Membrane Stability Index (MSI) was recorded at 35°C (34.15%), with the lowest

value (27.06%) measured at 40°C. However, no significant difference ($p < 0.05$) was detected between the MSI values at 35°C and 40°C.

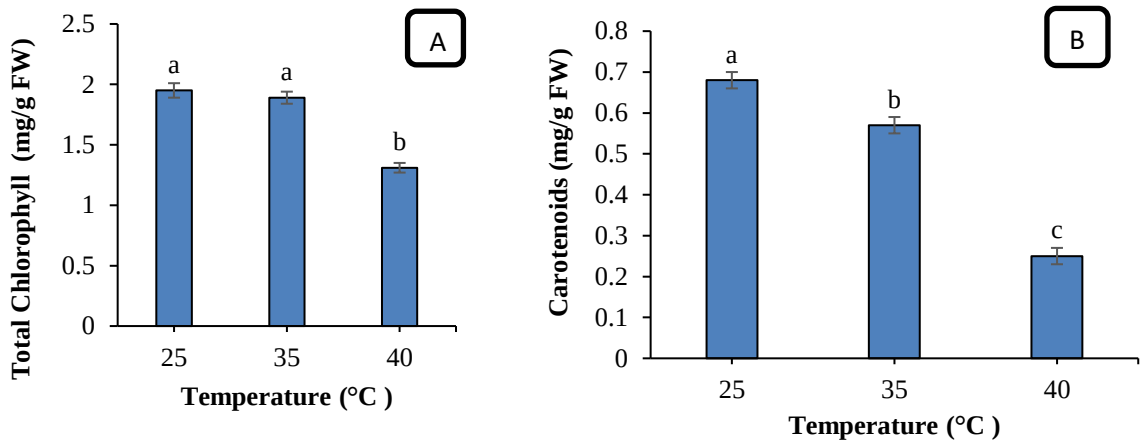

Fig. 3. Comparison of means effects of temperatures on leaf chlorophyll (A) and Leaf Carotenoids (B) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Temperature 25°C was Control treatment.

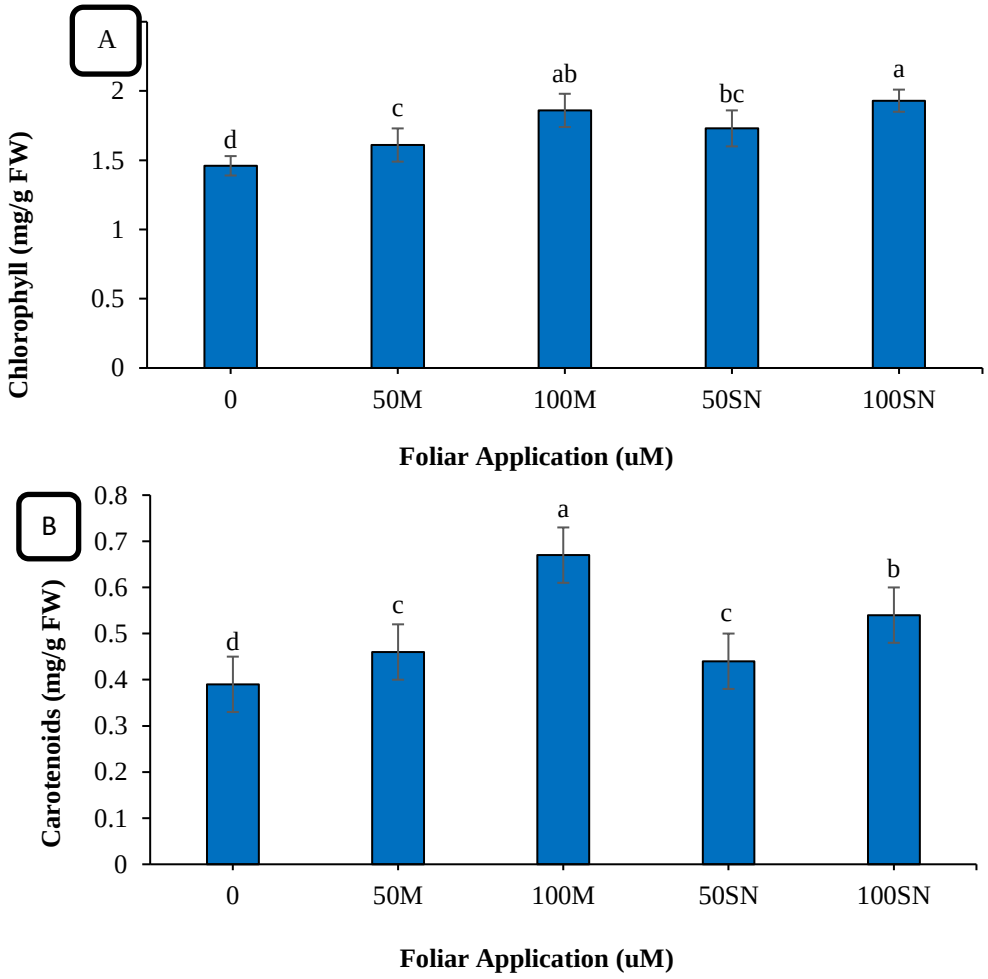

Fig. 4. Comparison of means effect of foliar application with melatonin (M) and sodium nitroprusside (SNP) on leaf chlorophyll (A) and Leaf Carotenoids (B) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside

