



## Application of Melatonin and Sodium Nitroprusside to Enhance the Physiological and Morphological Characteristics of Pepper

Dariush Ramezan<sup>a</sup>, Meisam Zargar<sup>b</sup>, Maryam Bayat<sup>b</sup> and Meysam Soltani Nejad<sup>c\*</sup>

<sup>a</sup> Department of Horticulture and Landscaping, Faculty of Agriculture, University of Zabol, Zabol 98613-35856, Iran

<sup>b</sup> Department of Agrobiotechnology, Institute of Agriculture, RUDN University, 117198 Moscow, Russia

<sup>c</sup> Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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Carbohydrates  
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Melatonin

### ABSTRACT

Heat stress arises when temperatures exceed a critical threshold for a specific duration during various stages of plant development. Consequently, it is crucial to devise effective strategies to overcome this obstacle in crop production. This research investigates the impact of melatonin and sodium nitroprusside on enhancing the heat resistance of *Capsicum annuum*. The Wonder cultivar of *Capsicum annuum* underwent various temperature treatments (25, 35 and 40°C) for a duration of 24h subsequent to being sprayed with melatonin and sodium nitroprusside at concentrations of 50 µM and 100 µM. The findings indicated that the application of melatonin during a temperature treatment resulted in an enhanced efficiency of leaf water consumption (16 percent). Additionally, pepper plants exposed to the same temperature treatment and treated with 100 µM melatonin exhibited a significant 13.5 % increase in fruit dry weight compared to the control group. The application of melatonin and sodium nitroprusside resulted in enhanced fruit characteristics and fruit marketability characteristics decreased. When exposed to a temperature of 40°C, there was a reduction of 18.50 % in the fruit's total soluble solids (TSS) compared to the control temperature of 25°C.

### ARTICLE

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\* Corresponding author: M. Soltani Nejad

E-mail address: [meysamsoltaninejad@gmail.com](mailto:meysamsoltaninejad@gmail.com)

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## 1. Introduction

Various meteorological models forecast that greenhouse gases will progressively elevate the atmospheric temperature, consequently resulting in global warming (Zargar *et al.*, 2025). In the event that plants are subjected to temperatures surpassing their tolerance threshold over a specific duration, their growth and development will incur irreversible harm (Soltani Nejad *et al.*, 2023; Orazalin *et al.*, 2024). Heat stress has a substantial impact on the growth and metabolic processes of plants (Zhou *et al.*, 2024). The primary indicators of heat stress in plants encompass diminished chlorophyll content and withered appearance of leaves and branches, scorching of plant organs, leaf aging, shedding of foliage, delayed seed germination and reduced germination success, disruption in the balance between photosynthesis and respiration, as well as a decline in stem dry weight, relative growth rate, and net absorption and production rate (Farooq *et al.*, 2009).

Heat stress in plants leads to an increased generation of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radical, consequently inducing oxidative stress (Sachdev *et al.*, 2021). Plants exhibit distinct reactions to both biotic and abiotic stresses (Mansha *et al.*, 2023). Despite the fact that plants have evolved various defense mechanisms to combat heat stress, these mechanisms often prove to be insufficient, leading to substantial damage inflicted upon the plant as a consequence of elevated temperatures (Roy *et al.*, 2022). In recent years, considerable attention has been paid to reducing the harmful effects of high temperatures in plants through the external application of some chemical compounds such as hydrogen peroxide, nitric oxide and melatonin (Alam *et al.*, 2018).

Melatonin is a molecule with an indole ring structure and a low molecular weight, which as a natural antioxidant plays an important role in the growth and development as well as the response to stresses in plants. Various studies have shown that treating plants with external melatonin improves tolerance to temperature stress (Liu *et al.*, 2015). In the conditions of high temperature stress, the plants of chamanwash (*Festuca arundinacea* Schreb) treated with melatonin had lower ion leakage and malondialdehyde levels, more chlorophyll, greater amount of total soluble proteins and higher activity of antioxidant enzymes compared to untreated plants. Elevated temperature stress leads to the peroxidation of membrane lipids and the degradation of photosynthetic pigments. Furthermore, such thermal stress adversely affects plant growth, resulting in diminished crop yields due to the disruption of carbohydrate and protein metabolism and transport (Alam *et al.*, 2018; Azizi *et al.*, 2024).

Nitric oxide (NO) as a molecular message, plays a vital role in various physiological processes of plants, such as regulating plant metabolism and senescence, inducing cell death, regulating stomatal movement, regulating photosynthesis, mitochondrial function, and regulating flowering (Sardar *et al.*, 2023). Research demonstrated that the external application of nitric oxide removes the free radicals and improves photosynthesis as well as cell water potential. Furthermore, enhancing photosynthetic efficiency by application of sodium nitroprusside can increase the yields (Zangani *et al.*, 2023). Also, sodium nitroprusside increases photosynthesis and increases dry matter under stress conditions (Farooq *et al.*, 2009).

It has been proven that nitric oxide is able to regulate many plant responses to a variety of biotic and abiotic stresses and reduce some consequences caused by oxidative stress (Zhou *et al.*, 2021). Numerous studies have shown that the application of melatonin and sodium nitroprusside through leaf spraying, particularly under high-temperature stress conditions, leads to a decrease in malondialdehyde levels and ion leakage in apple leaves. This treatment also enhances chlorophyll content, thereby improving the plant's photosynthetic capacity, as well as increasing protein levels and the activity of antioxidant enzymes. Consequently, these

effects contribute to a reduction in oxidative stress induced by elevated temperatures (Parankusam et al., 2017; Zangani *et al.*, 2023). In various plants, rapid production of nitric oxide has been observed during heat stress. Nitric oxide production increased in tobacco (*Nicotiana glauca*) and alfalfa (*Medicago sativa*) leaf cells exposed to heat stress (Parankusam *et al.*, 2017). It has been suggested that nitric oxide reduces the level of reactive oxygen species, during heat stress by activating antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase (Astier *et al.*, 2018).

It is hypothesized that the foliar application of melatonin and sodium nitroprusside could mitigate the unfavorable effects of heat stress on *Capsicum annuum* by sustaining photosynthesis parameters, promoting of mineral elements absorption and fruit quality. Therefore, this experiment aimed to investigate the impact of melatonin and sodium nitroprusside under heat stress on the fruit mineral element composition, leaf chlorophyll fluorescence, leaf gas exchange, fruit physicochemical characteristics and yield (fruit per plant) of *Capsicum annuum*. The objective of this study was to establish an experimental basis for the foliar application of melatonin and sodium nitroprusside in *Capsicum annuum* grown under temperature different regimes.

## 2. Materials and Methods

### 2.1. Test conditions

Experiments in a greenhouse were carried out during the autumn of 2022 at Zabol University, Zabol, Iran. This research was arranged as a factorial experiment with two factors (3x5) where the first factor- temperature had three levels (25, 35 and 40 °C) and the second factor- 5 types of foliar application (distilled water (control), melatonin 50µM, melatonin 100 µM, sodium nitroprusside 50 µM and sodium nitroprusside 100 µM). The treatments were randomized and each treatment was replicated three times. For this purpose, *Capsicum annuum* seedlings were obtained from one of the commercial seedling producers in Tehran province, Iran. The seedlings were planted in plastic pots with a diameter of 17 cm and a height of 16 cm, which contained a mixture of perlite, peat moss and soil in a ratio of 1:1:1 and in the greenhouses of the Faculty of Agriculture, Zabol University. The microclimate conditions of the greenhouse were as follows: day and night average temperature 25±2 and 20±2°C, respectively, 45 % relative humidity, and 14:10 h of light and dark. After two weeks of planting the seedlings and at the 5-leaf stage, the plants were sprayed with sodium nitroprusside and melatonin in concentrations of 50 µM and 100 µM, three times with a time interval of 24 h. At the same time, control samples were sprayed with distilled water. Following a 24h period after the last application, the plants were transferred to the growth chamber with relative humidity of 65 %, photo-period of 16 h of light and 8 h of darkness and light intensity of 270 µmol photons m<sup>-2</sup> s<sup>-1</sup>. In order to apply heat stress treatment, the temperature of the growth chamber (EYELA LTI-1000 SD) was gradually increased from 25°C temperature every 24 h by 5°C to reach temperatures of 35°C and 40°C temperatures. The plants in each temperature range remained in the growth chamber for 24 h and then at the end of the temperature treatment, the plants were transferred to the greenhouse at with a 25°C temperature.

### 2.2. Measurement of chlorophyll fluorescence parameters and photosynthetic characteristics

To measure chlorophyll fluorescence parameters, the clamps of the fluorescence chlorophyll measuring device (manufactured by Hansatech Instrument Ltd, England) were attached to the leaves. These clamps have a special shape that the diode of the device is easily fixed on them. The clamps have valves that, when closed, the desired part (leaf) is placed in the dark. After 30 min, the diode is connected to the clip and then the clip valve is opened by

pressing the button on the diode, the fluorescence parameters are displayed on the device screen. In this way, the  $F_v/F_M$  index is read from the device. The  $F_v/F_M$  index is obtained from the  $F_M - F_0/F_M$  formula, where in this formula,  $F_M$  is the maximum chlorophyll fluorescence and  $F_0$  is the minimum chlorophyll fluorescence in dark-acclimated leaves. The difference between  $F_M$  and  $F_0$  is called fluorescence Variable or  $F_v$ . The  $F_v/F_M$  index shows that the photosystem II efficiency is in the condition that all reaction centers of photosystem II are open (Maxwell and Johnson, 2000).

### 2.3. Measurement of gas exchange

Gas exchange parameters were assessed by measuring the upper leaves of three plants each, utilizing a portable photosynthesis measuring device (model ADC BioScientific Ltd, England, LCA4) between 11:00 and 12:00 AM. The measurements were conducted under a light intensity of 800-1000  $\mu\text{mol photons per m}^{-2}\text{s}^{-1}$  and at a temperature of  $25 \pm 2$  °C. Photosynthesis rate (net  $\text{CO}_2$  assimilation rate) per unit leaf area ( $P_n$ ) in  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ , Stomatal Conductance ( $G_s$ ) in  $\text{mmol CO}_2 \text{ m}^{-2}\text{s}^{-1}$  and transpiration (E) Rate (TR) in  $\text{mmol H}_2\text{O}$  were determined. Furthermore, Leaf Instantaneous Water Use Efficiency (WUE) in  $\text{mmol of CO}_2$  per mol  $\text{H}_2\text{O}$  was calculated using the following equation (Zhang *et al.*, 2018; Iqbal *et al.*, 2019).

$$\text{WUE} = \text{PN/TR}$$

### 2.4. Fruit morphological characteristics

The number of fruits per plant, fresh weight of each fruit (g), dry weight of each fruit (g) and fruit yield per plant (g) were computed.

### 2.5. Fruit Firmness, fruit total soluble solids, fruit acidity, appearance characteristics of fruit marketability and chemical characteristics of fruit

A penetrometer (Mc-Cornic-F327) was used to determine the firmness of fruit tissue. For this purpose, the skin layer on the fruit was removed and the pressure gauge tip with a diameter of 8 mm was pressed into fruit tissue and the penetrometer was calibrated in  $\text{kg.cm}^2$ . Total Soluble Solids (TSS) was measured with a digital refractometer (Euromex model: RD.5635). In this way, a piece of fruit flesh was compressed by a manual juicer and a few drops of its extract were placed on the prism of the refractometer. Total Soluble Solids of fruit pulp were recorded based on Brix. Fruit organic acids were determined by titration method. The titratable acidity percentage (TA) of the fruit extract was measured with a 0.1N NaOH until it reached the final pH of 8.2 (Thiex *et al.*, 2012).

### 2.6. Appearance characteristics of fruit marketability

The fruits marketability was assessed using a score index from 1 to 5 (Table 1). The key factors influencing marketability included color change, fruit appearance, and fruit tissue quality. The highest grade received a score of 1, while the least marketable fruit was given a score of 5 (Salarian *et al.*, 2018; Owens, 1950).

### 2.7. Fruit carbohydrates

Total sugar determination of the fruit solution was done by Anthrone reagent and using the method of McCready *et al.*, (1950). In order to make Anthrone solution, 150 mg of Anthrone was dissolved in 100 ml of dilute sulfuric acid, and to prepare 100 ml of dilute sulfuric acid, 76 ml of concentrated sulfuric acid was mixed with 38 ml of distilled water. To measure soluble sugars, 100  $\mu\text{l}$  of the extract was poured into a test tube and 3 ml of Anthrone solution was added to it. The obtained mixture was placed in a boiling water bath for 20 min at a temperature of 100 °C. Soluble sugars in fruits were quantified through spectrophotometric analysis at a wavelength of 620 nm. A standard curve was constructed using various glucose

concentrations to determine the sugar content, which was then reported in milligrams per gram of fruit dry weight (McCready *et al.*, 1950).

