Article

Not peer-reviewed version

Melatonin and Sodium Nitroprusside Impact on Vegetative Growth, Physiological, Biochemical Traits and Mineral Elements Contents of California Wonder Green Bell Pepper (*Capsicum annuum*) Under Different Temperatures

Dariush Ramezan *, Meisam Zargar *, Gani Stybayev, Zhenis Zharlygassov, Gulden Kipshakbayeva, Aliya Baitelenova

Posted Date: 25 December 2023

doi: 10.20944/preprints202312.1849.v1

Keywords: Foliage; vegetative growth; photosynthesis; temperature

Article

Melatonin and Sodium Nitroprusside Impact on Vegetative Growth, Physiological, Biochemical Traits and Mineral Elements Contents of California Wonder Green Bell Pepper (Capsicum annuum) Under Different Temperatures

Dariush Ramezan [1,*], Meisam Zargar [2,*], Gani Stybayev [3], Zhenis Zharlygassov [3], Gulden Kipshakbayeva [3] and Aliya Baitelenova [3]

[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
[3] Department of Plant Production, Faculty of Agronomy, S. Seifullin Kazakh Agrotechnical Research University, Astana 010000, Kazakhstan
* 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 a 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 Shoot dry weight without fruit (DWPWF) at 40°C in compared to 25°C, 21.54% decreased. Also, foliar application with 100µM melatonin in compared to control (0uM) caused a 13.26% an increased in dry weight of shoots without fruit (DWPWF). Also, leaf chlorophyll at 40°C temperature in compared to 25°C temperature 32.82% decreased and foliar application with 100µM melatonin and 100µM sodium nitroprusside improved leaf chlorophyll value in compared to other foliar application treatments. Foliar application with 50uM and 100uM sodium nitroperoxide at 40°C temperature caused decreased of 15.16% and 20.99% of leaf hydrogen peroxide in compared to 0uM foliar application treatment, respectively. Also, under 40°C temperature, foliar application with melatonin and sodium nitroprusside (50µM and 100µM) in compared to 0µM foliar application activity of leaf superoxide dismutase enzyme increased. Also, leaf nitrogen under with 50 µM and 100µM melatonin in compared to 0µM foliar application 9.61% and 23.72% increased. Leaf potassium in 40°C temperature, foliar application with 50uM melatonin, 100uM melatonin, 50uM sodium nitroprusside and 100µM sodium nitroprusside 55.20, 72, 25.60 and 70.40% in compared to 0uM foliar application increased respectively. leaf iron in foliar application with 100µM sodium nitroprusside in compared to 0µM foliar application 17.96% increased.

Keywords: Foliage; vegetative growth; photosynthesis; temperature

1. 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 will increase from 2°C to 5°C (Lal et al., 2012). 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,

 doi:10.20944/preprints202312.1849.v1

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 the result causes oxidative stress (Yin et al., 2008). 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. In recent years, a considerable attention has been paid in reducing the harmful effects of high temperatures in plants through the external application of some chemical compounds such as hydrogen peroxide, nitric oxide (Uchida et al., 2002) and melatonin (Alam et al., 2018).

Melatonin is a molecule with an indole ring structure and a low molecular weight, which has 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). Treatment with exogenous MEL has been shown to increase the expression of genes coding for antioxidant enzymes, enhancing SOD, POD and CAT activities, decreasing oxidative stress (Kostopoulou et al., 2015; Campos et al., 2019), lipid peroxidation and the concentrations of hydrogen peroxide (Reiter et al., 2015) and malondialdehyde (Jiang et al., 2016), and to increase photosynthesis rates, sugar metabolism and growth (Zhao et al., 2015).

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 (Siddiqui et al., 2011). 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 by 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). Regarding the global warming and high temperature stress and its harmful effects on plants under high temperatures, the aim of this study was to evaluate the various treatments of melatonin and sodium nitroprusside in the form of foliar application on growth and some physiological and biochemical characteristics of California wonder green bell pepper.

2. Materials and Methods

This research is a factorial experiment with two factors (3 x 5), where the first factor is three temperature levels (25°C, 35°C and 40°C) and the second factor is 5 types of foliar spraying (distilled water (control), melatonin 50uM, melatonin 100uM, sodium nitroprusside 50uM and sodium nitroprusside 100uM) was carried out in the form of a completely randomized design in three replications. California Wonder green bell pepper seedlings were obtained from one of commercial seedling producers in Tehran province. 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 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 5-leaf stage, plants were sprayed with sodium nitroprusside (nitric oxide releasing compound) and melatonin in concentrations of 50uM and 100uM, three times with a time interval of 24 hours. At the same time,

 doi:10.20944/preprints202312.1849.v1

control samples were sprayed with distilled water. 24 hours after last spraying, the plants were transferred to 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, temperature of 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. Plants in each temperature range remained in growth chamber for 24 hours and then at end of the temperature treatment, plants were transferred to greenhouse with a temperature of 25°C.

2.1. Measurement of Traits

2.1.1. Morphological Traits and plant apparent damage index

Height of plant, fresh weight of shoot without fruit, dry weight of shoot without fruit and number of leaf per plant were measured. Amount of plant apparent damage was calculated based on characteristics such as tuberization, chlorosis and necrosis of leaves and numbered from 1 to 5 (McNellie et al., 2018). In this method, number 1 is for healthy plants that have no visible symptoms, number 2 indicates twisting of leaf margin (less than 10%), number 3 for plants with necrotic leaf margin (less than 25%), number 4 indicates Chlorosis of leaves is moderate (less than 50 percent) and number 5 is for plants with severe necrosis (more than 50 percent).

2.1.2. Physiological and biochemical traits

Leaf Relative Water Content

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

Leaf Membrane Stability Index

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

Leaf Chlorophyll, Carotenoid and Spad

The samples (fresh weight of leaves) in amount of 0.2 g in 8ml of ethanol-acetone mixture (volume ratio 1:1) were placed at room temperature for 24 hours until the appearance of a white color in a dark environment. The absorbance of the solution was read using a spectrophotometer at wavelengths of 645 nm (A645), 663 nm (A663) and 440 nm (A440). The amounts of chlorophyll and carotenoids were calculated using the following relations and expressed in terms of milligrams per gram of fresh weight of leaves (Gratani, 1992):

To measure the spad, a hand-held chlorophyll meter model SPAD-502 Minolta, Japan was used.

$$\text{Chlorophyll}_a: 12.7 \times A_{663} - 2.69 \times A_{645} \quad (1)$$

$$\text{Chlorophyll}_b: 22.9 \times A_{645} - 4.86 \times A_{663} \quad (2)$$

$$\text{Total Chlorophyll}: 8.02 \times A_{663} + 20.20 \times A_{645} \quad (3)$$

 doi:10.20944/preprints202312.1849.v1

Carotenoids: $4.7 \times A_{440} - 0.27 \times$ Total Chlorophyll (4)

Leaf Anthocyanin

Anthocyanin content of leaves was measured according to the method described by Xu (Xu et al., 2005). Plant samples were heated with 20ml of 60% ethanol for 2 hours in a hot water bath, then the samples were filtered using a volumetric flask. The extract solution was read by a spectrophotometer at a wavelength of 535 nm. And it was expressed as milligrams per gram of leaf fresh weight.

