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Posted Date: 12 December 2023

doi: 10.20944/preprints202312.0861.v1

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The Effects of Melatonin and Sodium Nitroprusside on Characteristics of Chlorophyll Fluorescence, Leaf Gas Exchange, Physicochemical Characteristics and Mineral Elements of Pepper (*Capsicum annuum*) under Different Temperatures

Dariush Ramezan [1,*], Meisam Zargar [2,*], Maryam Bayat [2] and Elena Pakina [2]

[1] Department of Horticulture and landscaping, Faculty of Agriculture, University of Zabol, Zabol, Iran
[2] Department of Agrobiotechnology, Institute of Agriculture, RUDN University, 117198 Moscow, Russia
* Correspondence: drhorticul@uoz.ac.ir (D.R.); zargar_m@pfur.ru (M.Z.)

Abstract: In the present study, the effect of melatonin and sodium nitroprusside treatments on the improvement of high temperature tolerance in California Wonder green bell pepper plant was studied. For this purpose, a factorial experiment was conducted in form of a completely randomized design in three replications at Zabol University's Faculty of Agriculture. In this research, green bell pepper plants of Wonder cultivar were exposed to different temperature treatments (25°C, 35°C and 40°C) for 24 hours after being sprayed (foliar application) with 0µM, 50µM and 100µM concentrations of melatonin and Sodium nitroprusside. Results showed that Instantaneous leaf water consumption efficiency in 40°C temperature treatment with 50µM and 100µM melatonin increased. Also in 40°C temperature with 100µM melatonin, fruit dry weight in compared to control (0uM) 13.15% increased. Also, melatonin and sodium nitroprusside treatments in compared to control treatment improved fruit characteristics and reduced Appearance Characteristics of Fruit Marketability (ACFM) number. Under 40°C temperature treatment in compared to control temperature (25°C), fruit total soluble solids (TSS) 18.50% decreased also under 100µM melatonin treatment, fruit TSS 20.69% was increased compared to control treatment (0µM). Also fruit fructose at 40°C temperature in compared to 25°C temperature treatment 5.52% reduced. Fruit calcium at 35°C and 40°C temperature in compared to control temperature 6.96% and 15.65% decreased respectively. At temperature 35°C under foliar application of 100uM sodium nitroprusside, fruit copper in compared to control 28.93% increased.

Keywords: foliar application; vegetative growth; photosynthesis; temperature; minerals

Introduction

Different meteorological models predict that greenhouse gases will gradually increase the temperature of the air around the earth and lead to global warming. According to reports, until the end of this century, the temperature of the earth is warming (Marohasy, 2021). The increase in temperature associated with the world's climate changes is a limiting factor for the cultivation and performance of many plant species. If plants are exposed to temperatures higher than their tolerance threshold for a certain period of time, irreversible damage will be done to their growth and development. High temperature stress causes damage to plant tissues and significantly affects the growth and metabolism of plants. The main signs of heat stress in plants include less greenness and dead texture of leaves and branches, sunburn of plant organs, aging of leaves and their falling, delay in seed germination and loss of germination, imbalance in photosynthesis and respiration, and also, the reduction of stem dry weight, relative growth rate and net absorption and production rate. High temperature stress in plants accelerates the production and reaction of active oxygen species such as superoxide, hydrogen peroxide and hydroxyl radical and as a result causes oxidative stress (Sachdev et al., 2021). Although plants have developed defense strategies to deal with high temperature stress, these are often not enough and as a result, high temperatures cause significant damage to the plant.

 doi:10.20944/preprints202312.0861.v1

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 (Sachdev et al., 2021) 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. Melatonin acts as an absorber for free radicals and can directly destroy reactive oxygen species in the cell space and thus reduce oxidative stress in plants (Ding et al., 2017). Various studies have shown that treating plants with external melatonin improves tolerance to temperature stress. In the conditions of high temperature stress, the plants of chamanwash (*Festuca arundinacea* Schreb) treated with melatonin had lower ion leakage and malondialdehyde levels and more chlorophyll, amount of total soluble proteins and the activity of antioxidant enzymes compared to untreated plants (Alam et al., 2018). Nitric oxide as a molecular message, plays a vital role in various physiological actions of plants, such as inducing germination and reducing seed dormancy, regulating plant metabolism and senescence, inducing cell death, regulating stomatal movement, regulating photosynthesis, mitochondrial function, and regulating flowering. 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 (Ferranteet al., 2021). 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 the role of nitric oxide during heat stress may be reducing the level of reactive oxygen species, because it has been proven that this compound (nitric oxide) plays a role in activating antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase during heat stress. (Parankusam et al., 2017).

Materials and Methods

This research is a factorial experiment with two factors (3x5) where the first factor is three temperature levels (25°C, 35°C and 40°C) and the second factor is 5 types of foliar application (distilled water (control), melatonin 50µM, melatonin 100µM, sodium nitroprusside 50µM and sodium nitroprusside 100µM) was carried out in the form of a completely randomized design in three replications. For this purpose, California Wonder green bell pepper seedlings were obtained from one of the commercial seedling producers in Tehran province. 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, with relative average daily temperature and at night at 25±2°C and 20±2 °C, respectively, 45% relative humidity, and 14 to 10 hours of light and dark were kept. After 2 weeks of planting seedlings and at the 5-leaf stage, the plants were sprayed with sodium nitroprusside (nitric oxide releasing compound) and melatonin in concentrations of 50µM and 100µM, three times with a time interval of 24 hours. At the same time, control samples were sprayed with distilled water. 24 hours after the last spraying, the plants were transferred to the growth chamber with relative humidity of 65%, light period of 16 hours of light and 8 hours of darkness, and light intensity of 270 uM.m².s. In order to apply heat stress treatment, the temperature of the growth chamber (EYELA LTI-1000SD) was gradually increased from 25°C every 24 hours by 5°C to reach temperatures of 35°C and 40°C. The plants in each temperature range remained in the growth chamber for 24 hours and then at end of the temperature treatment, the plants were transferred to the greenhouse with a temperature of 25°C.