**Table 1. Marketability characteristics of *Capsicum annuum* investigated for classification, grade 1, 2, 3, 4 and 5.**

Grade	Quality	Color Uniformity	Health Status	Shape	Weight (g.F.W)
1	Excellent	100%	100%	Square Non-Square	60-45 more than 60
2	Good	(100-80) %	(100-80) %	Square Non-Square	50-40 60-45
3	Medium	(80-60) %	(80-60) %	Non-Square Non-Square	40-30 45-35
4	Bad	(50-40) %	(50-40) %	Square Non-Square	30-20 35-25
5	Brok	-	(0-20) %	-	less than 25

(-) do not have any of the characteristics.

## 2.8. Fruit glucose, fructose and sucrose

In order to measure and separate sugar, an HPLC (made by the withers company was used with a column (250x46 mm, DP=3 um) Carbohydrate C<sub>18</sub>) and amounts of sugar were expressed as milligrams per gram of fruit fresh weight.

## 2.9. Fruit Flavonoids and phenols

Flavonoid content of fruit extract was measured. To prepare the extract, one gram of the fruit tissue was ground using 80% methanol and brought to a volume of 8 ml. The volume was brought to 2.5 ml by adding NaNO<sub>2</sub>, AlCl<sub>3</sub> and NaOH. The absorbance of the solution was read after 5 minutes with a spectrophotometer at a wavelength of 507 nm and recorded as milligrams per 100 g of fruit fresh weight. The total phenol of fruit was read using Folin method and using a UV/VIS spectrophotometer model PG Instruments+T80 and expressed in µg.g F.W (McDonald *et al.*, 2001).

## 2.10. Fruit vitamin C and antioxidant capacity

Ascorbic acid concentration of fruit extract was measured based on color reduction of 2,6-dichlorophenol. In this method, 1 mg of fruit tissue was mixed with 3 ml of metaphosphoric acid (1 %) and after 30 min it was centrifuged at 4°C and 6000 rpm for 15 min. A volume 50 µL from supernatant solution brought to 200 µL and absorbance of samples was read at a wavelength of 520 nm. Ascorbic acid concentration of fruit was recorded using a calibration curve as milligrams per 100 g of fruit fresh weight (Chang *et al.*, 2002). The determination of antioxidant capacity of the fruit extract was done through DPPH free radical neutralization using a PG Instruments ItdT80+UV/VIS spectrophotometer (Miliauskas *et al.*, 2004).

## 2.11. Fruit Mineral Elements

Fruit nitrogen was measured by the combustion method with an elemental analyzer (CHNS-O Elemental Analyzer model ECS4010, Italy). The spectrophotometer method was used to measure phosphorus (Garvey *et al.*, 2023). One gram of dry matter was placed in an electric furnace at a temperature of 500°C for four hours. Then, 10 cc of hydrochloric acid (2 M) was added to samples and volume of 1000 cc with distilled water and read at 420 nm wavelength. The atomic absorption method was used to measure calcium, potassium, magnesium, iron, copper, zinc and manganese. Fruit samples weighing 0.5 g were desiccated and then dissolved in 10 ml of concentrated nitric acid. The mixture was maintained at a temperature of 70 °C for a duration of 24h to ensure complete dissolution of the samples in

the acid. Subsequently, the solutions were diluted to volume using deionized water, and their absorbance values were measured using an atomic absorption spectrophotometer model FSAA 240.

## 2.12. Statistical Analysis

The variance analysis of data was done with SAS 9.4 statistical software and RStudio-2023.12.0-369 software was used to draw correlation and principal component analysis (PCA). Means comparison was done with LSD test at 5 % significance level.

## 3. Results

### 3.1. Leaf Chlorophyll Fluorescence

Based on the information presented in Table 2, the maximum value of Minimum chlorophyll fluorescence ( $F_o$ ) was recorded as 0.46, while the minimum value was observed as 0.17. These values were associated with two different temperature treatments. Specifically, the highest  $F_o$  value of 0.46 was linked to a temperature treatment of 40°C with no foliar application of 0µM. On the other hand, the lowest  $F_o$  value of 0.17 was observed in a temperature treatment of 25°C with a foliar application of 50µM melatonin. Additionally, the highest (1.26) and lowest (0.89) Maximum chlorophyll fluorescence ( $F_m$ ) were respectively related to 25°C temperature treatment with 0 foliar application and 40°C temperature treatment with 0 foliar application, respectively, Table. 2 and the highest (1.04) and lowest (0.43) Maximal variable fluorescence ( $F_v$ ) were related to 25°C temperature treatment with 0 foliar application and 40°C temperature treatment with 0 µM foliar application, respectively, Table. 2.

**Table 2. Impact of melatonin, sodium nitroperoxide and temperature various levels on Minimum fluorescence, Maximum fluorescence, Variable fluorescence and photosystem II efficiency of California Wonder green bell pepper**

Temperature (°C)	Foliar Application (µM)	$F_o$	$F_m$	$F_v$	$F_v/F_m$
25	0	0.22±0.01e	1.26± 0.02a	1.04±0.03 a	0.82±0.01a
	50M	0.17±0.01f	1.23±0.1a	1.04 ±0.02a	0.85±0.01a
	100M	0.18±0.01f	1.21± 0.01ab	1.03±0.02 a	0.84±0.01a
	50NSP	0.22±0.01de	1.18± 0.01abc	0.96± 0.01b	0.82±0.01ab
	100NSP	0.23±0.01de	1.18± 0.01abc	0.95±0.01 b	0.80±0.01abc
35	0	0.35±0.01b	1.04± 0.02d	0.69±0.02 ef	0.67±0.01e
	50M	0.24±0.01de	1.06±0.02d	0.79 ±0.03d	0.76±0.01bcd
	100M	0.28±0.01c	1.08± 0.02cd	0.79± 0.02d	0.74±0.01cd
	50NSP	0.29±0.01c	1.17± 0.01abc	0.86 ±0.01c	0.74±0.01d
	100NSP	0.28±0.01c	1.03± 0.01de	0.74 ±0.01de	0.72±0.01de
40	0	0.46±0.01a	0.89± 0.01f	0.43± 0.02g	0.48±0.01f
	50M	0.30±0.01c	1.11± 0.01bcd	0.80± 0.01cd	0.72±0.01de
	100M	0.33±0.01b	1.04± 0.01de	0.70±0.01 ef	0.67±0.01e
	50NSP	0.24±0.01de	0.94± 0.01ef	0.68±0.01 f	0.73±0.01de
	100NSP	0.25±0.01d	1.05± 0.02d	0.78±0.02 d	0.74±0.01cd
LSD		0.02	0.10	0.06	0.06

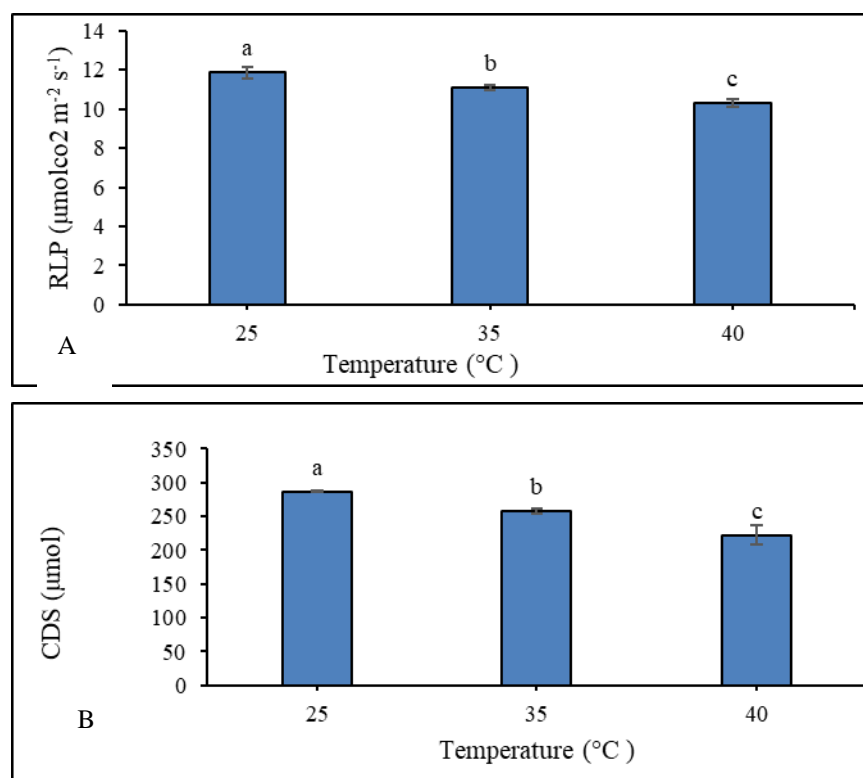
Melatonin (M) and Sodium Nitroperoxide (NSP), minimum fluorescence ( $F_o$ ), maximum fluorescence ( $F_m$ ), variable fluorescence ( $F_v$ ), photosystem II efficiency ( $F_v/F_m$ ), Error bars signify standard error (SE) three replicates, 25°C Temperature and 0µM foliar application: Control, Values in the same column with similar letters are not significantly different ( $p \leq 0.05$ ).

Based on the information presented in Table 2, there were no significant variations ( $p < 0.05$ ) observed among the 25°C temperature treatment with foliar application 0µM (control), melatonin 50µM and 100µM, sodium nitroprusside 50µM and 100µM, in terms of

photosystem II efficiency value. Consequently, these treatments can be categorized within the same statistical group. Furthermore, the lowest efficiency value of photosystem II (0.48) was recorded for the 40°C temperature treatment with foliar application 0µM (control).

### 3.2. Leaf Gas Exchange

The Fig. 1 (A) presents data indicating that the leaf photosynthesis rate values reached their highest level at 25°C (11.86 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and their lowest level at 40°C (10.29 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). Similarly, the Fig. 1 (B) shows that the carbon dioxide values under leaf stomata were highest at 25°C (287.20 µmol) and lowest at 40°C (222.36 µmol). These findings suggest a relationship between temperature and both leaf photosynthesis rate and carbon dioxide values.



**Fig. 1. Impact of various temperature levels on rate of leaf photosynthesis (Graph of A (RLP)) and carbon dioxide under leaf stomata (Graph of B (CDS)) of California wonder green bell pepper. Bars showing different letter(s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.**

According to data in Fig. 2, the highest (11.43 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and lowest (10.23 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) of leaf photosynthesis rate values were related to foliar application with 100 µM melatonin and 0 µM (control), respectively. Moreover, there was no statistically significant difference ( $p < 0.05$ ) in terms of leaf photosynthesis rate (RLP) value between foliar application treatments of 50µM and 100µM (Melatonin and nitroperoxide). The highest (10.43 µmol CO<sub>2</sub> per mol H<sub>2</sub>O) and lowest (5.21 µmol CO<sub>2</sub> per mol H<sub>2</sub>O) values of instant leaf water consumption Efficiency were related to 40°C temperature with 50µM melatonin foliar application and 25°C temperature with foliar application 100µM sodium nitroperoxide respectively, supplementary Fig. 3 (A). There was no statistically significant difference ( $p < 0.05$ ) between 40°C temperature treatment with 50µM melatonin foliar application and 40°C temperature treatment with 100µM melatonin foliar application. The data in supplementary Fig. 3 (B) shows that the highest (0.05 mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) leaf stomatal conductance was related to 25°C temperature treatment with 0µM foliar application, 25°C temperature treatment with 100µM melatonin foliar application and 35°C temperature treatment with foliar application 100µM melatonin whilst the lowest (0.01 mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)

leaf stomatal conductance was related to of 40°C temperature treatment with 0µM foliar application.