Leaf Ascorbic acid

The ascorbic acid concentration of leaf extract was measured based on the color reduction of 2,6-dichlorophenol by ascorbic acid. In this method, one milligram of leaf tissue was mixed with 3 ml of metaphosphoric acid (1%) and after 30 minutes, the mixture was mixe and centrifuged at 4°C and 6000 rpm for 15 min. From supernatant 50 microliters were removed and brought to volume of 200uL. The absorbance of samples was read at a wavelength of 520 nm. The ascorbic acid concentration of leaf was recorded using a calibration curve as mg. Fresh Weight (Chang et al., 2002).

Leaf Proline

To measure amount of proline, Bates et al. (1973) method was used with a little change. For this purpose, first, 500 mg of leaves were weighed and ground well in a Chinese oven in 5 ml of 10% sulfosalicylic acid aqueous solution. Then resulting mixture was centrifuged for 20 minutes at 15,000 g and 4 degrees Celsius.

Next, 2ml of supernatant were mixed with 2 milliliters of ninhydrin reagent and 2 milliliters of glacial acetic acid. Then the samples were placed in 100-degree Celsius water bath for one hour. After cooling samples, ml of toluene was added to each sample and vortexed for 30 seconds. Finally, the upper phase, which was red and contained proline dissolved in toluene, was removed and read at the same time as the standard samples in a spectrophotometer (model 160 UV-VIS, Shimadzu, made in Japan) at a wavelength of 520 nm. The concentration of proline was determined in terms of millimoles per gram of dry weight leaf with using a standard curve.

Leaf Carbohydrates

Measurement of total sugar leaf was done by anthrone reagent with using 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 100ml of dilute sulfuric acid, 76 ml of concentrated sulfuric acid was mixed with 38 ml of distilled water. To measure soluble sugars, 100uL of extract was poured into a test tube and three milliliters of anthrone solution was added to it. The obtained mixture was placed in a boiling water bath for 20 minutes at a temperature of 100°C. Leaf soluble sugars were measured using spectrophotometric method at a wavelength of 620 nm (McCready et al., 1950).

Leaf Glucose, Fructose and Sucrose

In order to measure and separate sugar, a HPLC manufactured by the American company withers was used with carbohydrate C_{18} column (250x46mm, DP=3um) and expressed as milligrams per gram of fresh leaf weight (Augustin et al., 1998).

Leaf Hydrogen peroxide

Amount of hydrogen peroxide in leaves was measured by the method of Loreto and Velikova (2001). Based on this method, 0.1 gram of leaf tissue was homogenized in a Chinese oven containing one milliliter of 0.1% trichloroacetic acid. Extract was placed in a centrifuge at 12,000g for 15 minutes at temperature of 4 degrees Celsius. Next, 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH= 7) containing 1 ml of 1 M potassium iodide, and then the

 doi:10.20944/preprints202312.1849.v1

absorbance of solution was read at a wavelength of 390 nm. Amount of hydrogen peroxide was determined in terms of micromoles per gram of fresh leaf with using a standard curve.

Leaf Membrane Lipid Peroxidation

Peroxidation of leaf membrane lipids was measured based on concentration of malondialdehyde (MDA) in leaves. For this purpose, 0.1 gram of leaf tissue was homogenized with 1 ml of 0.1% trichloroacetic acid with using liquid nitrogen. Extract was centrifuged for 20 minutes at 4 degrees Celsius at 12,000 g. Then, 3 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added to 1 ml of supernatant. Next, samples were placed in a hot water bath (temperature of 95 degrees Celsius) for 30 minutes, and then after cooling, samples were centrifuged for 5 minutes at 10,000 g. Finally, absorption of extract was read by a spectrophotometer at wavelengths of 532 and 600 nm. Amount of malondialdehyde was calculated in micromoles per gram of Fresh weight leaf based on extinction coefficient of 155 $mM^{-1}cm^{-1}$ (Shafeiee and Ehsanzadeh, 2019).

Leaf Glutathione

To measure glutathione, 0.2g of leaf was homogenized with 2 ml of cold 5% trichloroacetic acid. Extract was centrifuged for 10 minutes at 10,000g at temperature of 4 degrees Celsius. 0.5ml from supernatant was added to 0.5mL of phosphate buffer (100mM, pH 7) containing 0.5mM EDTA and 50uL of 3mM DTNB (5,5-dithiobis-2-nitrobenzoic acid). Absorbance of extract was read at wavelength of 512 nm. The standard curve was drawn with different glutathione concentrations and glutathione concentration of the leaf samples was determined in terms of mg per gram of Fresh Weight (AL-Huqail et al., 2017).

Leaf Phenylalanine Ammonialyase

The method of Saunders and McClure (Saunders and McClure, 1974) was used with a slight modification to measure activity of leaf phenylalanine ammonialyase enzyme. At first, 0.1 g of leaf was crushed using 1 ml of 50 mM phosphate buffer with pH=7 and centrifuged for 15 minutes at 14,000 rpm. After centrifugation, supernatant extract was used to measure the enzyme. Reaction mixture contained 250 microliters of enzyme extract, 250 microliters of 10 mM sodium borate buffer (pH=8.8), 250 microliters of distilled water, and 250 microliters of phenylalanine substrate (50 mM). Absorbance of sample was read at wavelength of 290 nm with using spectrophotometer. Enzyme activity was recorded using Beer and Lambert's law and with an extinction coefficient of 9630 $u^{-1}cm^{-1}$ in terms of nanomoles per gram of Fresh Weight per minute.

Leaf Super Oxide Dismutase

Superoxide dismutase enzyme activity was measured by spectrophotometric method. The reaction solution included 0.1 mM EDTA, 50 mM phosphate buffer, 13 mM methionine, 75 μM NBT, 2 μM riboflavin, and 10 μL of enzyme extract. The reaction solution was exposed to fluorescence light for 15 minutes. The reaction was stopped by transferring to dark conditions, and then absorbance of samples was read at wavelength of 560 nm and recorded in terms of micromoles per gram of Fresh Weight. (Giannopolitis and Ries, 1977).

Leaf Catalase Enzyme

Catalase enzyme activity of leaves was measured by spectrophotometric method. One milliliter of reaction mixture included 5uL of enzyme extract, 30uLof potassium phosphate buffer, 665 microliters of 0.5 mM ascorbic acid, and one microliter of 0.1mM hydrogen peroxide. Changes in absorbance at 240nm were measured by spectrophotometer and expressed in terms of micromoles per gram of leaf Fresh Weight (Beers and Sizer, 1952).

 doi:10.20944/preprints202312.1849.v1

Leaf Peroxidase

The activity of leaf peroxidase enzyme was measured by spectrophotometric method in such a way that enzyme activity was measured for 3 minutes in a reaction solution containing 490 microliters of 45 mM guaiacol and 490 microliters of 0.225 mM hydrogen peroxide and 20 microliters of enzyme extract and in wavelength of 470 nm was read with using a spectrophotometer and recorded as micromoles per gram of leaf Fresh Weight per minute (Hammerschmidt et al., 1982).