Measuring plant characteristics

Chlorophyll fluorescence and gas exchange

A fluorometer was used to measure leaf chlorophyll fluorescence and the components of chlorophyll fluorescence include Fm (maximum chlorophyll fluorescence in dark-adapted conditions), Fo (minimum chlorophyll fluorescence in dark-adapted conditions), Fv (variable chlorophyll fluorescence): Fm- Fo and Fv/Fm (Photosystem II Efficiency) were measured (Xia et al.,

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2023). Leaf gas exchange was performed by using a portable photosynthesis measuring device (model ADC BioScientific Ltd, England, LCA4). Photosynthesis Rate per unit leaf area (Pn) in micromoles of carbon dioxide per square meter per second, Stomatal Conductance (Gs) in millimoles of carbon dioxide per square meter per second, Rate of Transpiration (TR) in millimoles water, Also, Instantaneous Leaf Water Use Efficiency (WUE) in micromoles of carbon dioxide per mole of water was determined using the following equation (Zhang et al., 2018):

Relation1. WUE=PN/TR

Fruit morphological Traits

The number of fruits per plant, fresh weight of each fruit, dry weight of each fruit and fruit yield per plant were measured.

Fruit Firmness, Fruit Total soluble solids, Fruit acidity, Appearance Characteristics of Fruit Marketability and chemical characteristics of fruit

Firmness, Total soluble solids (TSS), acidity (TA)

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 degree of penetrometer was read in $kg.cm^2$. 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. Organic acids in fruit were determined by Titration method (AOAC, 2023).

Appearance Characteristics of Fruit Marketability

The marketability of fruits was determined by observation and in the range of 1 to 5. The basis of increase in marketability was amount of color change, lack of fruit appearance and the amount of shriveling of fruit tissue. So that a score of 1 was assigned to lowest and a score of 5 was assigned to the most non-marketable fruit.

Table 1. Marketability characteristics of bell pepper investigated for classification, grade 1, 2, 3, 4 and 5.

Grade	Quality	Color Uniformity	Health Status	Shape	Weight
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.

Fruit Carbohydrates

Total sugar measurement 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 100ml of dilute sulfuric acid, and to prepare 100 ml of dilute sulfuric acid, 76 ml of concentrated sulfuric acid was mixed with 38ml of distilled water. To measure soluble sugars, 100ul of the extract was poured into a test tube and 3ml of anthrone solution was added to it. The obtained

 doi:10.20944/preprints202312.0861.v1

mixture was placed in a boiling water bath for 20 minutes at a temperature of 100°. Fruit soluble sugars were measured using spectrophotometric method and at 620 nm wavelength, and by drawing a standard curve with specific glucose concentrations, the sugar content was expressed in mg. g fruit D.W (McCready et al., 1950).

Fruit Glucose, fructose and sucrose

In order to measure and separate sugar, an HPLC (made by the American company Withers was used with a column (250x46mm, DP=3um) Carbohydrate C_{18}) and amounts of sugar was expressed as milligrams per gram of fruit fresh weight.

Fruit Flavonoids and phenols

Flavonoid content of fruit extract was measured by the method of Kaijv et al. (2006). 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 $NaNo_2$, $AlCl_3$ and NaOH. The absorbance of the solution was read after 5 minutes with a spectrophotometer at a wavelength of 507nm and recorded as milligrams per 100 grams 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 Ug.g F.W (McDonald et al., 2001).

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, 1mg of fruit tissue was mixed with 3 ml of metaphosphoric acid (1%) and after 30 minutes was centrifuged at 4°C and 6000 rpm for 15 min. 50uL from supernatant solution brought to volume of 200 microliters and absorbance of samples was read at a wavelength of 520nm. Ascorbic acid concentration of fruit was recorded using a calibration curve as milligrams per 100g of fruit fresh weight (Chang et al., 2002). In order to determine antioxidant capacity of fruit extract was done through DPPH free radical neutralization using a PG Instruments ItdT80+UV/VIS spectrophotometer (Miliauskas et al., 2004).

Fruit Mineral Elements

Fruit nitrogen was measured by the combustion method with an elemental analyzer (CHNS-O Elemental Analyzer model ECS4010, Italy) (Carl et al., 1997). Spectrophotometer method was used to measure phosphorus (Rayan et al., 2001). One gram of dry matter was placed in an electric furnace at a temperature of 500°C for four hours. Then, 10cc of hydrochloric acid (2M) was added to samples and volume of 1000 cc with distilled water and read at 420 nm wavelength. Atomic absorption method was used to measure calcium, potassium, magnesium, iron, copper, zinc and manganese. 0.5 grams of dry samples (fruits) were dissolved in 10ml of concentrated nitric acid and suspension was placed at 70°C for 24 hours until samples were well dissolved in the acid and solutions were made up to volume with deionized water and their absorbance were read with an atomic absorption model FSAA 240 (White, 1976).