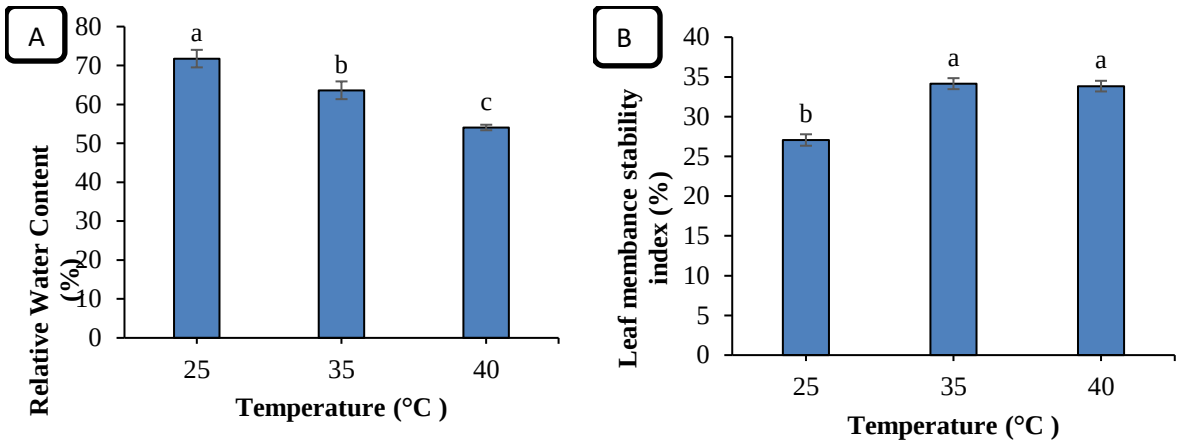

Fig. 5. Comparison of means effect of temperature on Leaf relative water content (A) and Leaf Membrane Stability Index (B) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Temperature 25 °C was control treatment.

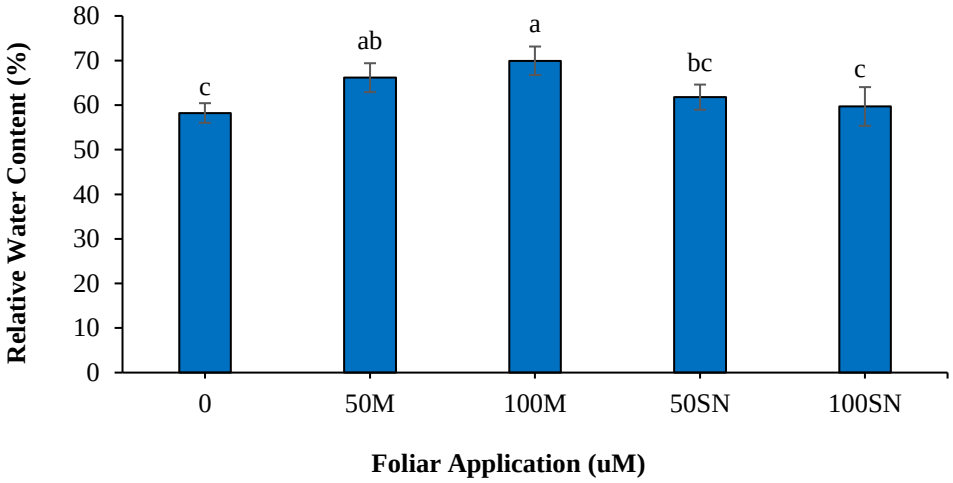

Fig. 6. Comparison of means effect of foliar application with melatonin (M) and sodium nitroprusside (SNP) on Leaf relative water content (A) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside.

3.6. Plant Height, Shoot Fresh Weight, Shoot Dry Weight, and Leaf Number

The results demonstrated that the greatest plant height (51.85 cm) occurred at 25°C, while the shortest plants (45.37 cm) were observed at 40°C, as shown in Fig. 7a. No significant difference ($p < 0.05$) was found between the plant heights at 25°C and 35°C. According to Fig. 7b, the tallest plants (54.82 cm) were recorded with 100 µM sodium nitroprusside treatment, and the shortest (45.38 cm) under the control (0 µM). Additionally, there was no significant difference ($p < 0.05$) between the 50 µM and 100 µM sodium nitroprusside treatments. Fig. 7c illustrates that shoot fresh weight was highest (302.53 g F.W.) at 25°C and lowest (250.55 g F.W.) at 40°C. Similarly, Fig. 8a shows the maximum shoot fresh weight (292.83 g F.W.) under 100 µM melatonin treatment and the minimum (264.89 g F.W.) in the control group (0 µM). There was no significant difference ($p < 0.05$) between shoot fresh weights in the 50 µM and 100 µM melatonin treatments, with both belonging to the same statistical group. Regarding shoot dry weight, the highest value (49.72 g D.W.) was observed at 25°C, and the lowest (39.01 g D.W.) at 40°C, according to Fig. 7d. Data in Fig. 8b indicate

that shoot dry weight peaked at 48.61 g D.W. with 100 µM melatonin treatment and was lowest (41.38 g D.W.) following 50 µM sodium nitroprusside application. Finally, Fig. 9 reveals that the greatest number of leaves (37.68) was recorded at 25°C with 50 µM sodium nitroprusside treatment, while the fewest leaves (24.86) occurred at 40°C under the control (0 µM) foliar application.