Leaf Ascorbate Peroxidase

Ascorbate peroxidase enzyme activity was measured by method of Cakmak and Marschner (1992) based on amount of ascorbate oxidation at 290 nm wavelength. The reaction mixture included 3 milliliters of 50 mM phosphate buffer (pH:7), 51.4 microliters of H_2O_2, 100 microliters of 5 mM ascorbate, and 50 microliters of enzyme extract. Enzyme reaction speed was recorded in the form of absorbance changes over time in one minute in terms of micromoles per gram of leaf fresh weight per minute.

Leaf Guaiacol Peroxidase0

The activity of leaf guaiacol peroxidase enzyme was measured based on oxidation of guaiacol with using H_2O_2 by method of Zhou et al. (2009). For this purpose, 3.35 microliters of guaiacol, 3.8 microliters of H_2O_2 and 50 microliters of enzyme extract were added to 3 milliliters of sodium phosphate buffer. Absorption changes were read in real time at a wavelength of 470 nm and a duration of 120 seconds. Each activity unit of guaiacol peroxidase enzyme was considered as an amount of enzyme that oxidizes one micromole of guaiacol per minute.

Leaf Mineral Elements

Leaf nitrogen was measured with 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 (Leaf) was placed in an electric furnace at a 500 °C temperature for four hours. Then, 10 cc 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 (Leaf) were dissolved in 10 ml of concentrated nitric acid and suspension was placed at 70 °C for 24 hours until samples were well dissolved in 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).

2.1.3. Statistical Analysis

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

3. Results

3.1. Plant growth

The results of this study showed that highest (51.85 cm) and lowest (45.37 cm) Plant Height are related to 25°C and 40°C temperature treatment, respectively (Table 1). Also, there was no significant difference ($p< 0.05$) between 25°C and 35°C temperature treatments in terms of plant height. According to Table 2, highest (54.82 cm) and lowest (45.38 cm) Plant Height are related to 100uM and 0uM sodium nitroprusside respectively. Also, there was no significant difference ($p< 0.05$) between 50 and 100uM sodium nitroprusside treatment. According to Table 1, highest (302.53g F.W.) and lowest (250.55g F.W.) shoot fresh weight without fruit are related to 25°C and 40°C temperature treatment respectively. Also, according to Table 2, highest (292.83g F.W.) and lowest (264.89g F.W.)

 doi:10.20944/preprints202312.1849.v1

shoot fresh weight without fruit are related to 100µM melatonin and control (0uM), respectively. Also, there was no significant difference ($p< 0.05$) in terms of shoot fresh weight without fruit between 50µM and 100µM melatonin and both were in the same statistical class. According to Table 1, highest (49.72g D.W) and lowest (39.01g D.W) shoot dry weight without fruit are related to 25°C and 40°C temperature treatment respectively. Also, data in Table 2 shows that highest (48.61g D.W.) and lowest (41.38g D.W.) shoot dry weight without fruit are related to 100µM melatonin and 50µM sodium nitroprusside, respectively. According to data in Table 3, highest (37.68) and lowest (24.86) leaf number were treated to 25°C temperature treatment with 50uM sodium nitroprusside and 40°C temperature treatment with of 0uM foliar application treatment, respectively.

3.2. Plant pigments

According to Table 1, Maximum (1.95Mg.g F.W) and Minimum (1.31Mg.g F.W) leaf chlorophyll was obtained from 25°C temperature treatment and 40°C temperature treatment, respectively. Also, there was not significant difference ($p< 0.05$) in terms of leaf chlorophyll value between 25 and 35°C temperature treatment. Data presented in Table 2 shows that The highest (1.93 $Mg.g^{-1}$ F.W) and the lowest (1.46 $Mg.g^{-1}$ F.W) amount of leave chlorophyll were related to 100uM sodium nitroprusside treatment and control (0uM) treatment, respectively. Also, there was not significant difference ($p<0.05$) between 100µM melatonin and 100µM sodium nitroprusside in terms of leave chlorophyll content and they are in the same statistical group. According to Table 1, the highest (0. 68 $Mg.g^{-1}$ F.W) and the lowest (0.25 $Mg.g^{-1}$ F.W) contents of leave carotenoids were obtained from 25°C and 40°C temperature treatments, respectively. also Table 2 shows that the highest (0.67 $Mg.g^{-1}$ F.W) and the lowest (0.39 $Mg.g^{-1}$ F.W) contents of leave carotenoid are related to foliar 100µM melatonin application and foliar 0µM application, respectively. According to the data in Table 3, the highest (2.83 $Mg.g^{-1}$ F.W) and the lowest (1.90 $Mg.g^{-1}$ F.W) amounts of leave anthocyanin were treated at 35°C temperature with foliar 0µM application and 25°C temperature with foliar 50uM sodium nitroprusside application respectively. Data presented in Table 3 shows that the highest (48.83) and lowest (27.31) leaf spad value were related to 25°C temperature with foliar spray of sodium nitroprusside (100µM) and 40°C temperature with foliar 0 uM application (control), respectively.

3.3. Physiological characteristics

According to Table 1, the highest (71.77%) and lowest (54.07%) RWC value of leaves are related to 25 and 40°C temperature treatment, respectively. Also, the data in Table 2 shows that the highest (69.94%) and lowest (58.23%) RWC values of leaves are related to foliar spray of melatonin (MT; 100 $\mu mol \cdot L^{-1}$) and 0uM foliar spraying treatment (control), respectively. These findings showed that there is not statistically significant difference ($p<0.05$) in terms of leave relative water content between application of melatonin at 100µM and 50µM. This study demonstrated that according to Table 1, the highest (34.15%) and lowest (27.06%) Membrane Stability Index of leave are related to 35°C and 40°C temperatures, respectively. Also there is not significant difference ($P<0.05$) between the temperature 35°C and 40°C temperatures in terms of Membrane Stability Index value.