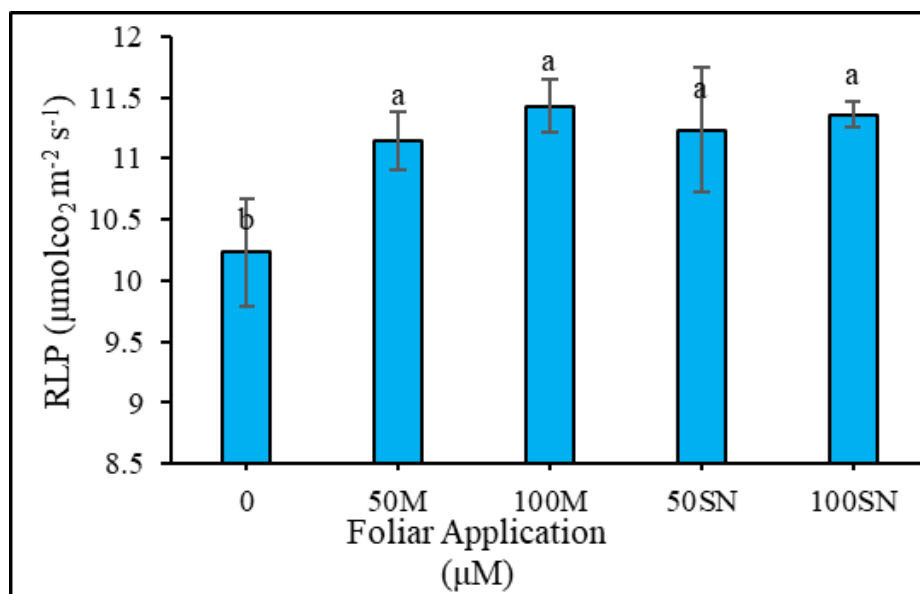


Fig. 2. Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on rate of leaf photosynthesis (RLP) of California wonder green bell pepper. Bars showing different letter(s) at p<0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

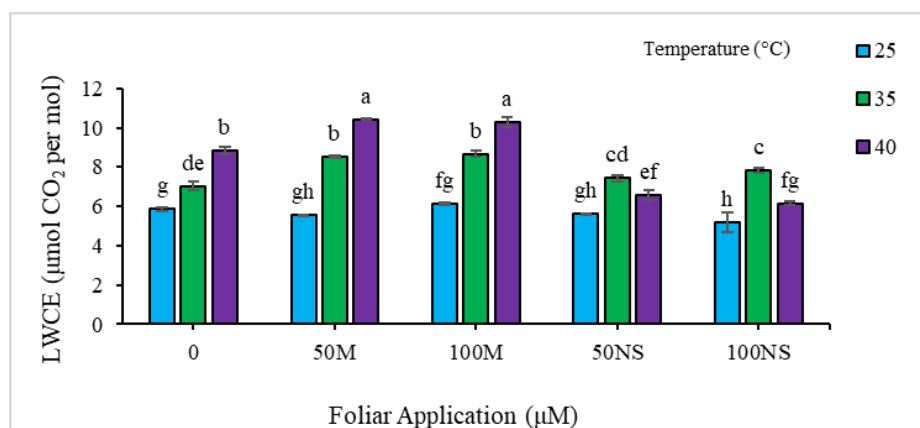
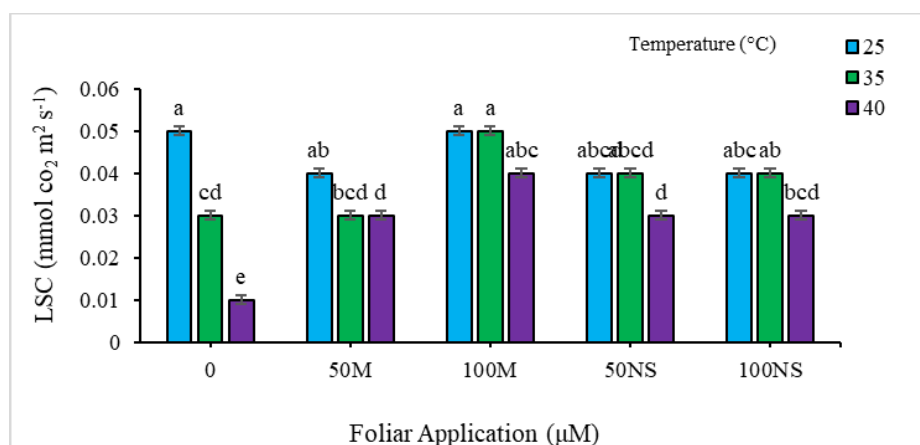


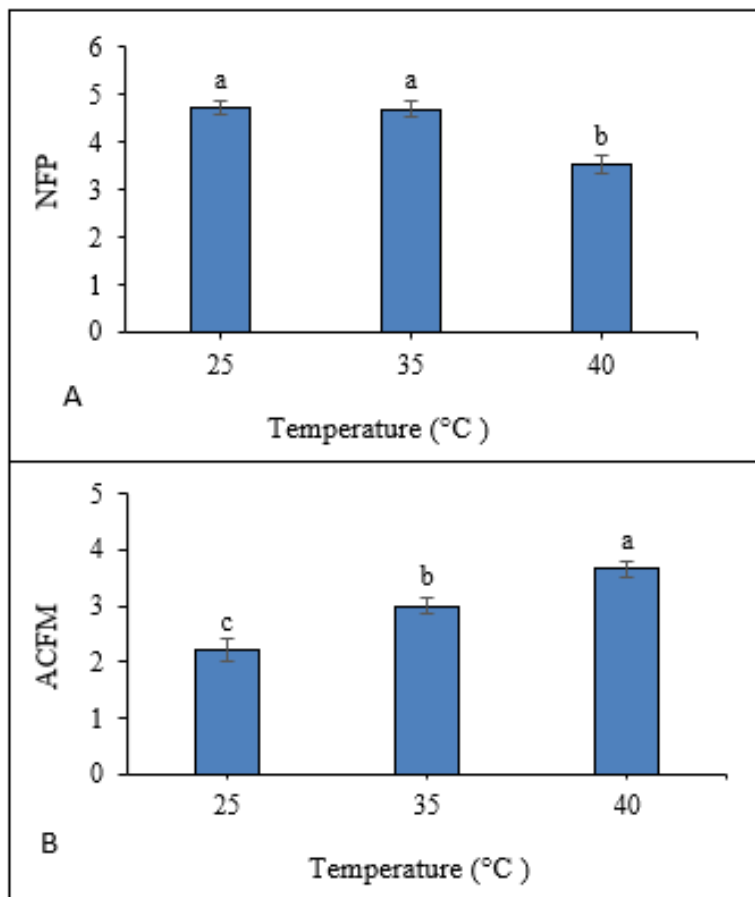
Fig. 3. Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on and Instant Leaf Water Consumption Efficiency (Graph of A (LWCE)) and Leaf stomatal conductance (Graph of B (LSC)) of California wonder green bell pepper. Bars showing different letter (s) at p<0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.



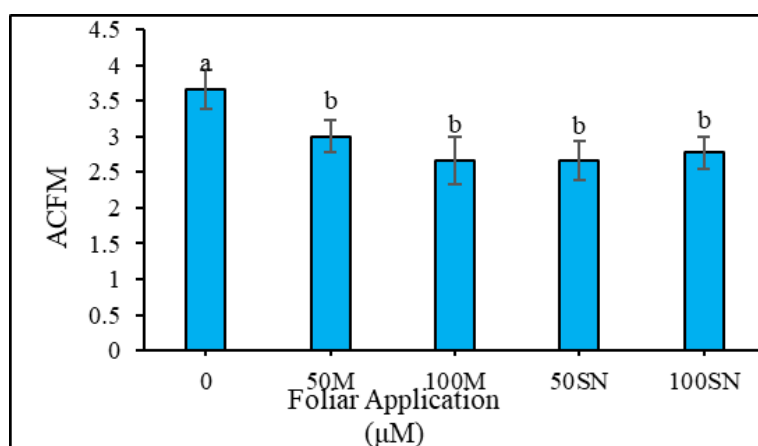
### 3.3. Fruit morphological characteristics

Supplementary Fig. 4 (A) demonstrates that the highest (4.71) and lowest (3.52) Number of Fruit Per Plant were related to 25°C and 40°C temperature treatments, respectively. There was no statistically significant difference (p< 0.05) between temperature treatments 25°C and 35°C. According to data in supplementary Fig. 4 (B), the highest (3.66) and lowest (2.20)

values of Appearance Characteristics of Fruit Marketability were related to temperature treatment of 40°C and 25°C, respectively. As shown in supplementary Fig. 5, with regards to foliar treatment, the highest (3.66) value of Appearance Characteristics of Fruit Marketability was related to 0µM treatment, and the lowest (2.66) value was related to 50 µM and 100µM melatonin and sodium nitroprusside treatments.



**Fig. 4.** Impact of temperature various levels on Number Fruit Per Plant (NFP, A) and Appearance Characteristics of Fruit Marketability (ACFM, B) of California wonder green bell pepper. Bars showing different letter(s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.



**Fig. 5.** Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on Appearance Characteristics of Fruit Marketability (ACFM) of California wonder green bell pepper. Bars showing different letter(s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

According to data in Table 3, the highest (74.91g) and lowest (54.63g) values of Single Fruit Weight were related to 25°C temperature treatment with 0µM foliar application and 40°C temperature treatment with 0µM foliar application, respectively. There was no statistically significant difference ( $p < 0.05$ ) between 25°C temperature treatment with 0µM foliar application and the 25°C temperature treatment with 50µM melatonin. As illustrated in

Table 3, the highest (8.78g) and lowest (5.80g) values of dry weight of single fruit were related to 35°C temperature treatment with 50µM sodium nitroperoxide and 40°C temperature treatment with melatonin 50µM respectively. Furthermore, there was no significant statistical difference ( $p < 0.05$ ) between the 35°C temperature treatment with 50 µM sodium nitroperoxide and 35°C temperature treatment with 0µM foliar application and 35°C temperature treatment with 50µM melatonin and 35°C temperature treatment with 100µM melatonin and 35°C temperature treatment with 100µM nitro peroxide. Table 3 shows that the highest (376.53 g) fruit yield per plant was related to treatment 25°C with 100µM sodium nitroperoxide foliar application and was statistically comparable with 25°C with 50µM sodium nitroperoxide application whereas the lowest (288.41g) fruit yield per plant was related to 40°C with 0µM foliar application.

**Table 3. Impact of melatonin, sodium nitroperoxide and temperature various levels on single fruit fresh weight, single fruit dry weight, fruit yield per plant of *Capsicum annuum***

Temperature (°C)	Foliar Application (µM)	SFW (g F.W)	DWSF (g D.W)	FYP (g F.W)
25	0	74.91±2.87a	8.03±0.07bc	320.77±6.4d
	50M	73.95±2.35a	7.47±0.26de	347.83±0.98c
	100M	70.44±0.78b	7.80±0.14cd	362.31±2.5b
	50NSP	70.40±0.39b	7.17±0.23ef	366.43±10.27ab
	100NSP	70.15±1.12bc	8.10±0.01bc	376.53±0.93a
35	0	67.18±0.64bcd	8.64±0.25a	297.94±1.13gh
	50M	66.44±0.47d	8.70±0.29a	311.26±1.48def
	100M	65.96±0.96d	8.31±0.14ab	313.53±6.55def
	50NSP	66.81±0.88cd	8.78±0.06a	306.70±0.71efg
	100NSP	67.04±0.14bcd	8.74±0.02a	308.82±0.92defg
40	0	54.63±0.93e	5.93±0.22h	288.41±1.54h
	50M	55.42±0.6e	5.80±0.21h	304.00±7.26fg
	100M	65.81±0.82d	6.71±0.08fg	316.95±2.62de
	50NSP	57.53±0.67e	6.23±0.06gh	307.50±0.33efg
	100NSP	56.31±0.72e	6.01±0.06h	302.92±0.9fg
LSD		3.42	0.49	12.15

Melatonin (M) and Sodium Nitroperoxide (NSP), single fruit fresh weight: SFW, single fruit dry weight: DWS, fruit yield per plant. Error bars signify standard error (SE) three replicates, 25°C Temperature and 0µM foliar application: Control, Values in the same column with similar letters are not significantly different ( $p \leq 0.05$ ).

### 3.4. Physicochemical characteristics of fruit

As shown in supplementary Fig. 6 the highest (2.84 kg.m<sup>2</sup>) and lowest (1.03 kg.m<sup>2</sup>) fruit firmness were related to 25°C with 100µM melatonin and 40°C with 0µM foliar application, respectively. In supplementary Fig. 7 the highest (6%) and lowest (4.89%) values of Fruit TSS were related to temperatures of 25°C and 40°C, respectively, and there was no statistically significant difference ( $p < 0.05$ ) between temperatures of 25°C and 35°C in terms of Fruit TSS value. According to supplementary Fig. 8 the highest (6.30%) and lowest (5.22%) values of Fruit TSS were related to 100µM and 0µM melatonin treatments, respectively. Fig. 9 shows that the highest (0.74) and lowest (0.35) values of Fruit TA were related to temperature treatments of 40°C and 35°C, respectively. As exhibited in Fig. 10, the highest (0.73) value of Fruit TA was associated with 100 µM sodium nitroperoxide foliar application. There was no statistically significant difference ( $p < 0.05$ ) between control (0µM), melatonin 50µM and 100 µM melatonin treatments in terms of Fruit TA value and all three were in the same statistical class.