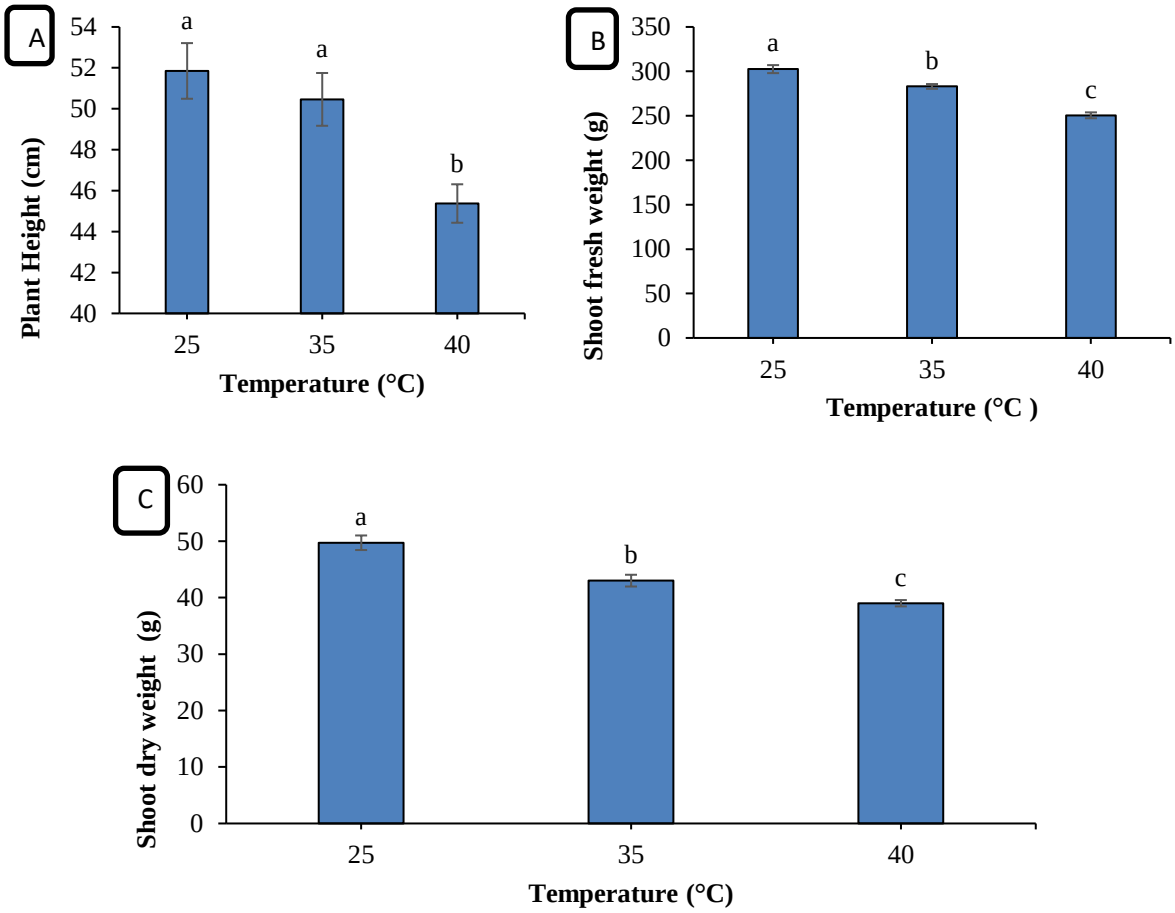

Fig. 7. Comparison of means effect of temperature on Plant Height (A), shoot fresh weight (B) and shoot dry weight (C) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Temperature 25°C was Control treatment.

3.7. Pearson's Correlation Analysis

A heat map based on Pearson's correlation was created to examine the relationships among growth, photosynthetic, oxidative stress, and antioxidant parameters following foliar application of melatonin and sodium nitroprusside under various temperature conditions (Fig. 10). The analysis revealed that shoot fresh and dry weights were positively correlated with leaf catalase and ascorbate peroxidase enzyme activities, as well as with leaf phosphorus, potassium, magnesium, zinc, and carotenoid contents. Conversely, shoot fresh and dry weights showed negative correlations with leaf hydrogen peroxide, glutathione, proline, and superoxide dismutase levels. Furthermore, leaf hydrogen peroxide exhibited negative correlations with leaf nitrogen, phosphorus, potassium, magnesium, manganese, zinc, and copper concentrations, while showing a positive correlation with leaf guaiacol peroxidase activity. Leaf nutrients such as nitrogen, phosphorus, and potassium were negatively correlated with superoxide dismutase activity, glutathione, and proline but positively correlated with manganese, iron, zinc, and copper (Fig. 10).