3.4. Biochemical characteristics

The results showed that the highest (0.77 $Mg.g^{-1}$ F.W.) and the lowest (0.40 $Mg.g^{-1}$ F.W.) leave Glutathione levels was obtained at the 40°C and 25°C temperature treatment, respectively (Table 1). The results showed that according to Table 1, the highest (25.46 mmol.g D.W) and the lowest (11.99mmol.g D.W) proline values in the leaves were related to 40°C and 25°C temperature treatment, respectively. According to Table 1, the highest (124.91 $Mg.g^{-1}$ F.W) and the lowest (89.25$Mg.g^{-1}$ F.W) content of leave carbohydrates was recorded to 40°C and 25°C temperatures, respectively. The results (Table 2) showed that the highest (116.10 $Mg.g^{-1}$ F.W) and the lowest (99.60 $Mg.g^{-1}$ F.W) values of leaf carbohydrates was recorded in 100uM of melatonin and control (0uM), respectively. Also, there was

 doi:10.20944/preprints202312.1849.v1

no significant difference ($p< 0.05$) between 100Mm.L^{-1} melatonin and 100µM.L^{-1} sodium nitroprusside treatments and they were in the same statistical class in terms of leaf carbohydrates. The results show that the highest (2.34 uM.g^{-1} F.W.) and the lowest (1.68uM.g^{-1} F.W.) values of lipid peroxidation of leaf membrane enzyme activity were observed on 0uM foliar application (control) and 100uM foliar application of sodium nitroprusside, respectively (Table 2). Also, there was no significant at the 5% probability level in terms of lipid peroxidation of leaf membrane enzyme activity values between control (0µM.L^{-1}) and 50µM.L^{-1} melatonin.

The results showed that the highest (3.57 uM.g^{-1} F.W) and the lowest (1.09uM.g^{-1} F.W) Hydrogen Peroxide values of leaves was observed at 40°C temperature with foliar treatment of melatonin with 100uM concentration and 25°C temperature with foliar treatment of melatonin with 100uM concentration, respectively (Table 3). Also, there was no significant at the 5% probability level between 40°C temperature treatment with 100uM foliar application of melatonin and 40°C temperature treatment with control. The results showed that the highest (89. 84uM.g^{-1} F.W) and the lowest (50.91uM.g^{-1} F.W) leaves superoxide dismutase enzyme activity values were treated at 40°C temperature with 100uM melatonin and 25°C temperature with 50µM sodium nitroprusside, respectively (Table 3). Also, there was no statistically significant difference ($p<0.05$) in terms of leaf superoxide dismutase enzyme activity values between 40°C temperature treatment with 50uM and100uM melatonin, 50uM and 100uM sodium nitroprusside and were in the same statistical class. The results showed that, the highest (3.14uM.g^{-1} F.W.) and the lowest (1.05uM.g^{-1} F.W.) leaves catalase enzyme activity values were treated at 25°C temperature with 0uM foliar spray (control) and at 40°C temperature with the 0uM foliar spray (control), respectively (Table 3) Also there was no significant difference between 25°C temperature treatment with 0uM foliar spray (control), 50uM melatonin, 100uM melatonin, 50uM sodium nitroprusside and 100uM sodium nitroprusside and were in the same statistical group. The results showed that the highest (53.25 uM.g^{-1} F.W) and the lowest (27.32 uM.g^{-1} F.W) ascorbate peroxidase enzyme activity values were related to at 25°C temperature treatment with 0uM foliar spray (control) and 40°C temperature treatment with 0uM foliar spray (control), respectively. (Table 3). According to Table 3, the highest (0.60 Unit mg^{-1} protein) of leave guayacol peroxidase enzyme activity values were related to 40°C temperature with melatonin foliar 100 µM. Also, there was no significant difference ($P< 0.05$) between control (0uM), 50 and 100 uM melatonin foliar spraying, sprayed with sodium nitroprusside (50 and 100 µM) in terms of leaves guayacol peroxidase enzyme activity.

3.5. Leaf Mineral Elements

The results showed that the highest (4.70% D.W.) and the lowest (3.05% D.W.) leaf nitrogen values were related to the 25°C and 40°C temperature treatment (Table 1), respectively. According to the data in Table 2, the highest (4.57% D.W.) and the lowest (3.33% D.W.) leaf nitrogen values were related to 100uMm sodium nitroprusside foliar spraying and 0uM foliar spraying (control treatment), respectively. According to the data in Table 1, the highest (0.36% D.W) and the lowest (0.21% D.W) leaf phosphorus values were related to 25°C and 40°C temperature treatment, respectively. According to the data in Table 2, the highest (0.31% D.W) leaf phosphorus values were related to foliar spray of 100µM sodium nitroprusside which was not significant difference ($P< 0.05$) in compared to 100µM melatonin foliar application. According to the data in Table 1, the maximum (2.35% D.W.) and the minimum (1.58% D.W.) leaf calcium values was observed in 25°C and 35°C temperature treatment, respectively. The results showed that the maximum (0.42% D.W.) and the minimum (0.28% D.W.) leaf magnesium values were observed in 25°C and 35°C temperature treatment, respectively (Table 1). According to the data in Table 1, the highest (0.42% D.W.) and the lowest (0.28% D.W.) leaf magnesium values were related to 25 and 40°C temperature treatment, respectively. The results showed that the highest (0.42% D.W) leaves magnesium values were related to application of 100µM melatonin (Table 2). Also, there was no significant difference between sprayed with melatonin foliar treatment with 50µM and sodium nitroprusside (50 and 100µM) in terms of leaf magnesium levels.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 December 2023 doi:10.20944/preprints202312.1849.v1

According to the data in Table 3, the highest (3.23%) and lowest (1.25%) amounts of leaf potassium were treated at 25°C temperature treatment with foliar spray of 100µM sodium nitroprusside and 40°C temperature treatment with control (0µM) respectively. Also, there was not significant difference between 25°C treatments with foliar application of 0uM, 50 and 100uM Melatonin foliar spraying and sodium nitroprusside foliar treatment with 50 and 100µM in terms of leaf potassium values. According to the data in Table 1, the highest (168.59 Mg.kg D.M) leaf iron amounts were related to 25°C temperature treatment. Also, there is no significant difference (P< 0.05) in terms of leaf iron values between 35 and 40°C temperature treatments. According to the data in Table 2, the highest (165.02 Mg.kg D.m) and the lowest (139.89 Mg.kg D.m) leaf iron amounts were related to foliar spray of 100 µM sodium nitroprusside and control (0 µM), respectively. The results showed that, the highest (44.49 Mg.kg D.m) leaves zinc values were related melatonin foliar treatment with 100µM (table 2). The results showed that the highest (87.28 Mg.kg D.) leaf manganese values were related to the 25°C temperature treatment with foliar spraying of the control (0µM) (table 3). According to the data in Table 3, there was no significant difference (P< 0.05) in terms of leaf copper values between 25°C temperature treatment with control, melatonin foliar treatment with 50 and 100 µM, Foliar spray of 50 and 100µM sodium nitroprusside.

Table 1. Comparison of means effects of temperature on plant height, fresh weight plant shoot without fruit, dry weight plant shoot without fruit, chlorophyll, carotenoids, RWC, membrane stability index, glutathione, proline, carbohydrates, nitrogen, phosphorus, calcium, magnesium and iron in California wonder green bell pepper.