Statistical Analysis

Variance analysis of data related was done with SAS 9.4 statistical software. Means comparison was done with LSD test at 5% probability level.

Results

Leaf Chlorophyll Fluorescence

According to data in Table 5, Highest (0.46) and lowest (0.17) minimum chlorophyll fluorescence (FO) were respectively to 40°C temperature treatment with 0mM foliar application and 25°C temperature treatment with 50uM melatonin foliar application. Also, highest (1.26) and lowest (0.89)

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maximum chlorophyll fluorescence (FM) were respectively related to 25°C temperature treatment with 0 foliar application and 40°C temperature treatment with 0 foliar application, respectively and highest (1.04) and lowest (0.43) variable chlorophyll fluorescence (FV) were related to 25°C temperature treatment with 0 foliar application and 40°C temperature treatment with 0 foliar application, respectively. According to data in Table 5, there was no statistically significant difference ($p<0.05$) between 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 and they are in the same statistical class as well as lowest (0.48) efficiency value of photosystem II were related to 40°C temperature treatment with foliar application 0µM (control).

Leaf Gas Exchange

According to data in Table 2, highest (11.86 uM co_2 m^{-2} s) and lowest (10.29 uM co_2 m^{-2} s) value of Rate of Leaf Photosynthesis are related to temperature of 25 and 40°C, respectively, also highest (287.20 uM) and lowest (222.36 uM) Carbon dioxide value under leaf stomata are related to treatment of 25°C and 40°C, respectively. According to data in Table 3, highest (11.43 uMco2 m-2 s) and lowest (10.23 uMco^2 m^{-2} s) value of Rate of Leaf Photosynthesis were related to foliar application with 100 mM melatonin and 0 (control), respectively. Also, there was no statistically significant difference (($p < 0.05$) in terms of Rate of Leaf Photosynthesis value between foliar application treatments of 50µMand 100µM (Melatonin and nitroperoxide). Mesophilic Conductance. Highest (10.43 uM CO_2 per mol H_2O) and lowest (5.21 uM CO_2 per mol H_20) value of Instant Leaf Water Consumption Efficiency was related to 40°C temperature with 50µM melatonin foliar application and 25°C temperature with foliar application 100µM sodium nitroperoxide respectively.

Also, 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 (Table 4). The data in Table 5 shows that highest (0.05 mM co_2 m^{-2} s) of leaf stomatal conductance was related to 25°C temperature treatment with 0 foliar application, 25°C temperature treatment with 100µM melatonin foliar application and 35°C temperature treatment with foliar application 100µM melatonin and also lowest (0.01 mM co2 m^{-2} s) of leaf stomatal conductance were related to of 40°C temperature treatment with 0 foliar application.

Table 2. Comparison of treatment means effect of temperature on rate of leaf photosynthesis, carbon dioxide under leaf stomata, number fruit per plant, appearance characteristics of fruit marketability, fruit TSS, Fruit TA, fruit vitamin C, fruit fructose, fruit antioxidant capacity, fruit calcium and fruit zinc of California Wonder green bell pepper .

T (°C)	RLP(uMco_2 m^{-2} s)	CDS (uM)	NFP	ACFM	TSS (%)	TA	Fructose (Mg.g F.W)	Vitamin C (Mg. 100g F.W)	FAC (%)	Nitrogen (%D.M)	Calcium (%D.M)	Zinc (Mg.kg D.M)
25	11.86a	287.20a	4.71a	2.20c	6a	0.53b	9.06a	109.86a	79.39a	3.79a	1.15a	19.87a
35	11.08b	258.05b	4.69a	3b	6.23a	0.35c	8.87ab	87.88b	67.49b	3.68a	1.07b	18.04b
40	10.29c	222.36c	3.52b	3.66a	4.89b	0.74a	8.56b	71.83c	57.23c	2.55b	0.97c	15.04c
LSD	0.58	23.43	0.50	0.44	0.28	0.03	0.37	5.91	4.18	0.26	0.06	1.72

Temperature:T, Rate of Leaf Photosynthesis:RLP, Carbon dioxide under leaf stomata:CDS, Number Fruit per Plant:NFP, Appearance Characteristics of Fruit Marketability:ACFM, Fruit Antioxidant Capacity:FAC, Differences letters indicate significantly different value at $p< 0.05$. 25°C Temperature: Control.

Table 3. Comparison of treatment means effect of foliar applicationing with melatonin and sodium nitroperoxide on rate of leaf photosynthesis, Appearance Characteristics of Fruit Marketability, Fruit Fructose, Fruit Vitamin C, Fruit Antioxidant Capacity, Fruit TSS, Fruit TA, Fruit Nitrogen and Fruit Zinc of California Wonder green bell pepper.

Foliar Application (mM)	RLP (uMco_2 m^{-2} s)	ACFM	TSS (%)	TA	Fructose (Mg.g F.W)	Vitamin C (Mg. 100g) F.W)	FAC (%)	Nitrogen (%D.M)	Zinc (Mg.kg D.M)

 doi:10.20944/preprints202312.0861.v1

0	10.23b	3.66a	5.22c	0.46c	8.72ab	79.36c	60.51b	2.68c	15.52c
50 M	11.14a	3.00b	5.83b	0.45c	8.64b	86.34bc	69.52a	3.06b	17.05bc
100 M	11.43a	2.66b	6.30a	0.45c	9.17a	94.89a	71.84a	4.46a	19.44a
50 SNP	11.23a	2.66b	5.58bc	0.60b	9.15a	92.37ab	68.34a	3.08b	17.13bc
100 SNP	11.36a	2.77b	5.60b	0.73a	8.47b	96.34a	69.97a	3.40b	19.10ab
LSD	0.75	0.57	0.37	0.04	0.48	7.63	5.40	0.33	2.22

Melatonin:M, Sodium nitroprusside:SNP, Rate of Leaf Photosynthesis:RLP, Appearance Characteristics of Fruit Marketability:ACFM, Fruit Antioxidant Capacity:FAC, Differences letters indicate significantly different value at $p < 0.05$. foliar application 0: Control.