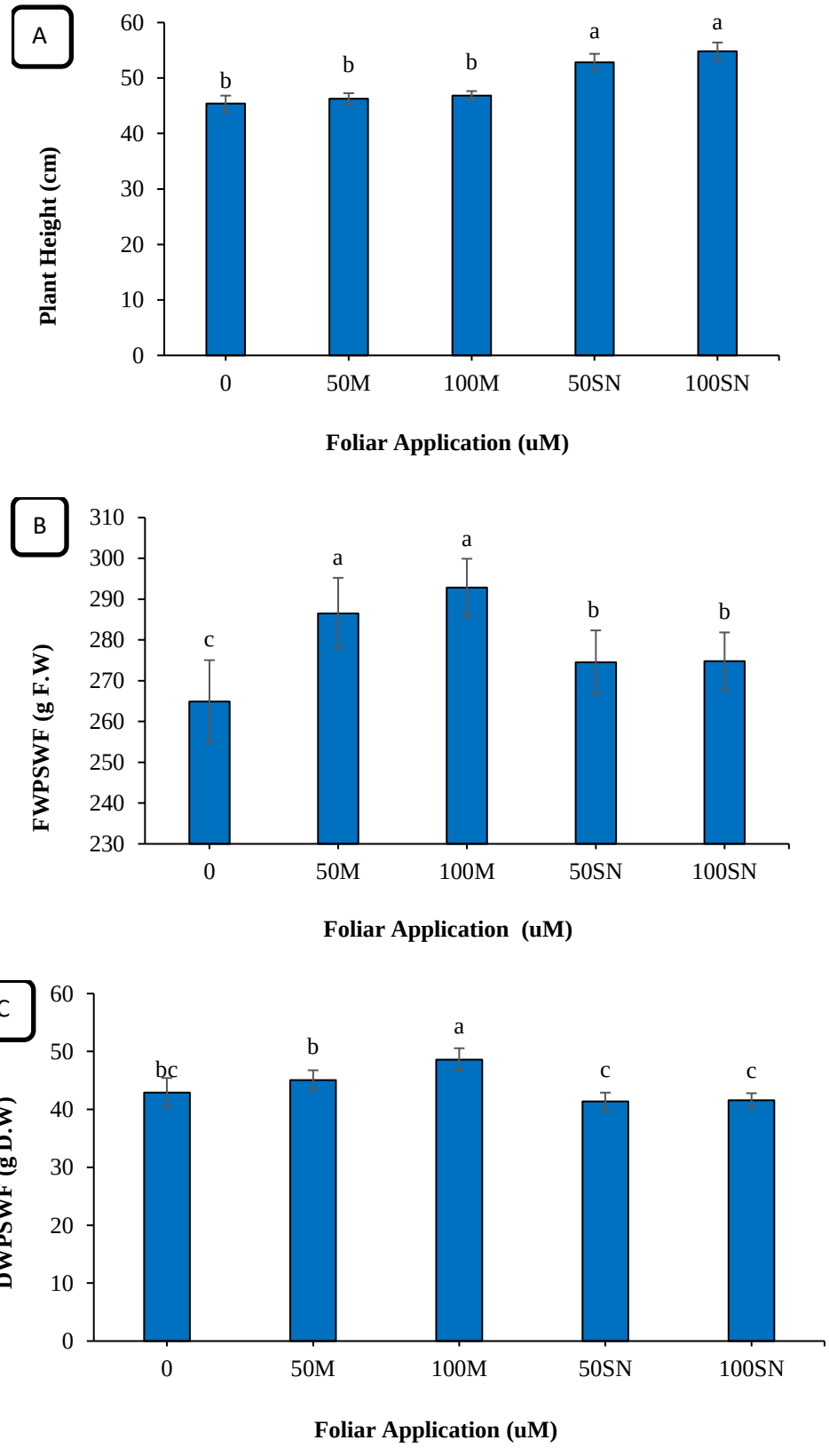

Fig. 8 .Comparison of means effect of foliar application with melatonin (M) and sodium nitroprusside (SNP) on plant height (A), shoot fresh weight (SFWWF, B) and shoot dry weight (SDWWF, C) of California Wonder green bell pepper, Differences letters indicate significantly different value at p< 0.05. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside.

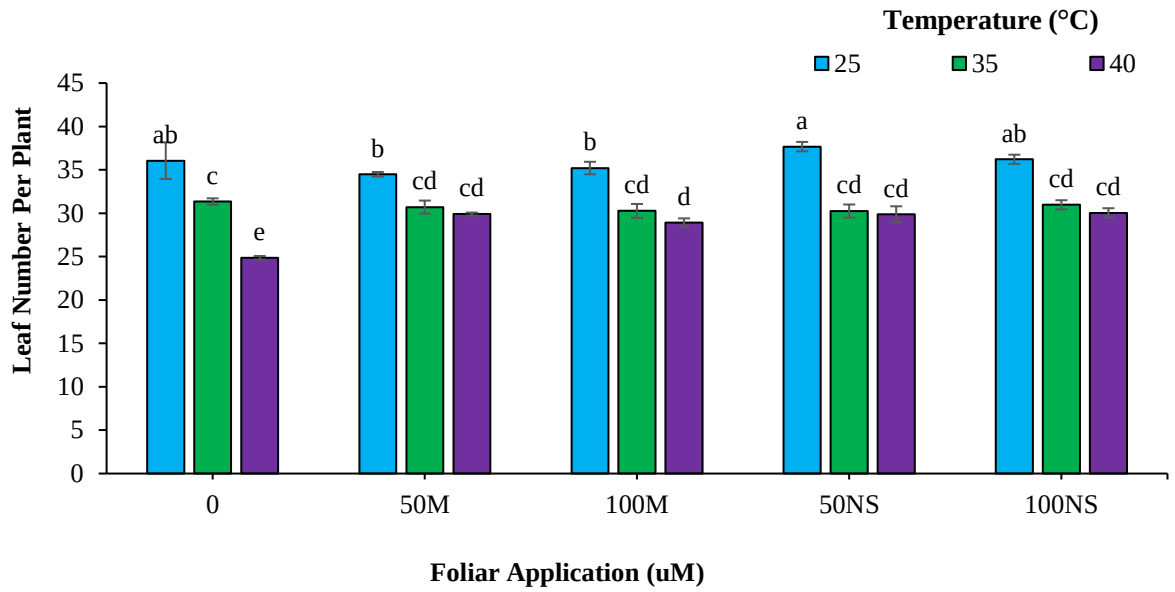

Fig. 9. Comparison of means interaction effect of foliar application with melatonin (M) and sodium nitroprusside (NS) and temperature on leaf number per plant (NLP) of California Wonder green bell pepper. Differences letters indicate significantly different value at p< 0.05. Temperature 25°C was Control treatment. Foliar treatments were including: 0 µM (control), 50 µM, and 100 µM concentrations of melatonin and sodium nitroprusside.