T (°C)	PH (cm)	FWPWF (g F.W)	DWPWF (g D.W)	Chlorophyll (Mg.g F.W)	Carotenoids (Mg.g F.W)	RWC (%)	MSI (%)	Glutathione (Mg.g F.W)	Proline (mMol.g D.W)	Carbohydrates (Mg.g F.W)	Nitrogen (% D.W)	Phosphorus (% D.W	Calcium (% D.W)	Magnesium (% D.W)	Iron (Mg.kg D.M)
25	51.85 a	302.53 a	49.72a	1.95a	0.68a	71.77a	27.06 b	0.40c	11.99c	89.25c	4.70a	0.36a	2.35a	0.42a	168.59a
35	50.46 a	283.03 b	43.01b	1.89a	0.57b	63.63b	34.15 a	0.63b	21.27b	110.51b	4.30b	0.29b	1.58c	0.38b	142.95b
40	45.37 b	250.55 c	39.01c	1.31b	0.25c	54.07c	33.84 a	0.77a	25.46a	124.91a	3.05c	0.21c	2.08b	0.28c	146.52b
LSD	2.02	6.92	2.01	0.11	0.02	4.73	1.83	0.11	2.84	6.90	0.18	0.01	0.12	0.01	7.33

Temperature: T, Plant Height: PH, Fresh Weight Plant Shoot Without Fruit: FWPWF, Dry Weight Plant Shoot Without Fruit: DWPWF, Membrane Stability Index: MSI, Differences letters indicate significantly different values at $p < 0.05$.

Table 2. Comparison of means effects of foliar spraying of melatonin and sodium nitroprusside on plant height, fresh weight plant shoot without fruit, dry weight plant shoot without fruit, chlorophyll, carotenoids, RWC, carbohydrates, lipid peroxidation of leaf membrane, nitrogen, phosphorus, magnesium, zinc and iron in California wonder green bell pepper.

Foliar Application (uM)	PH (cm)	FWPSWF (g F.W)	DWPSWF (g D.W)	Chlorophyll (Mg.g F.W)	Carotenoids (Mg.g F.W)	RWC (%)	Carbohydrates (Mg.g F.W)	LPLM (uMg F.W)	Nitrogen (% D.W)	Phosphorus (% D.W)	Magnesium (% D.W)	Zinc (Mg/kg D.m)	Iron (Mg.kg D.M)
0	45.38 b	264.89c	42.92bc	1.46d	0.39d	58.23c	99.60b	2.34a	3.33d	0.27c	0.30c	33.69c	139.89 c
50 M	46.28 b	286.48a	45.07b	1.61c	0.46c	66.15a b	105.26b	1.96a b	3.65c	0.27c	0.36b	41.49b	149.69 b

 doi:10.20944/preprints202312.1849.v1

100	M	46.85 b	292.83a	48.61a	1.86ab	0.67a	69.94a	116.10a	1.87b	4.12b	0.30ab	0.42a	44.49a	153.62 b
50	SNP	52.80 a	274.53b	41.38c	1.73bc	0.44c	61.78b c	104.85b	1.74b	4.42a	0.29bc	0.36b	35.53c	155.22 b
100	SNP	54.82 a	274.81b	41.59c	1.93a	0.54b	59.69c	115.30a	1.68b	4.57a	0.31a	0.36b	35.44c	165.02 a
LSD		2.61	8.93	2.60	0.14	0.03	6.11	8.91	0.43	0.23	0.02	0.02	2.48	9.41

Melatonin: M, Sodium nitroprusside: SNP, Plant Height: PH, Fresh Weight Plant Shoot Without Fruit: FWPSWF, Dry Weight Plant Shoot Without Fruit: DWPSWF, Lipid Peroxidation of Leaf Membrane: LPLM, Differences letters indicate significantly different values at $p < 0.05$.

Table 3. Comparison means effects of foliar spraying (melatonin and sodium nitroprusside) and temperature on number of leaf per plant, anthocyanins, spad, hydrogen peroxide, superoxide dismutase, catalase, ascorbate peroxidase, guayacol peroxidase, manganese, potassium and copper in California wonder green bell pepper.

T (°C)	Foliar Application (uM)	NLP	Anthocyanins (Mg.g F.W)	Spad	Hydrogen Peroxide (uM.g F.W)	Superoxide Dismutase (uM.g F.W)	Catalase (uM.g F.W)	Ascorbate Peroxidase (uM.g F.W)	Guayacol Peroxidase (Unit mg-1 protein)	Potassium (% D.W)	Manganese (Mg.kg D.M)	Copper (Mg.kg D.M)
25	0	36.06ab	2.16ef	35.33cd	1.34i	52.81fg	3.14a	53.25a	0.13f	2.99a	87.28a	13.69a
	50 M	34.49b	2.41bcd	35.80c	1.14i	51.41g	2.96ab	51.43a	0.13f	3.12a	80.56b	12.74a
	100 M	35.21b	2.14efg	35.40c	1.09i	51.46g	3.00ab	52.19a	0.14f	3.20a	77.05b	13.51a
	50 NSP	37.68a	1.90h	45.31b	1.14i	50.91g	2.98ab	51.83a	0.13f	3.00a	76.08b	13.39a
	100 NSP	36.22ab	1.99gh	48.83a	1.13i	51.06g	3.13a	52.26a	0.13f	3.23a	75.60b	12.49ab
35	0	31.35c	2.83a	35.19cd	2.42fe	60.48f	2.01fg	39.36b	0.30e	2.19bcd	68.61cd	6.77g
	50 M	30.71cd	2.49bc	34.01cde	2.03gh	72.75de	2.39de	33.56cd	0.41d	2.32bc	76.68b	9.20ef
	100 M	30.27cd	2.27de	33.62cde	1.92h	78.67cde	2.76bc	31.31def	0.45c	2.40bc	75.68b	9.42e
	50 NSP	30.25cd	2.34cd	32.71edf	2.37fg	79.51bcd	2.39de	31.91de	0.42cd	2.20bcd	75.03b	11.18bc
	100 NSP	30.97cd	2.34cd	33.27cdef	2.01h	80.50bcd	2.53cd	29.00ef	0.44c	2.43b	74.68bc	10.94cd
40	0	24.86e	2.06fgh	27.31h	3.43ab	70.86e	1.05h	27.32f	0.40d	1.25f	46.28f	3.36h
	50 M	29.90cd	2.52b	28.48gh	3.16bc	83.20abc	1.88fg	38.43b	0.50b	1.94d	45.16f	7.87fg
	100 M	28.92d	2.16ef	32.15ef	3.57a	89.84a	2.24def	3618bc	0.60a	2.15bcd	47.07f	7.75g
	50 NSP	29.88cd	2.09fg	32.40ef	2.91cd	83.72abc	1.86g	36.78bc	0.50b	1.57e	58.13e	10.18cde
	100 NSP	30.04cd	2.11efg	30.92fg	2.71de	87.48ab	2.13efg	33.87cd	0.50b	2.13cd	67.35d	9.61de
LSD		2.28	0.17	2.67	0.34	8.57	0.36	4.21	0.02	0.28	6.39	1.41

Temperature: T, Melatonin: M, Sodium nitroprusside: SNP, Number of leaf per Plant: NLP, Differences letters indicate significantly different values at $p < 0.05$.