Table 4. Comparison of treatment means interaction effect of foliar application with melatonin and sodium nitroperoxide and temperature on Leaf Transpiration Rate, Instant Leaf Water Consumption Efficiency, Fruit Firmness, Fruit Carbohydrates, Fruit Glucose, Fruit Sucrose, Fruit Flavonoids, Fruit Iron and Fruit Copper of California Wonder green bell pepper.

Temperature (°C)	Foliar Application (mM)	LTR (mM H_20)	ILWCE (uM CO_2 per mol H_20)	Firmness ($kg.m^2$)	Carbohydrates (Mg.g D.M)	Glucose (Mg.g F.W)	Sucrose (Mg.g F.W)	Flavonoids (Mg .100g F.W)	Iron (Mg.kg D.M)	Copper (Mg.kg D.M)
25	0	2.01bc	5.86g	2.33c	16.41fg	6.07f	10.41fg	11.96c	70.61b	13.01ab
	50 M	2.12ab	5.56gh	2.63b	15.95g	6.67ef	9.71gh	10.85d	70.46b	14.04a
	100 M	1.95bc	6.14fg	2.84a	20.70d	7.24e	8.98h	14.31a	77.27a	13.10ab
	50 NSP	2.10ab	5.62gh	2.16de	17.76ef	7.00ef	10.07gh	10.82de	74.18ab	13.72ab
	100 NSP	2.33a	5.21h	2.27cd	19.94d	6.79ef	10.43efg	13.38b	70.57b	12.43bc
35	0	1.44ef	7.04de	1.49h	23.84c	11.05a	13.64b	9.46f	53.47e	8.71fg
	50 M	1.32f	8.52b	1.66g	20.68d	9.85b	12.01cd	10.63de	56.41de	9.50ef
	100 M	1.35f	8.67b	1.73g	26.31b	7.36e	15.44a	11.88c	58.79cd	10.12de
	50 NSP	1.48ef	7.44cd	2.02ef	23.90c	6.75ef	11.68de	10.80de	57.02de	9.66ef
	100 NSP	1.43ef	7.84c	2.22cd	29.35a	7.61de	13.13bc	11.30cd	62.57c	11.23cd
40	0	0.99g	8.84b	1.03i	10.83i	7.49e	9.06h	7.11g	40.27h	7.26h
	50 M	0.98g	10.43a	1.39h	13.75h	8.51cd	10.38fg	9.30f	41.52gh	6.97h
	100 M	1.02g	10.29a	1.78g	15.43g	7.17e	12.01cd	10.57de	46.32fg	9.60ef
	50 NSP	1.65ed	6.57ef	1.72g	13.67h	9.22bc	10.88defg	9.49f	47.11f	7.15h
	100 NSP	1.79cd	6.15fg	1.96f	18.09e	7.66de	11.40def	10.00ef	52.51e	7.78gh
LSD		0.22	0.61	0.14	1.54	1.00	1.25	0.83	4.89	1.37

Melatonin: M, Sodium nitroprusside: SNP, Leaf Transpiration Rate: LTR, Instant Leaf Water Consumption Efficiency:ILWCE, Differences letters indicate significantly different value at $p < 0.05$. 25°C Temperature and 0 foliar application: Control.

Table 5. Comparisons of treatment means interaction effect of foliar applicationing with melatonin and sodium nitroperoxide and temperature on Chlorophyll fluorescence (FO, FM, FV and FV/FM), Leaf stomatal conductance, Single Fruit Weight, Dry Weight Single Fruit, Dry weight single Fruit and Fruit Manganese of California Wonder green bell pepper.

Temperature (°C)	Foliar Application (mM)	FO	FM	FV	FV/FM	LSC (mM co_2 m^2 s)	SFW (g F.W)	DWSF (g D.W)	FYP (g F.W)	Manganese (Mg.kg D.M)
25	0	0.22e	1.26a	1.04a	0.82a	0.05a	74.91a	8.03bc	320.77d	9.45c
	50M	0.17f	1.23a	1.04a	0.85a	0.04ab	73.95a	7.47de	347.83c	11.00a
	100M	0.18f	1.21ab	1.03a	0.84a	0.05a	70.44b	7.80cd	362.31b	10.32b
	50NSP	0.22de	1.18abc	0.96b	0.82ab	0.04abcd	70.40b	7.17ef	366.43ab	11.01a
	100NSP	0.23de	1.18abc	0.95b	0.80abc	0.04abc	70.15bc	8.10bc	376.53a	10.31b
35	0	0.35b	1.04d	0.69ef	0.67e	0.03cd	67.18bcd	8.64a	297.94gh	8.43e
	50M	0.24de	1.06d	0.79d	0.76bcd	0.03bcd	66.44d	8.70a	311.26def	8.96d
	100M	0.28c	1.08cd	0.79d	0.74cd	0.05a	65.96d	8.31ab	313.53def	9.51c
	50NSP	0.29c	1.17abc	0.86c	0.74d	0.04abcd	66.81cd	8.78a	306.70efg	8.80de
	100NSP	0.28c	1.03de	0.74de	0.72de	0.04ab	67.04bcd	8.74a	308.82defg	9.45c