3.8. Principal Component Analysis

The data were further analyzed using multivariate analysis to explore the relationships among various parameters and treatments (Fig. 11). The results showed that 76.7% of the total variance was explained by the principal component analysis (PCA), with the first principal component accounting for 65.3% and the second component for 11.4% of the variance. Leaf nitrogen, potassium, phosphorus, copper, zinc, iron, and catalase were grouped in the first quadrant, closely associated with the 35°C temperature and 100 µM melatonin treatment (t2m3). In contrast, antioxidant-related parameters such as leaf superoxide dismutase, proline, chlorophyll, glutathione, and anthocyanin clustered near the 40°C temperature combined with 100 µM sodium nitroprusside (t3m5). This suggests that the production of antioxidant enzymes increased under the 40°C treatment with 100 µM sodium nitroprusside. The proximity of hydrogen peroxide to the 40°C temperature with 50 µM melatonin treatment (t3m2) indicates an increase in leaf hydrogen peroxide levels under this high-temperature condition. Both hydrogen peroxide and malondialdehyde were located in the third quadrant, which is opposite to shoot fresh and dry weight, reflecting their negative correlation. Treatments t1m3, t1m5, and t1m4 appeared in the fourth quadrant, characterized by reduced antioxidant enzyme activity, while leaf relative water content, shoot fresh and dry weights, and leaf SPAD values were higher. The close association between treatment t1m5 (100 µM sodium nitroprusside under 25°C, no heat stress) and shoot fresh and dry weights highlights that the highest biomass accumulation occurred with this treatment (Fig. 11).

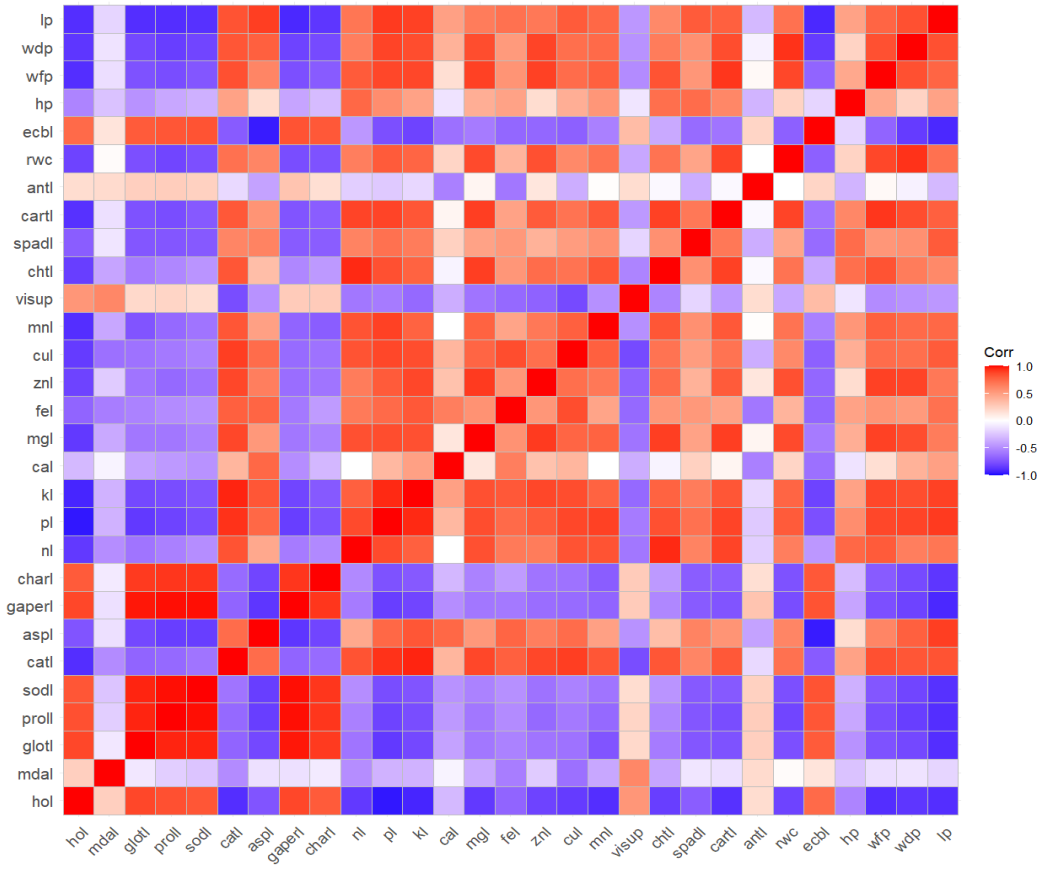

Fig. 10. Pearson's correlation between the studied parameters of California Wonder green bell pepper (*Capsicum annuum*) after exposure to foliar application with melatonin (M), sodium nitroprusside (NS) and different temperature regimes. hol—Leaf Hydrogen peroxide; mdal—leaf Membrane Lipid Peroxidation; glotl— Leaf glutathione; proll—Leaf proline; sodl—Leaf superoxide dismutase; catl—Leaf catalase; aspl—Leaf ascorbate peroxidase; gaperl—Leaf guayacol peroxidase; charl—Leaf soluble carbohydrates; nl—Leaf nitrogen; pl—leaf phosphorus; kl—leaf potassium; cal—Leaf calcium; mgl—Leaf magnesium; fel—Leaf iron; znl—Leaf Zinc; cul—leaf copper; mnl— leaf manganese; visup—plant visual damage index; chtl—Leaf total chlorophyll; spadl—Leaf spad; cartl—Leaf carotenoids; antl— leaf anthocyanins; rwc— Leaf relative water content; ecbl— Membrane stability index ; hp— plant height; wfp— shoot fresh weight; wdp— shoot dry weight; lp— Leaf number per plant.