4. Discussion

4.1. Effects of melatonin and sodium nitroprusside under different temperatures on plant growth

The plant height at 40°C temperature in compared with 25°C temperature 12.50% decreased. Foliar treatment with sodium nitroperoxide increased plant height in compared to other treatments, and there was not significant difference ($p<0.05$) between sprayed with sodium nitroperoxide (50 and 100µM). The shoots fresh weight without fruit (FWPWF) at 40°C temperature in compared to 25°C temperature 17.18% decreased. Melatonin foliar treatment with 50µM and 100µM in compared to other treatments increased the shoots fresh weight without fruit (FWPWF). Shoot dry weight without fruit (DWPWF) at 40°C temperature in compared to 25°C temperature 21.54% decreased. Foliar

 doi:10.20944/preprints202312.1849.v1

treatment with 100uM melatonin in compared to control (0uM), increasing 13.26% showed in shoots dry weight without fruit (DWPWF). Chen et al. (2009) showed that melatonin foliar treatment with 100µM on Arabidopsis plants increased the growth of aerial organs. Application of 50 and 100µM melatonin improved the aerial organs and roots weight of watercress. It seems that increased in the aerial weight was due to the effect of exogenous melatonin on absorption of nutrients from the roots (Oloumi et al., 2018). Melatonin foliar treatment increased the biomass and dry weight of shoot and root of watercress (Oloumi et al., 2018).

Studies have shown that melatonin foliar application increased the dry weight (Afreen et al., 2006). It seems that melatonin foliar treatment increases the plant's tolerance to stress by creating hormonal balance. Nitric oxide increases the rate of photosynthesis through increasing chlorophyll production, increasing the mitochondria and chloroplast function, increasing the activity of antioxidant enzymes such as superoxide dismutase and catalase, maintaining relative water content and reducing leaf water loss (Tian and Lei, 2007). Nitric oxide interferes with the rate of photosynthesis through interfering in the chlorophyll pigment biosynthesis (Arun et al., 2017). Nitric oxide increase photosynthesis through chlorophyll pigment increasing (Marvasi, 2017). Various reports indicate that nitric oxide protects the chloroplast membrane as a protective molecule. In addition, nitric oxide is also involved in the metabolic pathways of chlorophyll synthesis (Hemati et al., 2019). Nitric oxide, through the production of phenolic compounds such as salicylic acid, leads to the protection of chloroplasts and prevents degradation protein and increases the photosynthetic tissues. Researchers reported that external application of nitric oxide scavenges free radicals, develops cell membrane potential, improves photosynthesis and leaf water status. Probably, the photosynthetic efficiency increasing through foliar application of sodium nitroprusside can increase the dry matter (Farooq et al., 2009).

4.2. Effects of melatonin and sodium nitroprusside under different temperatures on plant pigments

Leaf chlorophyll values at 40°C in compared to 25°C temperature 32.82% decreased. Also, under 25°C temperature, foliar spraying with 100µM sodium nitroprusside 38.21% increased in leaf spad amount in compared to 0µM foliar spraying. Foliar spray of 100µM melatonin, in compared to control foliar spraying (0µM) 71.79% increased the leaves carotenoid values. Melatonin and nitric oxide play a role in inhibiting the decomposition of photosynthetic pigments and the leave chlorophyll content increasing (Yang et al., 2011). On the other hand, nitric oxide can play an important role in photosystem II protection through regulating the psbA gene expression and improve the photochemical efficiency II (Graziano et al., 2002). Melatonin through hormonal balance changing, increases cytokinin and auxin, and as a result improves plant growth (Sarropoulou et al., 2012). Melatonin inhibits the chlorophyllase and pheophorbide-a oxygenase enzymes activity and as a result preserves and increases the chlorophyll amount, postpones leaf senescence, improves the photosystem II performance and ultimately increases the photosynthesis efficiency (Turl et al., 2014).

4.3. Effects of melatonin and sodium nitroprusside under different temperatures on plant physiological characteristics

The leaves relative water content at 40°C temperature in compared to 25°C temperature 24.66% decreased. Sprayed with melatonin (50 and 100µM) improved the leaves relative water content in compared to foliar application of 0µM (control). temperatures of 35°C and 40°C increased Membrane Stability Index (MSI). Previous studies showed that melatonin prevents leaves senescence in high temperature through activating the cytokinin production and participating in inhibiting of abscisic acid production (Zhang et al., 2017). The results obtained from this study are in agreement with findings of Jahan et al. in tomato and Li et al. (2013) in corn. They showed that the foliar application of melatonin and nitric oxide reduced the temperature injuries (injuries through high temperature stress) and improved the survival percentage of these plants' seeds under high temperature stress. High temperature, increases the transpiration and as a result of the relative humidity decreased. To deal with water lack, plant cells accumulate osmolytes such as proline. The increase in the leaf water relative content of plants sprayed with melatonin and nitric oxide under

 doi:10.20944/preprints202312.1849.v1

high temperature stress is probably due to the increase in the proline production stimulation and water absorption improvement through the cells as a result the water balance maintaining. These result is similar to those reported by Jahan et al. (2019) in tomato plant. The results obtained from this study showed that improving photosynthesis has increased shoot dry matter. Melatonin through affecting on various physiological mechanisms, such as photosynthesis, respiration, ion absorption, membrane permeability, enzymes and hormones activities, effects on the growth and plant biomass production (Li et al., 2014; Janas & Posmyk, 2013; Zhang et al., 2015). Also, melatonin, as a ROS scavenger and activator of antioxidants, affects membrane stability and stabilizes chloroplasts and prevents chlorophyll decomposition (Wang et al., 2014). On the other hand, melatonin improves the photosystem II efficiency and increases the stomatal conductance and as a result of releasing carbon dioxide gas into the plant, it maintains the high capacity of carbon dioxide assimilation. Also, melatonin through affecting the enzyme activity such as rubisco, have a positive effect on primary net production and ultimately increased plant growth (Li et al., 2014; Janas & Posmy, 2013).

4.4. Effects of melatonin and sodium nitroprusside under different temperatures on plant biochemical characteristics

Leave Glutathione at 40°C temperature in compared to 25°C, 92.50% increased. Also, leaf proline amount 112.34% at 40°C temperature in compared to 25°C temperature, increased. Also, leaf carbohydrate at 40°C temperature in compared to 25°C, 39.96% increased. melatonin and sodium nitroprusside foliar treatment with 100µM, in compared to other foliar spray treatments increased leaf carbohydrates. Based on the results of this study foliar application with melatonin and sodium nitroprusside decreased the lipid peroxidation of leaf membrane (LPLM). Sprayed with sodium nitroperoxide (50µM and 100µM) at 40°C temperature in compared to control (0uM) 15.16 and 20.99% decreased the of leaves hydrogen peroxide amount, respectively. In general, foliar spraying with melatonin and sodium nitroprusside under temperature stress conditions reduced the leaves hydrogen peroxide amount. So that under 40°C temperature stress conditions, sodium nitroprusside more than melatonin decreased the leaves hydrogen peroxide amount.