 doi:10.20944/preprints202312.0861.v1

40	0	0.46a	0.89f	0.43g	0.48f	0.01e	54.63e	5.93h	288.41h	7.00g
	50M	0.30c	1.11bcd	0.80cd	0.72de	0.03d	55.42e	5.80h	304.00fg	8.48e
	100M	0.33b	1.04de	0.70ef	0.67e	0.04abc	65.81d	6.71fg	316.95de	7.83f
	50NSP	0.24de	0.94ef	0.68f	0.73de	0.03d	57.53e	6.23gh	307.50efg	8.56de
	100NSP	0.25d	1.05d	0.78d	0.74cd	0.03bcd	56.31e	6.01h	302.92fg	7.85f
	LSD	0.02	0.10	0.06	0.06	0.01	3.42	0.49	12.15	0.46

Melatonin: M, Sodium nitroprusside: SNP, Leaf stomatal conductance: LSC, Single Fruit Weight: SFW, Dry Weight Single Fruit:DWS, Fruit Yield per Plant:FYP, Differences letters indicate significantly different value at $p < 0.05$. 25°C Temperature and 0 foliar application: Control.

Morphological characteristics fruit

According to data in Table 2, highest (4.71) and lowest (3.52) Number Fruit Per Plant are related to 25°C and 40°C temperature treatments, respectively. Also, there was no statistically significant difference ($p < 0.05$) between temperature treatments 25°C and 35°C. According to data in Table 2, highest (3.66) and lowest (2.20) value of Appearance Characteristics of Fruit Marketability were related to temperature treatment of 40°C and 25°C, respectively. According to data in Table 3, highest (3.66) value of Appearance Characteristics of Fruit Marketability were related to 0µM foliar application treatment, and lowest (2.66) value were related to 50 and 100µM melatonin and sodium nitroprusside treatments. According to data in Table 5, highest (74.91g) and lowest (54.63g) value of Single Fruit Weight were related to of 25°C temperature treatment with 0µM foliar application and 40°C temperature treatment with 0µM foliar application, respectively. Also, 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. According to data in Table 5, highest (8.78g) and lowest (5.80g) value of dry weight of single fruit were treated to 35°C temperature treatment with 50µM sodium nitroperoxide and 40°C temperature treatment with melatonin 50µM respectively. Also, there is no a statistically significant 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. According to data in Table 5, highest (376.53 g) value of Fruit Yield per Plant were related to treatment 25°C with 100µM sodium nitroperoxide foliar application and statistically, it was at the probability level of 5% in a class with 25°C temperature treatment with 50µM sodium nitroperoxide foliar application and lowest (288.41g) value of Fruit Yield Per Plant were related to 40°C temperature treatment with 0µM foliar application.

Physicochemical characteristics of fruit

According to data in Table 4, highest (2.84 kg.m2) and lowest (1.03 kg.m2) Fruit Firmness are related to 25°C temperature treatment with 100uM melatonin and 40°C temperature treatment with 0uM foliar application, respectively. according to Table 2, highest (6%) and lowest (4.89%) value of Fruit TSS are related to temperatures of 25°C and 40°C, respectively, and there is no statistically significant difference ($p< 0.05$) between temperatures of 25°C and 35°C in terms of Fruit TSS value. According to Table 3, highest (6.30%) and lowest (5.22%) value of Fruit TSS are related to 100uM and 0uM melatonin treatment, respectively. According to Table 2, highest (0.74) and lowest (0.35) value of Fruit TA are related to temperature treatment of 40°C and 35°C, respectively. According to Table 3, highest (0.73) value of Fruit TA are related to 100 uM sodium nitroperoxide foliar applicationing treatment. Also, there was no statistically significant difference ($p<0.05$) between control (0uM), melatonin 50uM and 100 uM melatonin treatments in terms of Fruit TA value and all three are in the same statistical class. According to data in Table 2, highest (9.06 Mg.g F.W) fruit fructose are related to temperature treatment of 25°C. Also, statistically, there is no significant difference ($p<0.05$) between temperatures of 25°C and 35°C in terms of fruit fructose. Lowest fruit fructose value is related to temperature of 40°C, which was not different ($p< 0.05$) from fruit fructose value in 35°C treatment. According to data in Table 3, highest (9.17 Mg.g F.W) fruit fructose value are related to

 doi:10.20944/preprints202312.0861.v1

100uM melatonin foliar treatment. Also, in terms of fruit fructose value, there is no significant difference ($p<0.05$) between 0uM, 100uM melatonin and 0uM, 100uM sodium nitroperoxide foliar application treatments. According to data in Table 2, highest (109.86 Mg.100g F.W.) and lowest (71.83 Mg.100g F.W.) value of fruit vitamin C are related to temperature treatment of 25°C and 40°C respectively. According to data in Table 3, highest (96.34Mg.100g F.W.) amount of fruit vitamin C are related to foliar application treatment of 100uM sodium nitroperoxide. Also, there is no significant difference ($p<0.05$) between foliar application 100uM treatments of melatonin, 50uM sodium nitroperoxide and 100uM sodium nitroperoxide. The data from Table 2 shows that highest (79.39%) and lowest (57.23%) fruit antioxidant capacity related to temperature treatment of 25 and 40°C, respectively.