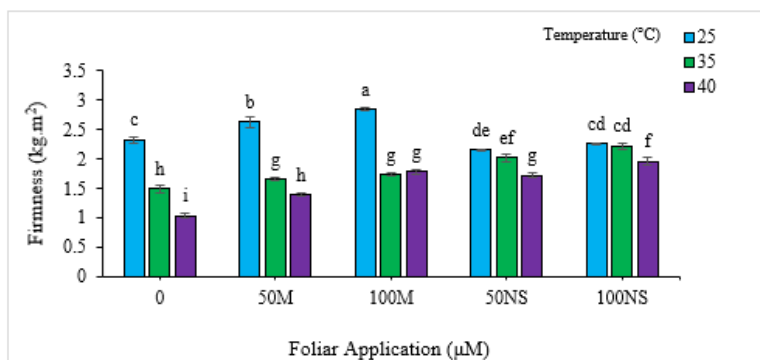


Fig. 6. Impact of melatonin (M), sodium nitroperoxide (SN) and temperature various levels on Fruit Firmness of California wonder green bell pepper. Bars showing different letter (s) at p < 0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

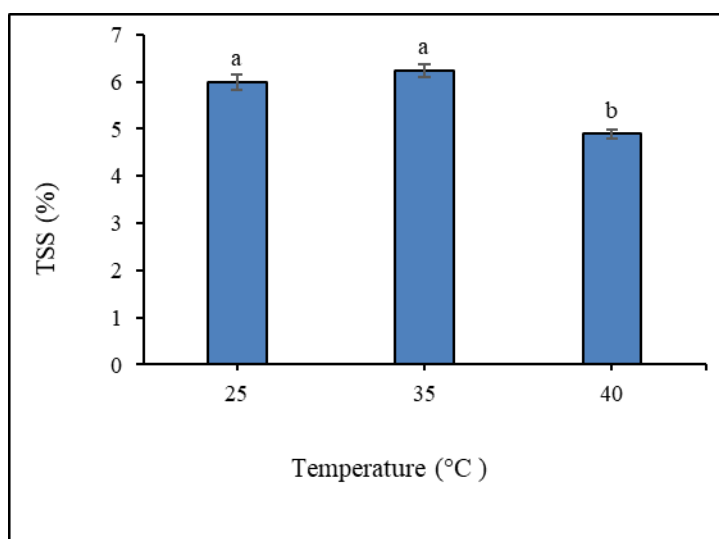


Fig. 7. Impact of Temperature various levels on Fruit TSS of California wonder green bell pepper. Bars showing different letter (s) at p < 0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

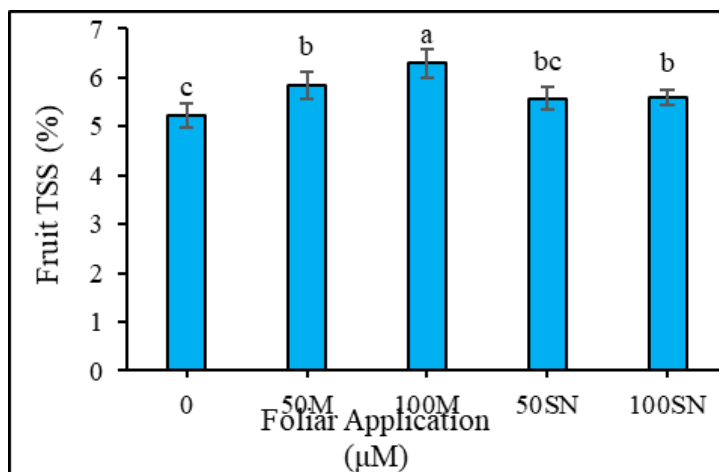
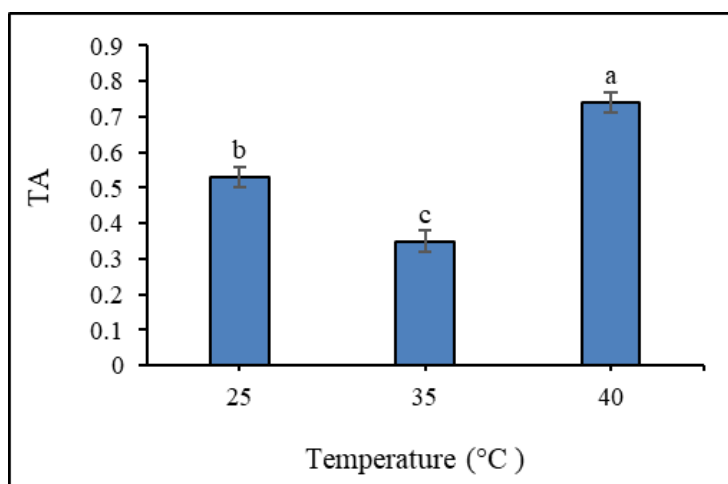
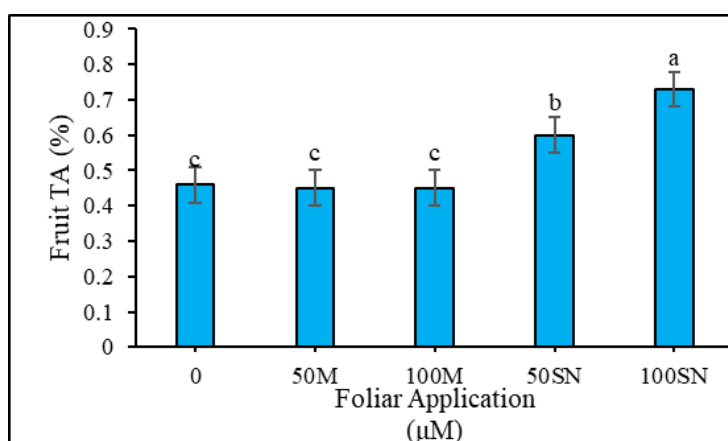


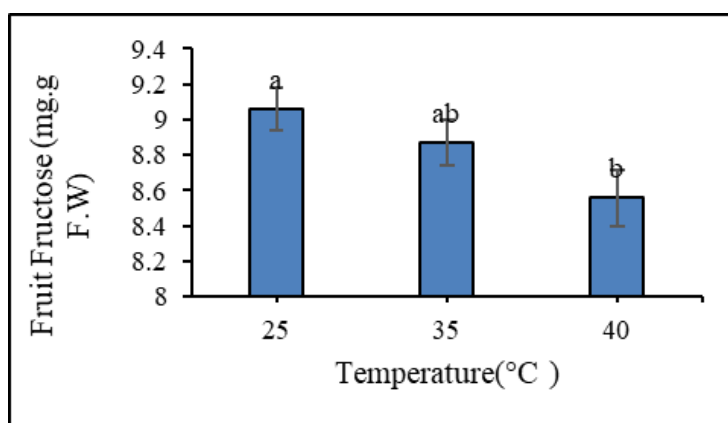
Fig. 8. Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on Fruit TSS of California wonder green bell pepper. Bars showing different letter (s) at p < 0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.



**Fig. 9.** Impact of temperature various levels on Fruit Total Acids (TA) of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control



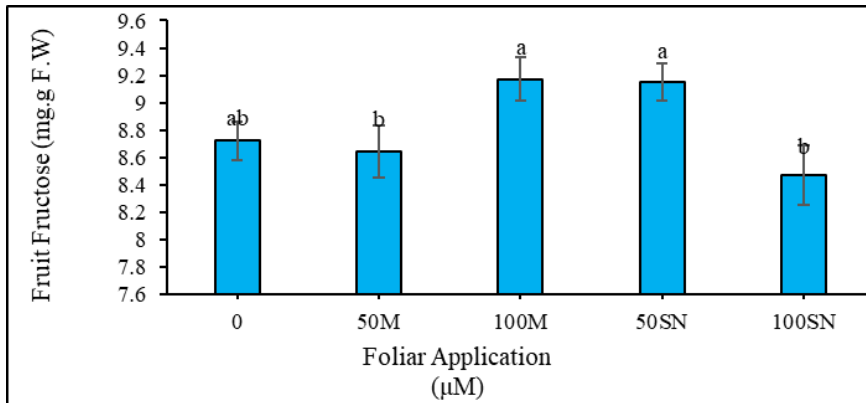
**Fig.10.** Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on Fruit Total Acids (TA) of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.



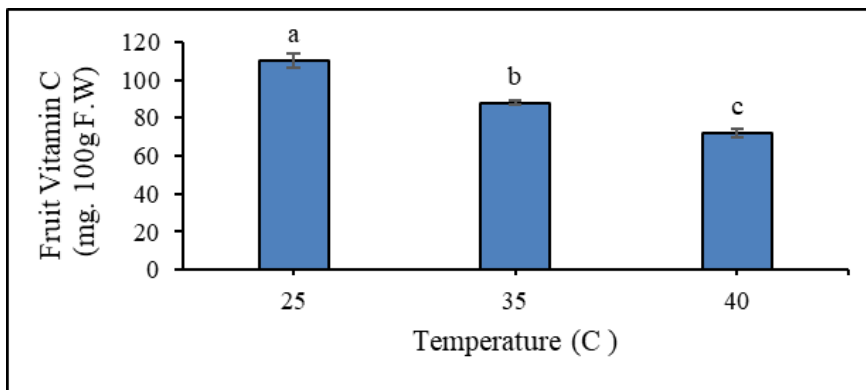
**Fig. 11.** Impact of temperature various levels on Fruit Fructose of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

Fig.10, 11 indicates that the highest (9.06 mg/g F.W) fruit fructose was associated with a temperature treatment of 25°C. Also, statistically, there was no significant difference ( $p < 0.05$ ) between temperatures of 25°C and 35°C in terms of fruit fructose. The lowest fruit fructose value was related to a temperature of 40°C, which was not statistically different ( $p < 0.05$ ) from fruit fructose of 35°C treatment. With regards to foliar application treatment, supplementary Fig. 12 shows that the highest (9.17 mg/g F.W) fruit fructose value was associated with 100μM melatonin foliar treatment. Moreover, in terms of fruit fructose value, there was no significant difference ( $p < 0.05$ ) between 0μM, 100μM melatonin and 0μM, 100μM sodium nitroperoxide foliar application treatments. In reference to data in Fig. 13, the highest (109.86 mg/100g F.W.) and lowest (71.83 mg/100g F.W.) values of fruit vitamin C were related to temperature treatments of 25°C and 40°C respectively. According to data in Fig. 14 the highest (96.34 m/g.100g F.W.) amount of fruit vitamin C was related to foliar application

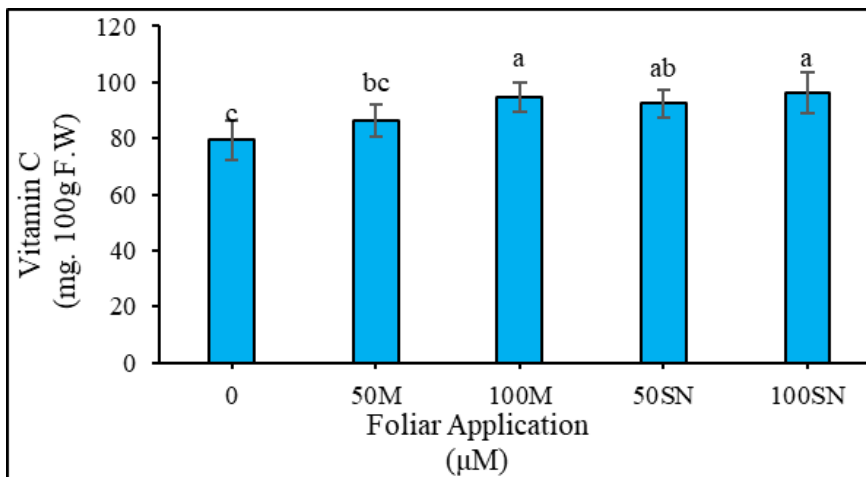
treatment of 100 $\mu$ M sodium nitroperoxide. There was no significant difference ( $p < 0.05$ ) between foliar application of 100 $\mu$ M melatonin, 50 $\mu$ M sodium nitroperoxide and 100 $\mu$ M sodium nitroperoxide. The data from Fig. 15 shows that the highest (79.39%) and lowest (57.23%) fruit antioxidant capacity were related to temperature treatments of 25°C and 40°C, respectively. Fig. 16 highlights that in terms of fruit antioxidant capacity no significant difference ( $p < 0.05$ ) was observed between foliar application treatments with melatonin and sodium nitroperoxide and also the lowest fruit antioxidant capacity was related to foliar application treatment 0 $\mu$ M (control).



**Fig. 12.** Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on fruit fructose of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.



**Fig.13.** Impact of temperature various levels on fruit vitamin C of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.



**Fig.14.** Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on fruit vitamin C of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

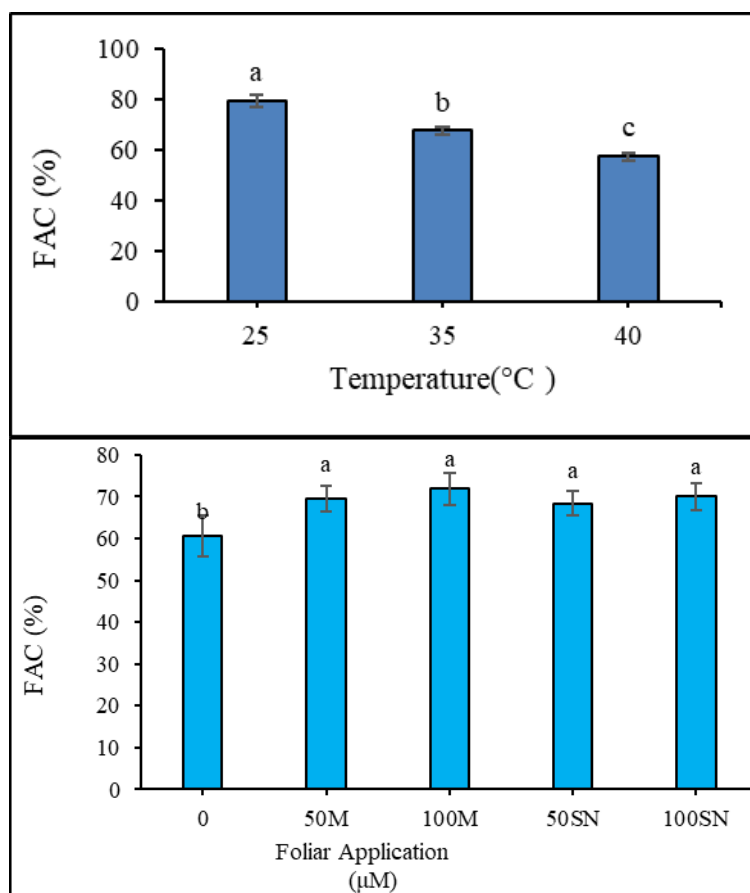


Fig. 15. Impact of temperature various levels on fruit antioxidant capacity of california wonder green bell pepper. Bars showing different letter (s) at p< 0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

Fig. 16. Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on fruit antioxidant capacity of california wonder green bell pepper. Bars showing different letter (s) at p< 0.05 for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

Table 4. Impact of melatonin, sodium nitroperoxide and temperature various levels on fruit carbohydrates, Glucose, Sucrose and Flavonoids of *Capsicum annum*.