Foliar spraying with melatonin and sodium nitroprusside (50 and 100µM) in compared to 0µM foliar spraying (control) at 40°C temperature increased the leave superoxide dismutase enzyme. It seems that melatonin and sodium nitroprusside reduces the temperature stress damage through the of superoxide dismutase enzyme activity increasing. Under 35°C and 40°C temperature stress with melatonin and sodium nitroprusside foliar treatment catalase enzyme activity increased. melatonin and sodium nitroprusside foliar treatment with 50µM and100µM increased the leaves ascorbate peroxidase enzyme activity under 40°C temperature. Also, under 40°C temperature stress conditions, sprayed with melatonin and sodium nitroprusside (50µM and 100µM) increased leaf guayacol peroxidase. During stress, the application of exogenous melatonin on soybean plants significantly reduced hydrogen peroxide content and increased the activity of ascorbate peroxidase, catalase and polyphenol oxidase (Wei et al, 2014). Osmotic regulators, including proline, play an important role in plants against stress through participating in cells osmotic regulation, stabilizing the enzymes and proteins activity, detoxifying active oxygen species and integrity of membranes maintaining. Proline as osmotic protector, plays an important role in maintaining cell integrity in stressful conditions and facilitates important proteins production that were necessary in response to stress (Iyer and Caplan, 1998).

Melatonin through proline biosynthesis (p5CS) gene expression increasing and also nitric oxide through proline production enzymes activity increasing, preventing the activity of the proline dehydrogenase enzyme that decomposes proline, increase the accumulation of proline in stressful conditions (Jahan et al., 2019). The proline amount in tomato and strawberry, which were treated with melatonin and sodium nitroprusside, increased significantly under high temperature and salinity stress in compared to the control plants (Jahan et al., 2019; Jamali et al., 2014). It has been reported that nitric oxide protects the plant from oxidative damage through regulating the homeostasis mechanisms of cellular oxidation-reduction reactions, increasing the conversion of

 doi:10.20944/preprints202312.1849.v1

superoxide radicals into water and oxygen, and also the hydrogen peroxide inhibiting enzymes activity increasing (Lamattina et al., 2003). The accumulation of hydrogen peroxide and malondialdehyde in rye plant (*Lolium perenne* L.) treated with nitric oxide was reduced significantly under temperature stress (Dong et al., 2014). The main mechanism of reduction of hydrogen peroxide accumulation in plants treated with melatonin, because of acts as an electron donor (melatonin) and detoxifies reactive oxygen species such as hydrogen peroxide (Alam et al., 2018). High temperature leads to oxidative stress, damage to biological macromolecules (proteins, lipids and nucleic acids) and membranes due to reactive oxygen species excessive accumulation (Yin et al., 2008). Therefore, increasing the tolerance to temperature stress was related to the antioxidant system stimulation. One of the most important defense mechanisms of plants to deal with damage caused through oxidative stress caused by excessive reactive oxygen species accumulation in temperature stress conditions is the of the antioxidant system activity increasing.

The antioxidant system includes non-enzymatic antioxidants such as ascorbic acid and glutathione and enzymatic antioxidants such as superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase. The results obtained from this study showed that non-enzymatic antioxidants such as leave glutathione and ascorbic acid increased at high temperatures. Glutathione has multiple roles and protects plants from high temperature stress through enzymes oxidation inhibiting (Ding et al., 2016). Ascorbic acid, due to strong antioxidant capacity, plays important role in protecting plants against oxidative stress. The results from the current study indicate that the use of melatonin and nitric oxide in high temperature stress led to the increase in pepper leave glutathione and ascorbic acid. These findings are in agreement with the results in other plants such as tomatoes (Wu et al., 2011). Nitric oxide plays an important role in the ascorbate-glutathione cycle. Nitric oxide plays a role in increasing the cells glutathione level trough the glutathione biosynthesis increasing or increasing cysteine formation, which is a precursor of glutathione (Parankusam et al., 2017). Increasing glutathione concentration regulate monohydroascorbate reductase and dehydroascorbate reductase enzymes activity, which can be responsible the of ascorbic acid formation (Wu et al., 2011). Also, glutathione and ascorbic acid higher levels in plants treated with melatonin, show that melatonin was a mediator in different antioxidative pathways such as ascorbate-glutathione cycle and its use can improve the plants antioxidant capacity.

The results obtained from this study showed that high temperature increased the superoxide dismutase and guaiacol peroxidase enzymes activity and decreased the catalase and ascorbate peroxidase enzymes activity. Superoxide dismutase is the first line of antioxidant defense and superoxide radicals converts into hydrogen peroxide. Next, hydrogen peroxide is decomposed into water and oxygen by the catalase, ascorbate peroxidase or guaiacol peroxidase enzymes in different parts of the cell (Arora et al., 2002). The results of present experiment showed that the plants treatment with melatonin and nitricoxide increased significantly in an antioxidant enzymes activity in comparison with control treatment. In addition to melatonin eliminate many free radicals directly, acts as a molecular signal and regulates the antioxidant enzymes activity (Ding et al., 2017). also previous studies have shown the role of melatonin in the antioxidant enzymes activity increasing to increase tolerance to adverse environmental conditions on plants such as pepper (Kaya and Doganlar, 2019). The nitric oxide antioxidant performance in response to oxidative stress caused by high temperature is due to its ability to neutralize free radicals (Yang et al., 2011). Improving of antioxidant enzymes activity during temperature stress is one of the common protective responses created through nitric oxide in most plant species. For example, wheat treatment with sodium nitroprusside increased the antioxidant enzymes activity such as superoxide dismutase, catalase, and soluble peroxidase (Parankusam et al., 2017). As an antioxidant, melatonin inhibits reactive oxygen species much more than glutathione, and also increases the antioxidant enzymes activity such as peroxidases, superoxide dismutase and catalase (Reiter et al., 2007).

4.5. Effects of melatonin and sodium nitroprusside under different temperatures on leaf mineral elements

Leaf nitrogen in 40°C temperature in compared to 25°C temperature 35.11% decreased. Sprayed with sodium nitroprusside 50 and 100µM, in compared to 0µM treatment 32.73% and

37.24% increased leaf nitrogen. Also, leaf nitrogen, under foliar application of melatonin 50μM and 100μM in compared to 0μM treatment 9.61% and 23.72% increased. Leaf phosphorus in 40°C temperature in compared to 25°C temperature 41.67% decreased. Also, leaf phosphorus under conditions of foliar application of melatonin 100uM and sodium nitroprusside 100uM in compared to 0uM foliar spray (control) 11.11 and 14.81% increased. Leaf potassium under 40°C temperature conditions, sprayed with melatonin 50μM, melatonin 100μM, sodium nitroprusside 50μM and sodium nitroprusside 100μM in compared to 0uM foliar spray (control) 55.20%, 72%, 25.60% and 70.40% increased, respectively. Leaf calcium values at 40°C temperature in compared to 25°C, 11.49% decreased. leaf magnesium values with melatonin foliar treatment 100μM in compared to 0μM foliar application 0.40% increased. Foliar treatment with 50uM and100uM melatonin in compared to 0μM foliar spraying, 23.15% and 32.06% leaf zinc levels increased, respectively.