The data obtained from Table 3 shows 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 lowest fruit antioxidant capacity was related to foliar application treatment 0uM (control). According to Table 4, highest (29.35 Mg.g D.M) and lowest (10.83 Mg.g D.M) fruit carbohydrate were related to temperature treatment of 35°C with foliar application of 100uM sodium nitroperoxide and temperature treatment of 40°C with foliar application of 0uM respectively. According to Table 4, highest (11.05 Mg.g F.W.) and lowest (6.07 Mg.g F.W.) fruit glucose were related to temperature treatment of 35°C with foliar application 0uM and temperature treatment of 25°C with foliar application 0uM respectively. According to Table 4, highest (15.44 Mg.g F.W) fruit sucrose are related to temperature treatment of 35°C with foliar application of melatonin 100uM. Also, there is no statistically significant difference ($p<0.05$) between temperature treatment 25°C with 50uM melatonin, temperature treatment 25°C with 100uM melatonin, temperature treatment 25°C with 50uM sodium nitroperoxide. According to Table 4, highest (14.31 Mg.100g F.W.) and lowest (7.11 Mg.100g F.W.) fruit flavonoid was related to temperature treatment 25°C with 100uM melatonin and temperature treatment 40°C with 0uM respectively.

Mineral Elements Fruit

According to Table 2, there was no statistically significant difference ($p<0.05$) between temperatures of 25°C and 35°C in terms of fruit nitrogen, also lowest (2.55% D.M) of fruit nitrogen was related to temperature of 40°C. According to Table 3, highest (4.46%D.M) and the lowest (2.68%D.M) were related to 100 uM melatonin and 0uM(control), respectively. According to Table 2, in terms of fruit calcium, highest (1.15% D.M) and lowest (0.97% D.M) were related to temperature treatment of 25°C and 40°C, respectively. According to Table 2, highest (19.87 Mg.kg D.M) and lowest (15.04 Mg.kg D.M) fruit zinc are related to temperature treatment of 25°C and 40°C, respectively. Also, according to Table 3, highest (19.44 Mg. kg D.M) and lowest (15.52 Mg.kg D.M) fruit zinc are related to 100uM melatonin and control (0uM), respectively. According to Table 4, highest (14.04 Mg.kg D.M) and lowest (6.97 Mg.kg D.M) fruit copper are related to temperature treatment of 25°C with 50 uM melatonin and temperature treatment of 40°C with 50 uM melatonin, respectively. According to data from Table 5, there is no statistically significant difference ($p<0.05$) in fruit manganese between temperature treatments 25°C with 50uM melatonin and treatment 25°C with 50uM sodium nitroperoxide. There was no molar, also lowest (7Mg.kg D.M) of fruit manganese was related to temperature treatment of 40°C with 0uM foliar spraying.

Discussion

Effects of melatonin and sodium nitroprusside under different temperatures on characteristics of chlorophyll fluorescence and leaf gas exchange.

High temperature stress (35°C and 40°C) significantly reduced the maximum fluorescence, variable fluorescence and efficiency of photosystem II, but increased the minimum fluorescence. highest efficiency of photosystem II was related to temperature treatment of 25°C. In many plant species, when photochemical efficiency of photosystem II reaches 0.83 or more, it means that there is no stress on plant, and therefore values lower than this value indicates presence of stress in plants

 doi:10.20944/preprints202312.0861.v1

(Mihaljevic et al., 2020). Findings of Yang et al. (2011) in chrysanthemum (*Chrysanthemum morifolium* L.) and Xu et al. (2016) in tomato showed that the use of nitric oxide and melatonin increased leaf chlorophyll fluorescence under high temperature stress conditions. Nitric oxide and melatonin by increasing activity of antioxidant enzymes and preventing accumulation of free radicals such as hydrogen peroxide, preserving integrity of the cell membrane and as a result reducing the damage to photosystem II have increased the performance of this photosystem. Also, maintaining function of photosystem II may be related to effect of melatonin and nitric oxide in inhibiting the decomposition of photosynthetic pigments and increasing the content of leaf chlorophyll (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 improve the photochemical efficiency of this photosystem (Gautamet al., 2022). melatonin and nitric oxide with adjusting accumulation of free radicals such as hydrogen peroxide, inhibit peroxidation of membrane lipids and increase efficiency of photosystem II, reducing oxidative damage caused by heat stress and as a result the symptoms of temperature stress such as tuberization, chlorosis and necrosis of leaves (Jahan et al., 2019: Siddiqui et al., 2011). High temperature stress reduces concentration of substomatal carbon dioxide (CDS) and reduces transpiration (LTR). With stomata closed and stomatal conductance (LSC) decreases, rate of photosynthesis (RLP) decreases. Also, according to equation 1, increasing numerator of the fraction (photosynthesis rate) and decreasing denominator of fraction (transpiration), instantaneous leaf water consumption efficiency (ILWCE) decreased. with increase of temperature stress, CDS values decreased so that a statistically significant difference was observed between all three temperature treatments at the probability level of 5%. With temperature stress due to closure of plant stomata, leaf stomatal conductance (LSC) decreased, which with reducing stomatal conductance, plant transpiration reduced. Due to reduction of carbon dioxide concentration under temperature stress conditions, rate of photosynthesis (RLP) decreased. Also, with use of melatonin and sodium nitroprusside, rate of leaf photosynthesis (RLP) changed compared to control, so that according to Table 3, there is a significant difference ($p<0.05$) between foliar application treatments and control treatment (0uM). With foliar application treatment of melatonin and sodium nitroprusside under temperature stress conditions of 40°C, the instantaneous leaf water consumption efficiency (ILWCE) increased so that in this temperature treatment (temperature of 40°C), melatonin 50µM and 100µM more than treatments others increased instantaneous water consumption efficiency (ILWCE). Yang et al. (Yang et al., 2018) reported that application of melatonin as a spray (foliar application) or with irrigation (fertigation) reduces damage to photosynthetic system of tomato.