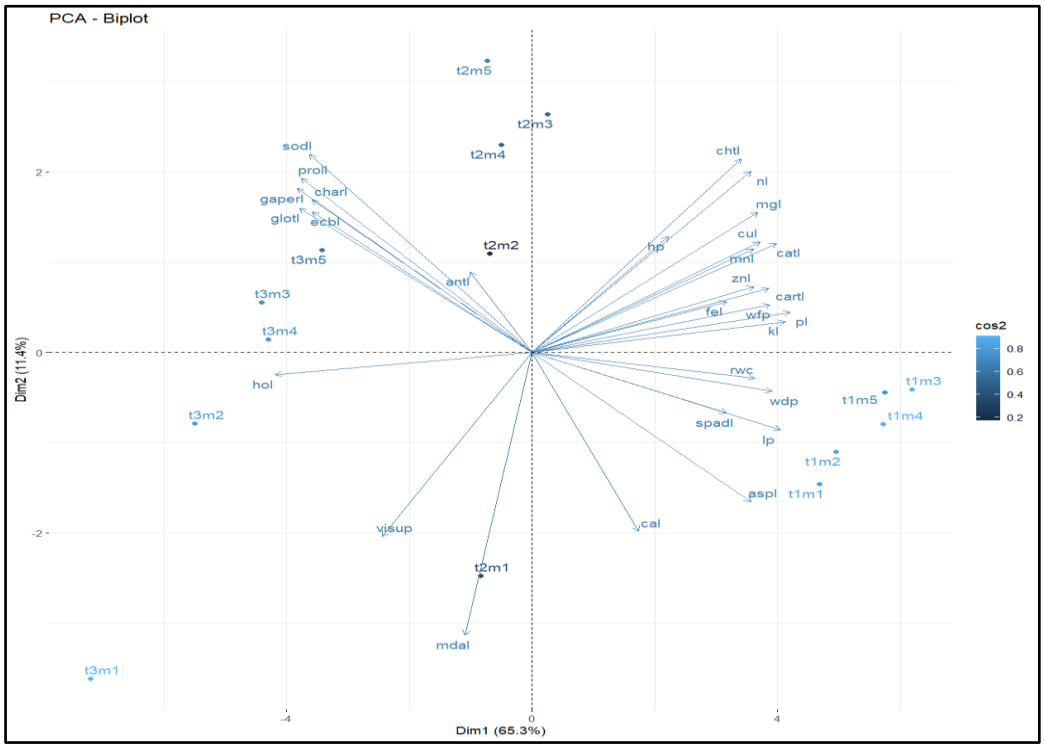

Fig. 11. Biplots of principal component analysis (PCA) represent the relationship between different variables and treatments in California Wonder green bell pepper (*Capsicum annuum*) treated with foliar application (m1: 0µM, m2: 50µM melatonin, m3:100µM melatonin, m4:50 µM sodium nitroprusside, m5:100 µM sodium nitroprusside) and different temperature regimes (t1:25°C, t2: 35°C, t3:40°C). The variables included hol—Leaf Hydrogen peroxide; mdal—leaf Membrane Lipid Peroxidation; glotl— Leaf glutathione; proll—Leaf proline; sodl—Leaf superoxide dismutase; catl—Leaf catalase; aspl—Leaf ascorbate peroxidase; gaperl—Leaf guayacol peroxidase; charl—Leaf soluble carbohydrates; nl—Leaf nitrogen; pl—leaf phosphorus; kl—leaf potassium; cal—Leaf calcium; mgl— Leaf magnesium; fel—Leaf iron; znl—Leaf Zinc; cul—leaf copper; mnl— leaf manganese; visup—plant visual damage index; chtl—Leaf total chlorophyll; spadl—Leaf spad; cartl—Leaf carotenoids; antl— leaf anthocyanins; rwc— Leaf relative water content; ecbl— Membrane stability index ; hp— plant height; wfp— shoot fresh weight; wdp— shoot dry weight; lp— Leaf number per plant.

3.9. Heat Map Analysis

A heat map was employed to examine the relationships among the measured parameters and to provide an overall perspective on the effects of foliar applications of melatonin and sodium nitroprusside under different temperature regimes on California Wonder green bell pepper (Capsicum annuum), as shown in Fig. 12. The analysis grouped the treatments into four distinct clusters. The first cluster consisted of treatments exposed to 40°C, the second included those at 25°C, and the third comprised treatments at 35°C. The traits assessed in this study were also categorized into four groups. The first cluster contained plant visual damage index, malondialdehyde, and leaf anthocyanin. The second cluster included leaf guaiacol peroxidase, superoxide dismutase, proline, glutathione, carbohydrate content, hydrogen peroxide, and membrane stability index. The third cluster grouped shoot fresh and dry weight, leaf carotenoids, relative water content, mineral elements such as nitrogen, magnesium, zinc, manganese, chlorophyll, plant height, and leaf SPAD values. Finally, the fourth cluster

consisted of leaf nutrient elements including phosphorus, potassium, calcium, copper, iron, along with catalase and ascorbate peroxidase enzyme activities, and leaf number (Fig. 12).