Temperature (°C)	Foliar Application (µM)	Carbohydrates (Mg.g D.M)	Glucose (Mg.g F.W)	Sucrose (Mg.g F.W)	Flavonoids (mg/.100g F.W)
25	0	16.41±0.27fg	6.07±0.29f	10.41±0.17fg	11.96±0.48c
	50 M	15.95±0.47g	6.67±0.11ef	9.71±0.55gh	10.85±0.32d
	100 M	20.70±0.56d	7.24±0.22e	8.98±0.38h	14.31±0.11a
	50 NSP	17.76±0.56ef	7.00±0.35ef	10.07±0.42gh	10.82±0.29de
	100 NSP	19.94±0.63d	6.79±0.32ef	10.43±0.33efg	13.38±0.03b
35	0	23.84±0.47c	11.05±0.19a	13.64±0.16b	9.46±0.51f
	50 M	20.68±0.55d	9.85±0.3b	12.01±0.41cd	10.63±0.06de
	100 M	26.31±0.64b	7.36±0.34e	15.44±0.69a	11.88±0.44c
	50 NSP	23.90±0.37c	6.75±0.64ef	11.68±0.56de	10.80±0.1de
	100 NSP	29.35±0.63a	7.61±0.45de	13.13±0.29bc	11.30±0.2cd
40	0	10.83±0.75i	7.49±0.09e	9.06±0.48h	7.11±0.15g
	50 M	13.75±0.14h	8.51±0.57cd	10.38±0.34fg	9.30±0.06f
	100 M	15.43±0.61g	7.17±0.38e	12.01±0.49cd	10.57±0.45de
	50 NSP	13.67±0.6h	9.22±0.21bc	10.88±0.48defg	9.49±0.11f
	100 NSP	18.09±0.29e	7.66±0.18de	11.40±0.34def	10.00±0.2ef
LSD		1.54	1.00	1.25	0.83

Melatonin (M) and Sodium Nitroperoxide (NSP), Error bars signify standard error (SE) three replicates, 25°C Temperature and 0µM foliar application: Control, Values in the same column with similar letters are not significantly different (p ≤ 0.05).

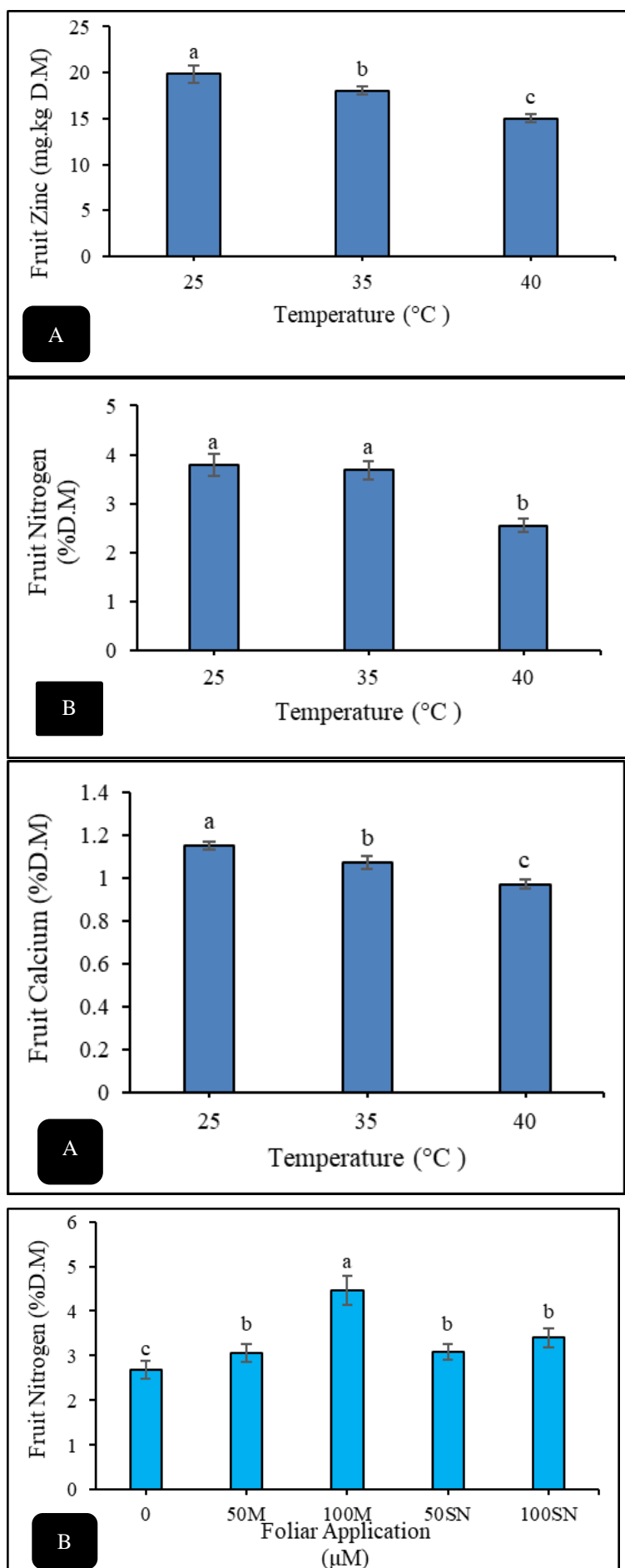
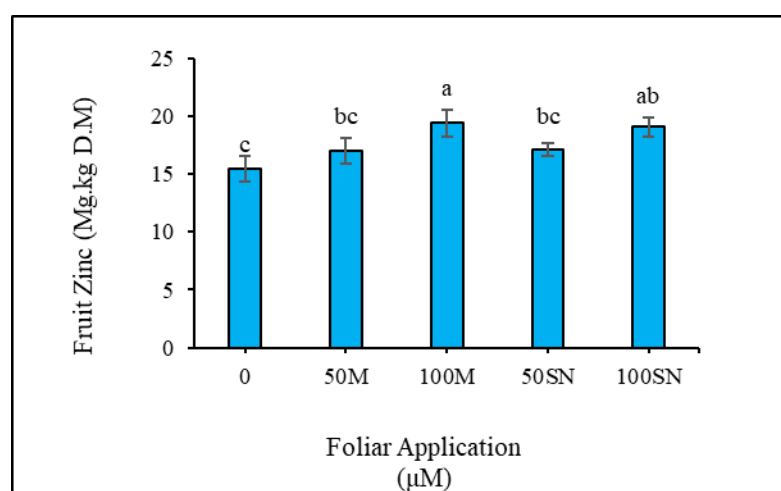


Fig.17. Impact of temperature various levels on Fruit Zinc (A) and Fruit Nitrogen (B) of california wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

Fig.18. Impact of melatonin (M), sodium nitroperoxide (SN) and temperature various levels on Fruit Calcium (A) and Fruit Nitrogen (B) of california wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.

### 3.5. Fruit mineral elements

As highlighted in Fig. 17 (A), there was no statistically significant difference ( $p < 0.05$ ) between temperatures of 25°C and 35°C in terms of fruit nitrogen, and the lowest fruit nitrogen content (2.55% D.M) was associated with a temperature of 40°C. Fig. 18 (B) indicates that the highest (4.46% D.M) and the lowest (2.68% D.M) nitrogen contents were related to 100µM melatonin and 0µM (control), respectively. According to Fig. 18 (A), in terms of fruit calcium, the highest (1.15% D.M) and lowest (0.97% D.M) contents were related to temperature treatments of 25°C and 40°C, respectively. As shown in supplementary Fig. 17 (B), the highest (19.87 Mg/kg D.M) and lowest (15.04 mg/kg D.M) fruit zinc contents were associated with temperature treatments of 25°C and 40°C, respectively. Furthermore, the highest (19.44 mg/kg D.M) and lowest (15.52 mg/kg D.M) fruit zinc contents were related to 100µM melatonin and control (0µM), respectively as shown in Fig. 19.



**Fig. 19. Impact of melatonin (M) and sodium nitroperoxide (SN) various levels on Fruit Zinc of California wonder green bell pepper. Bars showing different letter (s) at  $p < 0.05$  for each group imply significant difference. Error bars signify standard error (SE) of three replicates and 25°C Temperature: Control.**

**Table 5. Impact of melatonin, sodium nitroperoxide and temperature various levels on fruit Copper, Manganese and Iron of *Capsicum annum*.**

Temperature (°C)	Foliar Application (µM)	Copper (mg/kg D.M)	Manganese (mg/kg D.M)	Iron (mg/kg D.M)
25	0	13.01±1.44ab	9.45±0.33c	70.61±3.39b
	50M	14.04±0.12a	11.00±0.05a	70.46±3.57b
	100M	13.10±0.1ab	10.32±0.06b	77.27±0.66a
	50NSP	13.72±0.44ab	11.01±0.07a	74.18±0.68ab
	100NSP	12.43±0.3bc	10.31±0.04b	70.57±2.88b
35	0	8.71f±0.29g	8.43±0.09e	53.47±1.47e
	50M	9.50±0.17ef	8.96±0.04d	56.41±1.25de
	100M	10.12±0.05de	9.51±0.2c	58.79±1.3cd
	50NSP	9.66±0.27ef	8.80±0.15de	57.02±0.57de
	100NSP	11.23±0.45cd	9.45±0.16c	62.57±0.71c
40	0	7.26±0.6h	7.00±0.33g	40.27±1.21h
	50M	6.97±0.04h	8.48±0.09e	41.52±0.37gh
	100M	9.60±0.21ef	7.83±0.08f	46.32±0.66fg
	50NSP	7.15±0.08h	8.56±0.07de	47.11±0.67f
	100NSP	7.78±0.4gh	7.85±0.04f	52.51±0.87e
LSD		3.42	1.37	0.46

Melatonin (M) and Sodium Nitroperoxide (NSP), Error bars signify standard error (SE) three replicates, 25°C Temperature and 0µM foliar application: Control, Values in the same column with similar letters are not significantly different ( $p \leq 0.05$ ).

As illustrated by Table 4, 5 the highest (14.04 mg/kg D.M) and lowest (6.97 mg/kg D.M) fruit copper contents were related to temperature treatment of 25°C with 50µM melatonin and temperature treatment of 40°C with 50 µM melatonin, respectively. According to data from Table 5, there was no statistically significant difference ( $p < 0.05$ ) in fruit manganese content between temperature 25°C with 50µM melatonin and treatment 25°C with 50µM sodium nitroperoxide. Moreover, the lowest (7mg/kg D.M) fruit manganese content was related to temperature treatment of 40°C with 0µM foliar spraying. Table 5 demonstrates that, at 40°C, the highest fruit iron content (52.51mg/kg D.M) was related to 100µM sodium nitroprusside application.