Effects of melatonin and nitroprusside under different temperatures on fruit morphological characteristics

With increasing temperature, number of fruits per plant (NFP) decreased, and maximum decrease was at 40°C. Also, with increasing temperature, fruit fresh weight per plant (SFW) decreased. Under temperature condition of 40°C, 100µM melatonin foliar application treatment improved fruit fresh weight (SFW) compared to other foliar application treatment, so that 20.46% increased fruit fresh weight (SFW) compared to control treatment (0µM). Fruit dry weight (DWSF) decreased with increasing temperature. Under temperature condition of 35°C, melatonin and sodium nitroprusside foliar application treatment had no significant ($p<0.05$) effect on fruit dry weight (DWSF). With increasing stress temperature to 40°C, 100µM melatonin treatment increased dry weight of fruit (13.15%) compared to control foliar application treatment (0uM). Results of fruit yield in plant (FYP) showed that with foliar application of melatonin and sodium nitroprusside under temperature conditions of 35°C and 40°C, fruit yield in plant (FYP) in compared to control treatment (0uM) increased. High temperature reduces synthesis of growth stimulators such as cytokinins and increases growth inhibitors such as abscisic acid, which reduces plant growth. Melatonin and sodium nitroprusside, due to their essential role in reducing amount of lipid peroxidation and damage to pigments against oxidative stress, as well as strengthening biological activities such as growth and photosynthesis, absorbing and transferring ions and changing activity of some enzymes, improve accumulation dry matter of plant and consequently yield is improved (Jahan et al., 2019). With increase in temperature, Appearance Characteristics of Fruit Marketability (ACFM) values increased

 doi:10.20944/preprints202312.0861.v1

so that due to destructive effects of temperature on fruit, the highest ACFM values were less than 40°C. Also, melatonin and sodium nitroprusside treatment compared to control treatment improved fruit characteristics and reduced ACFM number. High temperature stress causes morphological damage such as twisting, chlorosis and necrosis of leaves and use of melatonin and nitric oxide leads to reduction of these damages, consequently it will improve photosynthetic characteristics of plant and increase yield. On the other hand, melatonin may improve overall growth of plant and maintain number of leaves in stressful conditions, and in this way, dry weight of plant is also better maintained under stressful conditions (Wang et al., 2016).

Effects of melatonin and nitroprusside under different temperatures on fruit physicochemical characteristics

Under 40°C temperature treatment in compared to control temperature (25°C), fruit total soluble solids (TSS) 18.50% decreased also under treatment of 100µM melatonin, fruit total soluble solids (TSS) 20.69% in compared to control treatment (0µM) increased. An increase in temperature causes disturbances in plant's photosynthetic system, as well as disrupting plant sink-source relationships, as well as changing plant hormonal balance. These changes will reduce fruit total soluble solids (TSS). Low concentrations of melatonin can increase carbon dioxide conversion efficiency and dry weight accumulation (Liu et al., 2015). Melatonin and sodium nitroprusside treatment increase the carbohydrate storage by improving the photosynthetic capacity of the plant and thus increase the fruit total soluble solids (TSS) under temperature stress conditions (35°C and 40°C). Fruit TA at 40°C temperature in compared to the control temperature treatment (25°C) 39.62% increased which can be attributed to the reduction of fruit TSS. Also, with increase in temperature (35°C and 40°C), rate of photosynthesis decreases and respiration pathway becomes more active, which results in production of more organic acids. Sodium nitroprusside 100uM caused 58.70% increase in fruit TA, which can be pointed to the production of nitrogenous acidic compounds by sodium nitroprusside in plant. In addition to the general effect of temperature stress on the photosynthetic system of the plant and the reduction of basic carbohydrate production in plants due to increase of other cycles that work based on respiration and metabolism of primary compounds, cause more production of organic acids in plant, as well as the presence of heat stress in plants, cause the activation of enzymes that leads the main path and the production of basic compounds to the consumption of sugars and the production of organic acids. Fruit carbohydrate decreased with increasing temperature. Under 35°C temperature, 100µM sodium nitroprusside treatment fruit carbohydrate in compared to the control treatment (0µM) 23.11% increased. Also, at 40°C temperature, 100µM sodium nitroprusside treatment 67.04% fruit carbohydrate (0µM) increased in compared to control treatment. Percentage of fruit fructose reduction at 40°C temperature compared to 25°C temperature treatment was 5.52. Under 35°C temperature treatment, foliar application with 100µM melatonin 13.20% increased fruit sucrose in compared to control (0µM). High temperature stress produces negative radicals, which increases the activity level of enzymes to deal with these free radicals, and the use of sodium nitroprusside causes the production of nitric oxide, which combines with these free radicals and destroys them. Melatonin acts as an absorber for free radicals produced in stressful conditions, which can directly destroy reactive oxygen species in the intercellular space and thus reduce oxidative stress in plants, Melatonin also increases chlorophyll and the activity of antioxidant enzymes that production of primary photosynthetic compounds in plants under high temperature stress conditions decrease less and adjusts the stressful conditions for plant, so by maintaining the plant's photosynthetic capacity, the amount of sugars is less affected by high temperature stress. At a temperature condition of 40°C , foliar application of 100µM melatonin 48.66% increased fruit flavonoids in compared to 0µM foliar application. Vitamin C of fruit decreased by 34.62% at 40°C compared to the control temperature (25°C). As the temperature increased, the antioxidant capacity of fruit decreased so that it decreased by 27.91% at 40°C in compared to 25°C. Also, all four foliar application treatments (melatonin 50µM and 100µM and sodium nitroprusside 50µM and 100µM) increased the fruit antioxidant capacity in compared to 0µM foliar application treatments (control). It has been reported that melatonin stimulates the biosynthesis of phytohormones, antioxidant compounds, facilitates the absorption of nutrients and increases product quality (Liu et al., 2015).