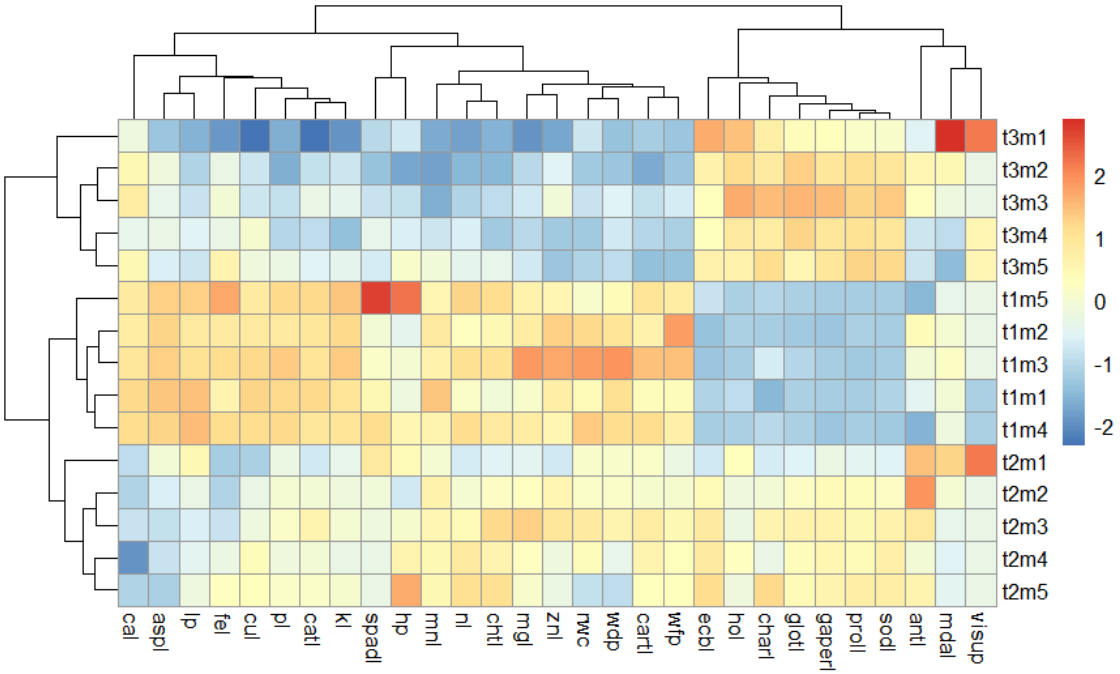

Fig. 12. Cluster heat map analysis of California Wonder green bell pepper (*Capsicum annuum*) grown under a combination of foliar application (m1: 0µM, m2: 50µM melatonin, m3:100µM melatonin, m4:50 µM sodium nitroprusside, m5:100 µM sodium nitroprusside) and different temperature regimes (t1:25°C, t2: 35°C, t3:40°C). The parameters (dependent variables) included hol—Leaf Hydrogen peroxide; mdal—leaf Membrane Lipid Peroxidation; glotl—Leaf glutathione; proll—Leaf proline; sodl—Leaf superoxide dismutase; catl—Leaf catalase; aspl—Leaf ascorbate peroxidase; gaperl—Leaf guayacol peroxidase; charl—Leaf soluble carbohydrates; nl—Leaf nitrogen; pl—leaf phosphorus; kl—leaf potassium; cal—Leaf calcium; mgl— Leaf magnesium; fel—Leaf iron; znl—Leaf Zinc; cul—leaf copper; mnl— leaf manganese; visup—plant visual damage index; chtl—Leaf total chlorophyll; spadl—Leaf spad; cartl—Leaf carotenoids; antl— leaf anthocyanins; rwc— Leaf relative water content; ecbl— Membrane stability index ; hp— plant height; wfp— shoot fresh weight; wdp— shoot dry weight; lp— Leaf number per plant.

3.10. Discussion

Nutrient uptake is crucial for maintaining the physiological, biochemical, and metabolic functions of plant cells. High temperature stress disrupts the activity of enzymes involved in nutrient metabolism, thereby reducing the absorption and accumulation of essential mineral elements in plants (Hungria and Kaschuk, 2014; Klimenko *et al*., 2006). Under heat stress, the uptake of minerals typically decreases compared to non-stressed controls. However, foliar applications of sodium nitroprusside and melatonin significantly enhanced leaf mineral nutrient content relative to controls. This improvement likely results from the direct and indirect effects of melatonin and sodium nitroprusside on various physiological and biochemical processes that enhance root nutrient absorption and translocation to aerial parts. Melatonin treatment has been shown to promote root growth and improve water and mineral uptake under stressful conditions (Iqbal *et al*., 2021). Increased root activity enhances absorption of key nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium, thereby improving plant performance during high temperature stress (Shaimaa Mohammed Elsayed *et al*., 2021). Other studies confirm that exogenous melatonin application boosts uptake of potassium, magnesium, phosphorus, and nitrogen under heat stress (Abd El-