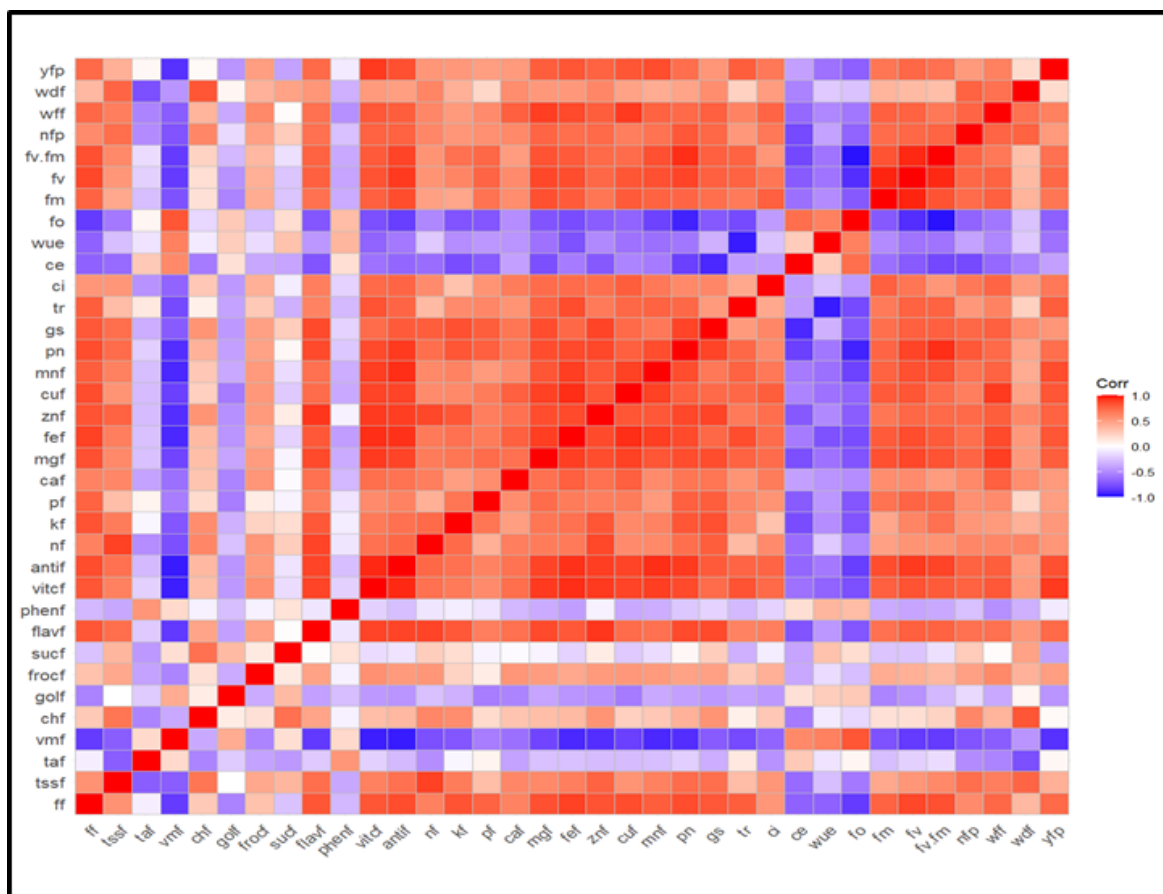


Fig. 20. Pearson's correlation between the studied parameters of *Capsicum annum* after exposure to foliar application melatonin and sodium nitroprusside under different temperature regimes. ff—fruit firmness; tssf—fruit total soluble solids; vmf—fruit marketability; chf—fruit carbohydrates; golf—fruit glucose; frocf —fruit fructose; sucf—fruit sucrose; flavf —fruit flavonoid; phenf—fruit phenol; vitcf—fruit vitamin C; antif—fruit antioxidant activity; nf —fruit nitrogen; kf—fruit potassium; pf—fruit phosphorus; caf—fruit calcium;mgf—fruit magnesium; fef —fruit iron; znf—fruit zinc; cuf —fruit copper; mnf—fruit manganese; pn—photosynthesis rate; gs—leaf stomatal conductance; tr—transpiration rate; ci—sub stomatal carbon dioxide concentration; ce—mesophilic conductance; wue—leaf water consumption efficiency; fo—minimum fluorescence;fm —maximum fluorescence; fv—variable fluorescence; fv.fm—photosystem 2 efficiency; nfp— fruits number per plant; wff— fruit fresh weight; wdf— fruit dry weight; yfp—fruit yield per plant.

### 3.6. Correlation and Principal Component Analysis

The results of the correlation analysis showed that the trait of fruit yield per plant (yfp) had a significant positive correlation with the traits of fruit vitamin C (vitcf), fruit antioxidant activity (antif), fruit copper (cuf), fruit manganese (mnf) and leaf transpiration rate (tr). Also, fruit yield per plant trait (yfp) has a significant negative correlation with fruit marketability traits (vmf), leaf water use efficiency (wue) and leaf minimum fluorescence (fo) (Fig. 20). A

PCA was conducted to analyze the variability of collected information and the interrelation between the different treatments and attributes. The results showed that the first dimension explained 61.90 percent and the second dimension explained 12 percent of the variances. The treatments of 35°C temperature with 50µM sodium nitroprusside (t2m4), 35 °C temperature with 100 µM sodium nitroprusside (t2m5) and 35°C temperature with 100 µM melatonin (t2m3) had the best conditions due to being in cluster one. Also, according to Fig. 21, the treatment of 35 °C temperature with 100 µM sodium nitroprusside (t2m5) increased the fruit dry weight (wdf) due to being close to traits such as fruit carbohydrate (chf) and fruit total soluble solids (tssf). The 40°C temperature treatment with 100 µM melatonin (t3m3) had a higher leaf water use efficiency (wue) due to the short distance with traits such as fruit marketability (vmf) and leaf minimum fluorescence (fo) (Fig. 21).

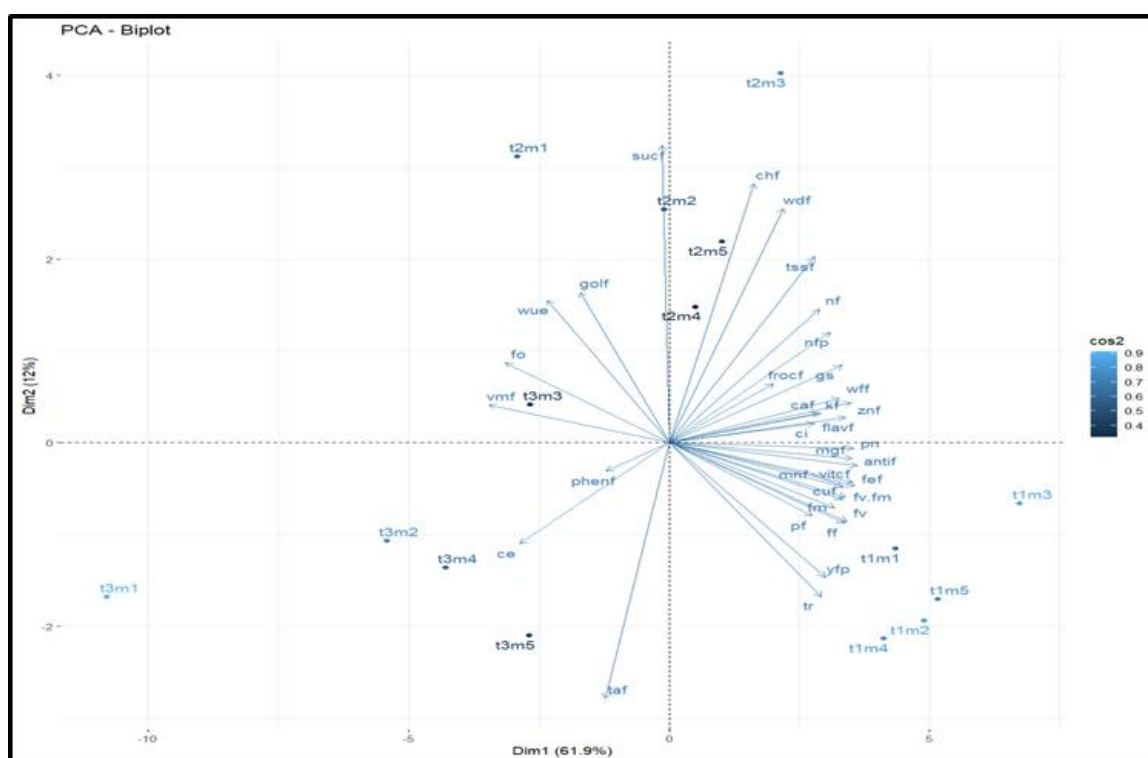


Fig. 21. Biplots of principal component analysis (PCA) represent the relationship between different variables and treatments in *Capsicum annum* treated with foliar application (m1:0 µM, m2:50mel, m3:100mel, m4:50snp, m5:100snp) under different temperature regimes (t1:25, t2:35, t3:40°C). The variables included ff—fruit firmness; tssf—fruit total soluble solids; vmf—fruit marketability; chf—fruit carbohydrates; golf—fruit glucose; frocf —fruit fructose; sucf—fruit sucrose; flavf —fruit flavonoid; phenf—fruit phenol; vitcf—fruit vitamin C; antif—fruit antioxidant activity; nf —fruit nitrogen; kf—fruit potassium; pf—fruit phosphorus; caf—fruit calcium;mgf—fruit magnesium; fef —fruit iron; znf—fruit zinc; cuf —fruit copper; mnf—fruit manganese; pn—photosynthesis rate; gs—leaf stomatal conductance; tr—transpiration rate; ci—sub stomatal carbon dioxide concentration; ce—mesophilic conductance; wue—leaf water consumption efficiency; fo—minimum fluorescence;fm —maximum fluorescence; fv—variable fluorescence; fv.fm—photosystem 2 efficiency; nfp— fruits number per plant; wff— fruit fresh weight; wdf— fruit dry weight; yfp—fruit yield per plant.

#### 4. Discussion

As temperature increased from 25 to 40°C, fruit nitrogen content decreased by 32.72%. It seems that by high temperature stress, certain genes were activated, which induce stomatal closure, thus reducing transpiration rate, consequently decreasing nutrient uptake. Fruit calcium decreased by 6.96% and 15.65% at 35°C and 40°C respectively in comparison with control temperature (25°C) and also Fruit zinc decreased by 9.21% and 24.31% at 35°C and 40°C respectively compared to the control temperature. High temperature stress reduces

synthesis of growth stimulators such as cytokinins and increases growth inhibitors such as abscisic acid, which retards plant growth, and reduces the plant's ability to take up water and nutrients (Li *et al.*, 2021).

Melatonin plays a crucial role in enhancing various biological processes including growth and development, photosynthesis, ion absorption and transfer, modulation of enzyme activity, as well as improving transpiration and translocation within the plant. Indeed, the application of 100  $\mu\text{M}$  melatonin on leaves seems to stimulate the activation of particular plant genes responsible for synthesizing specific enzymes that enhance photosynthetic mechanisms during periods of stress, leading to elevated levels of carbohydrates. Furthermore, the enhancement of photosynthetic efficiency facilitates the transportation of essential nutrients (Mineral Elements) from the roots to aboveground plant parts (such as stems and fruits). Foliar application with 100  $\mu\text{M}$  melatonin increased the fruit zinc content. Melatonin allows leaves to have a greater capacity for carbon dioxide absorption and stomatal conduction (Arnao *et al.*, 2014), which increases photosynthetic capacity and carbohydrate reserves. Furthermore, melatonin increases net rate of photosynthesis, stomatal conductance and simultaneously increases relative water content, which increases plant metabolism thus enhancing plant tolerance to stress (Arnao *et al.*, 2014; Eshghi *et al.*, 2024). Better growing conditions and carbon production (carbohydrate reserves) facilitate nutrient absorption from soil and their transfer to plant organs. It has also been reported that low concentrations of melatonin stimulate biosynthesis of indole acetic acid (IAA) and root growth (Roosta *et al.*, 2024; Arnao *et al.*, 2014).

Application of 100 $\mu\text{M}$  sodium nitroprusside at 40°C resulted in a 30.39% increase in fruit iron content compared to the control. Similarly, treatment with 50 $\mu\text{M}$  sodium nitroprusside at the same temperature led to a 22.29% increase in fruit manganese content compared to the 0 $\mu\text{M}$  treatment. The application of either 100 $\mu\text{M}$  sodium nitroprusside or ABA effectively alleviated the detrimental impacts of heat stress on the photosynthetic process and overall growth of wheat (*Triticum aestivum* L.) plants. These plants were subjected to a temperature of 40°C for a duration of six hours daily over a period of 15 days (Iqbal *et al.*, 2022). The maximum fluorescence, variable fluorescence, and photosystem II efficiency ( $F_v/F_m$ ) were significantly diminished under heat temperature stress (35 and 40°C). However, the minimum fluorescence exhibited an increase. When the photochemical efficiency of photosystem II in various plant species exceeds 0.83, it signifies the absence of stress on the plant. Consequently, any values below this threshold indicate the presence of stress in plants (Mihaljević *et al.*, 2020). The efficiency of Photosystem II serves as a key parameter for assessing the operational status of the photosynthetic system (Manafi *et al.*, 2021). The activity of photosystem II, is significantly decreased under high temperatures stress (Christou *et al.*, 2014; Rowland *et al.*, 2010). A reduction in photosystem II efficiency serves as a sign of photoinhibitory damage in plants under various stress conditions (Li *et al.*, 2013; Moradi *et al.*, 2017).