 doi:10.20944/preprints202312.0861.v1

Melatonin acts as a strong antioxidant under stress conditions, and by increasing the content of chlorophyll, carotenoids and photosynthesis, finally, in addition to increasing products quantity, improves quality under stress conditions (Zhang et al., 2015).

Effects of melatonin and nitroprusside under different temperatures on content of fruit mineral elements

Fruit nitrogen with temperature increasing from 25°C to 40°C 32.72% decrease, Also, there was 66.42% increase in fruit nitrogen with 100µM melatonin in compared to control treatment (0µM).

It seems that by increasing temperature and causing stress to plant, certain genes are activated, which causes close the stomata, and with the stomatal system closing and transpiration decreasing, water and nutrients absorb decrease, also according to attention to high temperature stress conditions, nutrients flow between source and sink (leaves and fruits) is reduced. High temperature reduces synthesis of growth stimulators such as cytokinins and increases growth inhibitors such as abscisic acid, which causes decrease in plant growth, and decrease in plant growth and plant's ability to water and nutrients absorb decreased. Melatonin and sodium nitroprusside, due to their essential role in strengthening biological activities such as growth and development, photosynthesis, absorption, ions transfer and changing activity of some enzymes, improve absorption and transfer of water and nutrients in plant. In fact, with 100uM melatonin foliar application inducing expression of specific genes plant that enzymes are produced that strengthen photosynthetic system under stress conditions and cause increase carbohydrate, as well as an increase photosynthetic capacity improves flow of nutrients (mineral elements) from root to aerial organs. Fruit calcium at 35°C and 40°C in compared to control temperature 6.96% and 15.65% decreased respectively. Fruit zinc at 35°C and 40°C in compared to control temperature 9.21% and 24.31% decreased respectively. Also, foliar application 100µM melatonin and 100µM sodium nitroprusside increase fruit zinc. At temperature 35°C under foliar application of 100uM sodium nitroprusside, fruit copper in compared to control 28.93% increased. Also, at temperature of 25°C under foliar application 100µM melatonin, fruit copper 32.23% increase in compared to control (0uM). At temperature 40°C under foliar application of 100uM sodium nitroprusside, iron copper in compared to control 30.39% increased. Fruit manganese at 35°C temperature, under 100µM melatonin and 100uM sodium nitroprusside, 12.81% and 12.10%) increased respectively in compared to control (0µM). Fruit manganese content at temperature of 40°C, under 50µM melatonin treatment and 50µM sodium nitroprusside treatment, in compared to 0µM treatment 21.14 and 22.29% increased respectively. Melatonin allows leaves to have a greater capacity for carbon dioxide absorption and stomatal conduction (Arnao and Hernandez-Ruiz, 2014) which increases photosynthetic capacity and more carbohydrate reserves, also melatonin increases net rate of photosynthesis, stomatal conductance and simultaneously increases relative water content, which increases plant metabolism and plants tolerate stress conditions (Ahmad et al., 2019) .Better growing conditions and carbon production facilitate nutrients 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 stimulates root growth. But relationship between IAA and melatonin is still not fully understood (Arnao and Hernandez-Ruiz, 2014). Root growth stimulating causes more water and mineral elements to be absorbed and transferred to plant aerial parts.

Conclusions

In the present study, high temperature stress caused morphological damage such as twisting, chlorosis and necrosis of leaves and the application of melatonin and sodium nitroprusside led to the reduction of these damages. Under 40°C temperature, 50µM and 100µM melatonin, increased instantaneous leaf water consumption efficiency. Also at a temperature range of 40°C , 100µM melatonin treatment increased fruit fresh weight in compared to control treatment (0µM) 20.46%. With temperature increasing to 40°C, 100µM melatonin treatment increased dry weight of fruit (13.15%) in compared to control. under 100µM melatonin treatment, fruit tolal soluble solids (TSS) 20.69% in compared to control (0µM) increased. Under 35°C temperature, 100µM sodium nitroprusside treatment in compared to control treatment (0µM) 23.11% increased the fruit

carbohydrate. Fruit nitrogen 66.42% with 100µM melatonin in compared to control(0µM) increased. Fruit calcium at 35°C and 40°C in compared to control temperature 6.96% and 15.65% decreased respectively. Fruit manganese at 35°C temperature, under 100µM melatonin and 100uM sodium nitroprusside in compared to control (0µM).12.81% and 12.10% increased respectively.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by RUDN University Strategic Academic Leadership Program.

Conflicts of Interest: The authors declare no conflict of interest.

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