Naby *et al*., 2020). Additionally, melatonin stimulates biosynthesis of indole acetic acid, which promotes root development and, consequently, water and nutrient absorption (Bajwa *et al*., 2014). For example, foliar application of 100 and 200 µM melatonin enhanced nutrient uptake in strawberry plants (Zahedi *et al*., 2020). The highest leaf chlorophyll content was observed with 100 µM sodium nitroprusside. Melatonin, acting as a free radical scavenger and antioxidant activator, preserves membrane integrity and stabilizes chloroplasts, preventing chlorophyll degradation (Wang *et al*., 2014). Both melatonin and nitric oxide contribute to the inhibition of chlorophyll breakdown (Yang *et al*., 2011a). Melatonin suppresses enzymes such as chlorophyllase and pheophorbide-a oxygenase, thereby maintaining chlorophyll levels and delaying leaf senescence. Foliar sprays of melatonin and sodium nitroprusside increased pigment content and reduced heat-induced leaf senescence in tomato plants by downregulating senescence-related genes and chlorophyll degradation pathways (Hansika Sati *et al*., 2023). Sodium nitroprusside similarly mitigated chlorophyll loss under heat stress, likely due to its positive effect on iron metabolism, as iron availability is closely linked to chlorophyll biosynthesis (Zhang *et al*., 2012). Nitric oxide reduces oxidative damage caused by heat stress in two ways: by stimulating antioxidant production to neutralize free radicals and by preventing carotenoid degradation, which protects against oxidative stress. Elevated carotenoid levels under heat stress improve plant tolerance, as demonstrated in Chrysanthemum morifolium treated with sodium nitroprusside (Yang *et al*., 2011a). High temperature stress suppresses growth-promoting hormones like cytokinins and increases inhibitors such as abscisic acid, thereby limiting growth and impairing water and nutrient uptake (Li *et al*., 2021). Foliar applications of melatonin and sodium nitroprusside increased leaf relative water content by maintaining cell metabolic balance and reducing water loss. Heat stress disrupts cellular water balance, reducing root water uptake, increasing leaf water loss, and causing electrolyte leakage (Machado and Paulsen, 2001; Wahid and Close, 2007; Young *et al*., 2004). Maintaining membrane integrity is critical for heat tolerance; oxidative stress enhances membrane lipid peroxidation, weakening membrane stability and increasing electrolyte leakage (Hasanuzzaman *et al*., 2013). Melatonin treatments have been reported to reduce electrolyte leakage and water loss in tomato plants under heat stress (Golam Jalal Ahammed *et al*., 2019; Barman *et al*., 2019; Kharbech *et al*., 2020). Similarly, sodium nitroprusside enhances membrane stability by boosting enzymes involved in stress signaling, reducing electrolyte leakage and increasing survival in heat-stressed maize and wheat seedlings (Li *et al*., 2020). Melatonin and sodium nitroprusside foliar sprays also increased leaf carbohydrate content while decreasing malondialdehyde and hydrogen peroxide levels. Plants deploy non-enzymatic antioxidants such as glutathione, proline, and soluble sugars to counteract free radicals generated under heat stress. Proline acts as an osmoprotectant, stabilizes enzymes and proteins, detoxifies reactive oxygen species, and maintains membrane integrity. Its synthesis is upregulated as a stress adaptation mechanism (Sehar *et al*., 2022). Glutathione similarly protects plants by inhibiting oxidative enzymes (Ding *et al*., 2016). Melatonin enhances proline biosynthesis through upregulation of the p5CS gene, while nitric oxide increases proline levels by stimulating biosynthetic enzyme activities and inhibiting proline degradation (Shah Jahan *et al*., 2019). Increased proline accumulation in tomato and strawberry treated with melatonin and sodium nitroprusside under heat stress has been documented (Jamali *et al*., 2014). Sodium nitroprusside alone or combined with calcium increased proline production in Solanum lycopersicum under heat stress (Siddiqui *et al*., 2017), and similar trends were seen in Vicia faba (Das and Roychoudhury, 2014). External melatonin treatment also promotes proline accumulation (Xing *et al*., 2021). Sodium nitroprusside application increased glutathione levels in wheat seedlings exposed to 38°C for 48 hours, likely through enhanced glutathione biosynthesis or increased cysteine availability (Iqbal *et al*., 2022). By modulating osmoprotectants, nitric oxide helps plants manage oxidative stress induced by heat. Oxidative stress raises malondialdehyde levels, disrupting

membrane fluidity and protein polymerization essential for membrane structure (Narayanan *et al*., 2016). Melatonin reduces membrane damage by limiting H2O2 overproduction and lowering malondialdehyde content. Nitric oxide similarly decreased H2O2 accumulation in rye plants under temperature stress (Dong *et al*., 2013). Foliar treatments with 50 and 100 µM melatonin and sodium nitroprusside under 40°C increased activities of antioxidant enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). SOD and GPX activities increased with temperature stress, and their activity was further enhanced by these treatments. While catalase and APX activity declined with rising temperature, foliar sprays mitigated this reduction. High temperature-induced oxidative stress disrupts cellular redox balance by generating harmful free radicals such as superoxide anions and hydrogen peroxide, damaging membranes and macromolecules. Elevated H2O2 levels under heat stress stimulate antioxidant enzyme production as a defense mechanism (Sehar *et al*., 2023). SOD acts as the primary defense by converting superoxide radicals to H2O2, which is subsequently broken down by catalase, APX, or GPX enzymes in different cellular compartments (Arnao and Hernández-Ruiz, 2007). Melatonin minimized heat-induced oxidative stress in tomato seedlings by enhancing antioxidant pathways and gene expression related to the ascorbate-glutathione cycle. In terms of growth, sodium nitroprusside led to greater plant height compared to melatonin, while 100 µM melatonin produced the highest shoot fresh and dry weights. Under heat stress, both melatonin and nitric oxide improved water and nutrient uptake, increasing relative water content, chlorophyll concentration, photosynthetic capacity (carbohydrates), and ultimately shoot biomass. Heat stress causes protein and enzyme denaturation, reduces water and nutrient absorption, and limits growth (Hassan *et al*., 2020). It also elevates respiration and photorespiration rates while decreasing photosynthesis, reducing assimilate production and biomass accumulation. Foliar applications of 50 and 100 µM melatonin improved both shoot and root biomass in watercress, likely by enhancing nutrient uptake (Hakimeh Oloumi *et al*., 2018). Melatonin supports diverse physiological processes including growth, photosynthesis, ion uptake and transport, enzyme activity regulation, and transpiration/translocation. It likely activates genes encoding enzymes that enhance photosynthetic efficiency during stress, leading to increased carbohydrate accumulation and improved nutrient transport from roots to shoots. Exogenous nitric oxide application improves photosynthesis and leaf water status, thereby promoting dry matter accumulation (Farooq *et al*., 2009). Under heat stress, foliar melatonin and sodium nitroprusside applications boosted mineral uptake, chlorophyll content, and photosynthetic rate, enhancing carbohydrate production and growth compared to untreated plants.

Conclusions

This study demonstrated that foliar spraying with 100 µM melatonin and sodium nitroprusside at the five-true-leaf stage improved shoot fresh weight of California Wonder bell pepper seedlings under 40°C heat stress. Melatonin treatment also increased leaf relative water content and reduced hydrogen peroxide accumulation under stress. Both treatments enhanced mineral nutrient content in above-ground tissues. Therefore, foliar application of 100 µM melatonin or sodium nitroprusside could be a practical strategy to mitigate heat stress during seedling production of California Wonder bell pepper. Further research is recommended to evaluate repeated foliar sprays at various seedling growth stages to optimize stress tolerance. Ultimately, incorporating melatonin and sodium nitroprusside treatments during seedling production may promote greater resilience to high temperatures once plants are transplanted to greenhouses or fields by stimulating the synthesis of primary and secondary metabolites that support stress resistance.

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