The findings of this experiment are consistent with the results of Zhou *et al.*, (2020), who reported that high temperature decreased the  $F_v/F_m$  of tomato seedlings. In the present study, the sodium nitroprusside foliar application significantly prevented the decrease of photosystem II efficiency induced by high temperature stress. The maintenance of photosystem II efficiency in sodium nitroprusside treated plants under high temperature stress was observed in a previous study (Song *et al.*, 2013). These results indicated that sodium nitroprusside reduced heat-induced photoinhibition by protecting photosystem II. Sodium nitroprusside may improve photosystem II efficiency by increasing photosynthetic pigments and hindering pigment deterioration resulting in progressing the overall photosynthetic process (Sahay *et al.*, 2020). The findings of Yang *et al.*, (2011) in chrysanthemum (*Chrysanthemum morifolium* L.) and Xu *et al.*, (2016) in tomato showed that the use of

melatonin and nitric oxide increased leaf chlorophyll fluorescence under high temperature stress conditions. Melatonin and Nitric oxide enhance the activity of antioxidant enzymes thus preventing accumulation of free radicals and hydrogen peroxide as well as preserving integrity of the cell membrane, consequently reducing damage to photosystem II and retaining its efficiency. Also, maintaining the photosystem II function under heat stress may be related to the effect of melatonin and nitric oxide in inhibiting the decomposition of photosynthetic pigments and increasing leaf chlorophyll content (Yang *et al.*, 2011). On the other hand, nitric oxide can play an important role in protection of photosystem II by regulating the expression of the *psbA* gene and improving the photochemical efficiency of this photosystem (Gautam *et al.*, 2022). Nitric oxide probably improves the chlorophyll status of plant cells by the removal of the reactive oxygen species produced under stress (Laspina *et al.*, 2005).

Sodium nitroprusside treatment also helped to retain the photosystem II efficiency and prevented the increase in  $F_0$  (Yang *et al.*, 2011). It was documented that prior exposure to 100 $\mu$ M sodium nitroprusside led to improved photosynthetic electron transport in *Festuca arundinacea* when subjected to a temperature of 44°C (Laspina *et al.*, 2005). By utilizing sodium to recover the concentration of chlorophyll in heat stressed leaves of *Gingiber officinale*, the fluorescence components of chlorophyll are subsequently normalized (Laspina *et al.*, 2005). Heat stress reduces the substomatal carbon dioxide (CDS) and transpiration (LTR). Stomatal closure due to heat stress leads to a decrease in leaf stomatal conductance (LSC), triggering reduction in plant transpiration rate (Chen *et al.*, 2013). Due to the reduction of carbon dioxide concentration under temperature stress conditions, rate of photosynthesis (RLP) decreased. Moreover, with use of melatonin and sodium nitroprusside, rate of leaf photosynthesis (RLP) changed compared to control. The instantaneous leaf water consumption efficiency (ILWCE) was enhanced through the application of melatonin and sodium nitroprusside via foliar method at a temperature of 40°C. Under 40°C temperature and foliar application of with 50 $\mu$ M and 100 $\mu$ M melatonin instantaneous water consumption efficiency (ILWCE) was better than other pre-treated. It was also reported that pretreat plants with sodium nitroprusside can improve the photosynthesis under heat stress (Sehar *et al.*, 2023). And also reported that the exogenous application of sodium nitroprusside under heat stress improved the photosynthesis system (Hassan *et al.*, 2022).

The fruit's total soluble solids (TSS) decreased by 18.50% when exposed to a temperature of 40°C, as compared to the control temperature of 25°C. Increase in temperature disrupts the plant photosynthetic system, as well as disharmonize plant sink-source relationships, and alters plant hormonal balance. These changes result in reduced fruit total soluble solids (TSS). Nevertheless, when exposed to 100  $\mu$ M melatonin, there was a 20.69% rise in fruit total soluble solids (TSS) compared to the control treatment of 0  $\mu$ M. Low concentrations of melatonin can increase carbon dioxide conversion efficiency and dry weight accumulation (Zhang *et al.*, 2015). Melatonin and sodium nitroprusside treatment increased the carbohydrate storage by improving the photosynthetic capacity of the plant and thus increased the fruit total soluble solids (TSS) under temperature stress conditions (35 °C and 40 °C).

At a temperature of 40°C, the fruit TA increased by 39.62% compared to the control temperature of 25°C, which can be linked to the reduction in fruit TSS. The photosynthesis rate decreased under temperature stress conditions (35 and 40°C), leading to a more active respiration pathway and the production of excessive organic acids, thus increasing fruit TA. Furthermore, aside from the overall impact of temperature stress on the photosynthetic system and the decrease in primary carbohydrate synthesis, the heightened activity of other metabolic pathways results in an increased production of organic acids. Heat stress in plants, also causes the activation of enzymes that take part in the production of basic compounds which leads to the sugars consumption and subsequent organic acids production (Siddiqui *et al.*, 2017). The application of 100 $\mu$ M sodium nitroprusside resulted in a 58.70% increase in fruit titratable

acidity (TA), potentially attributed to the synthesis of nitrogenous acidic compounds induced by sodium nitroprusside within the plant. At a temperature of 35°C, the application of 100µM sodium nitroprusside resulted in a 23.11% increase in fruit carbohydrate content compared to the control treatment (0 µM). Similarly, at 40°C, the same treatment led to a 67.04% increase in fruit carbohydrate content. Furthermore, at 40°C, there was a 5.52% decrease in fruit fructose content compared to the treatment at 25°C. At a temperature of 35 °C, the application of 100µM melatonin through foliar treatment resulted in a 13.20% increase in fruit sucrose levels compared to the control group which received 0 µM of melatonin. Plants possess non-enzymatic antioxidant defense system (carbohydrates) to cope with the destructive effects of heat-induced ROS (Hasanuzzaman *et al.*, 2013). Melatonin increases chlorophyll content and promotes the photosynthetic compounds production in plants under heat stress thus maintaining a considerable amount of sugars under high temperature stress (Paulsen, 1994). Sodium nitroprusside reduced the high temperature stress in tomato plants by increasing, carbohydrates (Sarropoulou *et al.*, 2012). Several lines of evidence indicate NO as a key signaling molecule in mediating various plant responses such as photosynthesis and osmolyte accumulation (carbohydrate) under heat stress. Furthermore, nitric oxide is related to other messenger molecules (signaling molecules) such as phytohormones in relation to high temperature stress. Several lines of evidence have reinforced the importance of accumulation of osmolytes such as soluble sugars to maintain osmotic balance in heat stressed plants (Li *et al.*, 2020).

At a temperature of 40°C, the application of 100µM melatonin through foliar spraying resulted in a significant increase of 48.66% in fruit flavonoid content compared to the control group with 0 µM melatonin application. Conversely, at the same temperature, there was a notable decrease of 34.62% in fruit Vitamin C levels compared to the control temperature of 25°C. The fruit antioxidant capacity decreased by 27.91% when the temperature was raised to 40°C, as compared to the antioxidant capacity at 25°C. Foliar application treatments (50, 100µM melatonin and sodium nitroprusside) increased the fruit antioxidant capacity in comparison with 0µM foliar application treatment (control). According to reports, the application of 100µM sodium nitroprusside has been shown to alleviate heat damage by regulating non-enzymatic antioxidants, including vitamin C. Studies have indicated that melatonin can enhance the production of phytohormones and antioxidants, improve nutrient absorption, and enhance the quality of products, such as increasing fruit Vitamin C content and antioxidant capacity.

The quantity of fruits per plant (NFP), the weight of fresh fruits per plant (SFW), and the weight of dry fruits (DWSF) exhibited a decline as the temperature rose. At 40°C temperature, 100µM melatonin, 20.46% and 13.15% increased the fruit fresh and dry weight (SFW) in compared to control (0µM) respectively. Fruit marketability characteristics decreased with temperature increasing. Heat stress denatures proteins and enzymes, and reduces nutrient and water uptake, which is also a major reason for the reduction in plant growth (dry weight loss) under Heat stress (Oloumi *et al.*, 2018). Heat stress decreases the photosynthesis rate, while increasing respiration and photo-respiration, which in turn reduces assimilates production and causes reduction in growth and biomass production (dry weight loss) (Paulsen, 1994). Melatonin through changes in the amounts and types of plant hormones, increases cytokinin and auxin and as a result improves plant growth. Additionally, the application of melatonin and sodium nitroprusside resulted in enhanced fruit marketability traits and decreased compared to the control group. In a recent study, it was found that melatonin elevates biomass production in tea plants and improves tea quality under high temperature (Li *et al.*, 2020). An exogenous supply of melatonin also maintains the nutrient and water uptake and results in significant improvements in growth and development. The findings revealed that melatonin treatment promotes the dry and fresh weight. In another study, it was determined that

melatonin foliar application increased the biomass and dry weight of shoot and root of watercress (Oloumi *et al.*, 2018). Under conditions of elevated temperature stress, the application of melatonin and sodium nitroprusside to the leaves leads to enhanced absorption of mineral elements. This subsequently results in an increase in the amount of chlorophyll present in the leaves, thereby elevating the rate of photosynthesis. Furthermore, this increase in photosynthesis leads to a higher production of plant carbohydrates, ultimately leading to improved growth and biomass values when compared to the control group.

## Conclusions

The findings of this study indicated that applying melatonin and sodium nitroprusside through foliar spraying on *Capsicum annuum* with five leaves helped mitigate heat-induced stress by high temperatures. The research findings indicated that the stress induced by elevated temperatures was alleviated through the impact of melatonin and sodium nitroprusside on various biochemical, physiological, and morphological processes. Furthermore, the application of melatonin and sodium nitroprusside resulted in an enhancement of fruit quality by influencing the synthesis of primary metabolites, ultimately leading to an increase in the soluble solids content of the fruit. Foliar application of melatonin and sodium nitroprusside has been shown to alleviate the stress induced. The results indicate that the concurrent foliar application of melatonin and sodium nitroprusside was not carried out during the seedling production phase of the *Capsicum annuum* under high temperature stress. Therefore, spraying the foliage with concentrations of 50 and 100 micromolar melatonin and sodium nitroprusside proves to be an efficient method for mitigating heat stress in regions where this crop is cultivated in both greenhouse and field settings under high temperature conditions. The examination of various moments for applying foliage spray can be cited in relation to the growth and maturity phases of the plant within both field and greenhouse settings. Further investigation is warranted in order to gain a deeper understanding of the chemical and molecular processes that govern the behavior of melatonin and sodium nitroprusside following their application.

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## Conflicts of Interest

The authors declare no conflict of interest.

## References

- Alam, M. N., Zhang, L., Yang, L., Islam, M. R., Liu, Y., Luo, H., P. Yang, Q. Wang, Z. Chan, and Chan, Z. 2018. Transcriptomic profiling of tall fescue in response to heat stress and improved thermotolerance by melatonin and 24-epibrassinolide. *BMC genomics*, 19, 1-14. <https://doi.org/10.1186/s12864-018-4588-y>.
- Arnao, M. B. and Hernández-Ruiz, J. 2014. Melatonin: plant growth regulator and/or biostimulator during stress?. *Trends in plant science*, 19(12), 789-797. <https://doi.org/10.1016/j.tplants.2014.07.006>.
- Astier, J., Gross, I., & Durner, J. 2018. Nitric oxide production in plants: an update. *Journal of Experimental Botany*, 69(14), 3401-3411. <https://doi.org/10.1093/jxb/erx420>.
- Azizi, S., Lastochkina, O.V., Seyed Hajizadeh, H and Aliniaiefard, S. 2024. Proper quality Citation of LED light to produce high-quality ornamental plants in controlled environment agricultural systems: A review. *Greenhouse Plant Production Journal*, 1(2): 35-50.

- Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, 10(3). <https://doi.org/10.38212/2224-6614.2748>.
- Chen, K., Chen, L., Fan, J. and Fu, J., 2013. Alleviation of heat damage to photosystem II by nitric oxide in tall fescue. *Photosynthesis research*, 116, 21-31. <https://doi.org/10.1007/s11120-013-9883-5>.
- Christou, A., Filippou, P., Manganaris, G.A. and Fotopoulos, V., 2014. Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. *BMC Plant Biology*, 14, 1-11. <https://doi.org/10.1186/1471-2229-14-42>.
- Eshghi, S., Sarikhani, S., Shirdel, M., Davarzani, M. and Hosseini, S. S. 2024. The Citationsignificance of controlled environments in shaping the future of fruit cultivation. *Greenhouse Plant Production Journal*, 1(4): 11–35.
- Farooq, M., Basra, S. M. A., Wahid, A. and Rehman, H. 2009. Exogenously applied nitric oxide enhances the drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science*, 195(4), 254-261. <https://doi.org/10.1111/j.1439-037x.2009.00367.x>.
- Garvey, S. M., Templer, P. H., Bhatnagar, J. M. and Hutryra, L. R. 2023. Soils at the temperate forest edge: An investigation of soil characteristics and carbon dynamics. *Science of The Total Environment*, 891, 164320.
- Gautam, H., Fatma, M., Sehar, Z., Mir, I.R. and Khan, N.A., 2022. Hydrogen sulfide, ethylene, and nitric oxide regulate redox homeostasis and protect photosynthetic metabolism under high temperature stress in rice plants. *Antioxidants*, 11(8),1478. <https://doi.org/10.3390/antiox11081478>.
- Hasanuzzaman, M., Nahar, K., Alam, M.M., Roychowdhury, R. and Fujita, M., 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International journal of molecular sciences*, 14(5), 9643-9684. <https://doi.org/10.3390/ijms14059643>.
- Hassan, M.U., Ghareeb, R.Y., Nawaz, M., Mahmood, A., Shah, A.N., Abdel-Megeed, A., Abdelsalam, N.R., Hashem, M., Alamri, S., Thabit, M.A. and Qari, S.H., 2022. Melatonin: a vital pro-tectant for crops against heat stress: mechanisms and prospects. *Agronomy*, 12(5),1116. <https://doi.org/10.3390/agronomy12051116>.
- Iqbal, N., Hussain, S., Raza, M. A., Yang, C. Q., Safdar, M. E., Brestic, M., A. Aziz, M.S. Hayyat, M.A. Asghar, X.C. Wang, J. Zhang, W. Yang. and Liu, J. 2019. Drought tolerance of soybean (*Glycine max* L. Merr.) by improved photosynthetic characteristics and an efficient antioxidant enzyme activities under a split-root system. *Frontiers in physiology*, 10, 786.<https://doi.org/10.3389/fphys.2019.00786>.
- Iqbal, N., Sehar, Z., Fatma, M., Umar, S., Sofo, A. and Khan, N. A. 2022. Nitric oxide and abscisic acid mediate heat stress tolerance through regulation of osmolytes and antioxidants to protect photosynthesis and growth in wheat plants. *Antioxidants*, 11(2), 372. <https://doi.org/10.3390/antiox11020372>.
- Laspina, N.V., Groppa, M.D., Tomaro, M.L. and Benavides, M.P., 2005. Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant science*, 169(2), 323-330. <https://doi.org/10.1016/j.plantsci.2005.02.007>.
- Li, S. M., Zheng, H. X., Zhang, X. S. and Sui, N. 2021. Cytokinins as central regulators during plant growth and stress response. *Plant cell reports*, 40, 271-282. <https://doi.org/10.1007/s00299-020-02612-1>.
- Li, X., Li, M.H., Deng, W.W., Ahammed, G.J., Wei, J.P., Yan, P., Zhang, L.P., Fu, J.Y. and Han, W.Y., 2020. Exogenous melatonin improves tea quality under moderate high temperatures by increasing epigallocatechin-3-gallate and theanine biosynthesis in *Camellia sinensis* L. *Journal of plant physiology*, 253, 153273. <https://doi.org/10.1016/j.jplph.2020.153273>.
- Li, Z.G., Yang, S.Z., Long, W.B., Yang, G.X. and Shen, Z.Z., 2013. Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. *Plant, Cell & Environment*, 36(8),1564-1572. <https://doi.org/10.1111/pce.12092>.
- Liu, J., Wang, W., Wang, L. and Sun, Y. 2015. Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant growth regulation*, 77, 317-326. <https://doi.org/10.1007/s10725-015-0066-6>.
- Manafi, H., Baninasab, B., Gholami, M. and Talebi, M., 2021. Nitric oxide induced thermotolerance in strawberry plants by activation of antioxidant systems and transcriptional regulation of heat shock

- proteins. *The Journal of Horticultural Science and Biotechnology*, 96(6), 783-796. <https://doi.org/10.1080/14620316.2021.1927206>.
- Mansha, M. Z., Aatif, H. M., Ikram, K., Hanif, C. M. S., Sattar, A., Iqbal, R., Q. uz Zaman, Salem Mesfir Al-Qahtani, Nadi Awad Al-Harbi, W.A. Omar. and Ibrahim, M. F. 2023. Impact of various salinity levels and *Fusarium oxysporum* as stress factors on the morpho-physiological and yield attributes of onion. *Horticulturae*, 9(7), 786. <https://doi.org/10.3390/horticulturae9070786>.
- Maxwell, K. and Johnson, G. N. 2000. Chlorophyll fluorescence—a practical guide. *Journal of experimental botany*, 51(345), 659-668.. <https://doi.org/10.1093/jexbot/51.345.659>.
- McCready, R. M., Guggolz, J., Silveira, V. and Owens, H. S. 1950. Determination of starch and amylose in vegetables. *Analytical chemistry*, 22(9), 1156-1158.
- McDonald, S., Prenzler, P. D., Antolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive extracts. *Food chemistry*, 73(1), 73-84. [https://doi.org/10.1016/s0308-8146\(00\)00288-0](https://doi.org/10.1016/s0308-8146(00)00288-0).
- Mihaljević, I., Lepeduš, H., Šimić, D., Vuletić, M.V., Tomaš, V., Vuković, D., Dugalić, K., Teklić, T., Babojelić, M.S. and Zdunić, Z., 2020. Photochemical efficiency of photosystem II in two apple cultivars affected by elevated temperature and excess light in vivo. *South African journal of botany*, 130, 316-326. <https://doi.org/10.1016/j.sajb.2020.01.017>.
- Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, 85(2), 231-237. <https://doi.org/10.1016/j.foodchem.2003.05.007>.
- Moradi, S., Baninasab, B., Gholami, M. and Ghobadi, C., 2017. Paclobutrazol application enhances antioxidant enzyme activities in pomegranate plants affected by cold stress. *The Journal of Horticultural Science and Biotechnology*, 92(1), 65-71. <https://doi.org/10.1080/14620316.2016.1224605>.
- Oloumi, H., F. Nasibi. and H. Mozaffari., 2018. Investigation of the growth rate and secondary metabolites content of *Lepidium sativum* under exogenous melatonin treatment, Graduate University of Advanced Technology. 5, 144–154. <https://doi.org/10.29252/nbr.5.2.144>.
- Orazalin, N. S., Ntim, C. G. and Malagila, J. K. 2024. Board sustainability committees, climate change initiatives, carbon performance, and market value. *British Journal of Management*, 35(1), 295-320. <https://doi.org/10.1111/1467-8551.12715>.
- Owens, R.M. M. 1950. Determination of starch and amylose in vegetables. *Anal Chem*, 22, 1156-1159.
- Parankusam, S., Adimulam, S. S., Bhatnagar-Mathur, P. and Sharma, K. K. 2017. Nitric oxide (NO) in plant heat stress tolerance: current knowledge and perspectives. *Frontiers in Plant Science*, 8, 1582. <https://doi.org/10.3389/fpls.2017.01582>.
- Paulsen, G.M. High temperature responses of crop plants. *Physiology and determination of crop yield*. 1994 Oct 1:365-89. <https://doi.org/10.2134/1994.physiologyanddetermination.c25>.
- Roosta, H.R, Sharifinejad, S. and Azizi Ilami, M. R. and Bikeloo, M. 2024. The effect of Citationdifferent ratios of vermicompost in various planting substrates on the growth and physiological characteristics of *Spathiphyllum (Spathiphyllum wallisii)*. *Greenhouse Plant Production Journal*, 1(4): 49-72. <https://10.61186/gppj.1.4.49>
- Rowland, J.G., Pang, X., Suzuki, I., Murata, N., Simon, W.J. and Slabas, A.R., 2010. Identification of components associated with thermal acclimation of photosystem II in *Synechocystis* sp. PCC6803. *PLoS One*, 5(5), p.e10511. <https://doi.org/10.1371/journal.pone.0010511>.
- Roy, S., Mathur, P., Chakraborty, A. P., & Saha, S. P. (Eds.). 2022. *Plant stress: Challenges and management in the new decade*. Springer Nature.
- Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M. and Hasanuzzaman, M. 2021. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, 10(2), 277. <https://doi.org/10.3390/antiox10020277>.
- Sahay, S., Torres, E.D.L.C., Robledo-Arratia, L. and Gupta, M., 2020. Photosynthetic activity and RAPD profile of polyethylene glycol treated *B. juncea* L. under nitric oxide and abscisic acid application. *Journal of Biotechnology*, 313, 29-38. <https://doi.org/10.1016/j.jbiotec.2020.03.004>.
- Salarian, M., Alizadeh, A., Davari, K. and Ansari, H. 2018. Water and Salinity stress on morphological characteristics, quality and quantity of Sweet Pepper in smart drip irrigation system. *Iranian Journal of Irrigation & Drainage*, 11(6), 1049-1063.
- Sardar, H., Khalid, Z., Ahsan, M., Naz, S., Nawaz, A., Ahmad, R., K. Razzaq, S.M. Wabaidur, C. Jacquard, I. Širić, P. Kumar, S. Abou Fayssal. and Abou Fayssal, S. 2023. Enhancement of salinity

- stress tolerance in lettuce (*Lactuca sativa* L.) via foliar application of nitric oxide. *Plants*, 12(5), 1115. <https://doi.org/10.3390/plants12051115>.
- Sarropoulou, Virginia N., Ioannis N. Therios, and Kortessa N. Dimassi-Theriou. "Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* × *P. canescens*), and MxM 60 (*P. avium* × *P. mahaleb*)." *Journal of Pineal Research* 52, no. 1 (2012): 38-46. <https://doi.org/10.1111/j.1600-079x.2011.00914.x>.
- Sehar, Z., Mir, I.R., Khan, S., Masood, A. and Khan, N.A., 2023. Nitric oxide and proline modulate redox homeostasis and photosynthetic metabolism in wheat plants under high temperature stress acclimation. *Plants*, 12(6),1256. <https://doi.org/10.3390/plants12061256>.
- Siddiqui, M.H., Alamri, S.A., Al-Khaishany, M.Y., Al-Qutami, M.A., Ali, H.M., AL-Rabiah, H. and Kalaji, H.M., 2017. Exogenous application of nitric oxide and spermidine reduces the negative effects of salt stress on tomato. *Horticulture, Environment, and Biotechnology*, 58, 537-547. <https://doi.org/10.1007/s13580-017-0353-4>.
- Soltani Nejad, M., Samandari Najafabadi, N., Aghighi, S., Zargar, M., Stybayev, G., Baitelenova, A. and Kipshakbayeva, G. 2023. Application of Biosynthesized Silver Nanoparticles from Oak Fruit Exudates against *Pectobacterium carotovorum* subsp. *carotovorum* Causing Postharvest Soft Rot Disease in Vegetables. *Agronomy*, 13(6), 1624. <https://doi.org/10.3390/agronomy13061624>
- Song, L., Yue, L., Zhao, H. and Hou, M., 2013. Protection effect of nitric oxide on photosynthesis in rice under heat stress. *Acta physiologiae plantarum*, 35, 3323-3333. <https://doi.org/10.1007/s11738-013-1365-z>.
- Thiex, N., Novotny, L. and Crawford, A. 2012. Determination of ash in animal feed: AOAC official method 942.05 revisited. *Journal of AOAC international*, 95(5), 1392-1397. <https://doi.org/10.5740/jaoacint.12-129>.
- Xu, W., Cai, S.Y., Zhang, Y., Wang, Y., Ahammed, G.J., Xia, X.J., Shi, K., Zhou, Y.H., Yu, J.Q., Reiter, R.J. and Zhou, J., 2016. Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. *Journal of Pineal Research*, 61(4),457-469. <https://doi.org/10.1111/jpi.12359>.
- Yang, W., Sun, Y., Chen, S., Jiang, J., Chen, F., Fang, W. and Liu, Z., 2011. The effect of exogenously applied nitric oxide on photosynthesis and antioxidant activity in heat stressed chrysanthemum. *Biologia plantarum*, 55, 737-740. <https://doi.org/10.1007/s10535-011-0178-4>.
- Zangani, E., Angourani, H. R., Andalibi, B., Rad, S. V. and Mastinu, A. 2023. Sodium nitroprusside improves the growth and behavior of the stomata of *Silybum marianum* L. subjected to different degrees of drought. *Life*, 13(4), 875. <https://doi.org/10.3390/life13040875>.
- Zargar, M., Ramezan, D., Bayat, M and Soltani Nejad, M. 2025. Alleviation of High Temperature Stress in Bell Pepper through Foliar Application of Melatonin and Sodium Nitroprusside. *Greenhouse Plant Production Journal*, 2(1): 18-43.
- Zhang, J., Jiang, H., Song, X., Jin, J. and Zhang, X. 2018. The Responses of Plant Leaf CO<sub>2</sub>/H<sub>2</sub>O Exchange and Water Use Efficiency to Drought: A Meta-Analysis. *Sustainability*, 10(2), 551. <https://doi.org/10.3390/su10020551>.
- Zhang, N., Sun, Q., Zhang, H., Cao, Y., Weeda, S., Ren, S. and Guo, Y.D., 2015. Roles of melatonin in abiotic stress resistance in plants. *Journal of experimental botany*, 66(3), 647-656. <https://doi.org/10.1093/jxb/eru336>.
- Zhou, J., Huang, L., Wang, P., and Zhao, L. 2024. Root morphology and transcriptomic analyses of the response to heat stress in *Paspalum wettsteinii* hack. *Genetic Resources and Crop Evolution*, 71(7), 3219-3239. <https://doi.org/10.1007/s10722-023-01828-w>.
- Zhou, R., Yu, X., Li, X., Dos Santos, T.M., Rosenqvist, E. and Ottosen, C.O., 2020. Combined high light and heat stress induced complex response in tomato with better leaf cooling after heat priming. *Plant physiology and biochemistry*, 151,1-9. <https://doi.org/10.1016/j.plaphy.2020.03.011>.
- Zhou, X., Joshi, S., Khare, T., Patil, S., Shang, J. and Kumar, V. 2021. Nitric oxide, crosstalk with stress regulators and plant abiotic stress tolerance. *Plant Cell Reports*, 40(8), 1395-1414. <https://doi.org/10.1007/s00299-021-02705-5>.