Foliar spraying with 100μM sodium nitroprusside in compared to 0μM foliar spraying caused a 17.96% increase in leaf iron levels. leaf manganese under 40°C temperature conditions with application of 50μM and 100μM sodium nitroprusside in compared to foliar spraying with 0μM, 25.61% and 45.53% increased. leaf copper with foliar spraying with 100μM sodium nitroprusside in compared to 0μM foliar spraying (control) 202.98% increased. According to melatonin and sodium nitroprusside physiological effects, it seems that the leaves chlorophyll amounts increasing increases the photosynthetic capacity, which in turn increases the growth, which increases the nutrients absorption from roots and its transfer to the air organ. Melatonin stimulates the biosynthesis of indole acetic acid (IAA) and thus stimulates root growth (Bajwa et al., 2014). The roots growth and development will increase the absorption of water and nutrients. Sarropoulou et al. (2012) reported that melatonin treatment, in addition to being able to expand the root system, is to increase the porphyrin and chlorophyll biosynthesis. In a research, it was found that 100μM and 200μM melatonin foliar spraying weekly, increased the nutrients absorption in strawberry (Zahedi et al., 2020).

5. Conclusions

Based on the results of this study, high temperature stress caused morphological damage such as twisting, chlorosis and necrosis of leaves. Foliar application of sodium nitroperoxide increased the height of the plant. Sprayed with melatonin (50μM and 100μM) increased the shoots fresh weight without fruit (FWPWF) in compared to other foliar application treatments. Also, with 50μM and 100μM melatonin foliar application improved the leaves relative water content in compared to foliar spray of 0μM (control).

Foliar spraying with 50μM and 100μM sodium nitroperoxide at 40°C temperature in compared to 0uM foliar spraying caused a decrease of 15.16% and 20.99% in the leave hydrogen peroxide amounts, respectively. Also, leaf nitrogen with foliar application of 50μM and 100μM sodium nitroprusside in compared to 0uM foliar spraying 32.73% and 37.24% increased, respectively. Also, leaf phosphorus under the conditions of foliar spraying with 100μM melatonin and 100μM sodium nitroprusside, in compared to 0μM foliar spraying (control), 11.11% and 14.81% increased, respectively. Leaf manganese content under of 40°C temperature, foliar spraying with 50μM and 100μM sodium nitroprusside in compared to 0μM foliar spraying (control), of 25.61% and 45.53% increased, respectively.

Reference

Afreen, F., Zobayed, S. & Kozai, T. (2006). Melatonin in spectral quality of light and UV-B radiation. Journal of Pineal Research, 41(2), 108-115

Alam, M.N., L. Zhang, L. Yang, M.R Islam, Y. Liu, H. Luo, P. Yang, Q. Wang, and Z. Chan. 2018. Transcriptomic profiling of tall fescue in response to heat stress and improved thermotolerance by melatonin and 24-epibrassinolide. BMC Genom. 19:1–14.

AL-Huqail, A.A., S.A. AL-Rashed, M.M. Ibrahim, G.A. EL-Gaaly, and M.I. Qureshi. 2017. Arsenic induced eco-physiological changes in Chickpea (Cicer arietinum) and protection by gypsum, a source of sulphur and calcium. Sci. Hort. 217:226–233.

AOAC. 2000. Official methods of analysis.17th ed. Association of official analytical chemists. Washington, DC
Arora, A., R.K. Sairam, and G.C. Srivastava. 2002. Oxidative stress and antioxidative system in plants. Curr. Sci. 82:1227–1238.
Arun, M., Naing, A.H., Jeon, S.M., Ai, T.N., Aye, T. and. Kim, C.K. 2017. Sodium nitroprusside stimulates growth and shoot regeneration in chrysanthemum. Horticulture, Environment and Biotechnology, 58(1): 78-84.
Ashraf, M., Karim, F. and Rasul, E. (2002) Interactive effects of gibberlic acid and salt stress on growth, ion accumulation and photosynthetic capacity of two-spring wheat cultivars differing in salt tolerance. Plant Growth Regulatiaon 36: 49-59.
Augustin M A, Osman A, Azudin M O and Mohamed S A (1998) Physico-Chemical Changes in muskmelons (Cucumis melo, L.) during Storage. Pertanika. 11(2):203-209.
Bajwa, V.S., Shukla, M.R., Sherif, S.M., Murch, S.J. and Saxena, P.K. 2014. Role of melatonin in alleviating cold stress in Arabidopsis thaliana. J. Pineal. Res.56: 238-245.
Baker, N. R. & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. Journal Experimental of Botany 55, 607-621.
Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39:205–207.
Beers, R.F, Sizer, I.W, 1952, Aspectrometric method for measuring the breakdown of the hydrogen peroxide by catalase,The Journal of biological chemistry, 195, 133-140.
Cakmak, I. and H. Marschner.1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 98:1222–1227.
Campos, C.N., Ávila, R.G., de Souza, K.R.D., Azevedo, L.M., Alves, J.D., 2019. Melatonin
reduces oxidative stress and promotes drought tolerance in young Coffea arabica L. plants. Agric. Water Manag. 211, 37–47.
Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. G. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10: 178-182.
Chen, Q., Qi, W. B., Reiter, W., Wei, W. & Wang, B. M. (2009). Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of
Brassica juncea. Journal of Plant Physiology, 166, 324-328.
Ding, F., B. Liu, and S. Zhang. 2017. Exogenous melatonin ameliorates cold-induced damage in tomato plants. Sci. Hort. 219:264–271.
Ding, X., Y. Jiang, L. He, Q. Zhou, J. Yu, D. Hui, and D. Huang. 2016. Exogenous glutathione improves high root-zone temperature tolerance by modulating photosynthesis, antioxidant and osmolytes systems in cucumber seedlings. Sci. Rep. 6:1–12.
Dong, Y., L. Xu, Q. Wang, Z. Fan, J. Kong, and x. Bai. 2014. Effects of exogenous nitric oxide on photosynthesis, antioxidative ability, and mineral element contents of perennial ryegrass under copper stress.J. Plant Interact. 9:402–411.
Farooq. M., Basra, S.M.A., Wahid, A., Rehman ,H., 2009. Exogenously applied mitric oxide enhances the drought tolerance in fine grain aromatic rice (Oryza sativa L.). Journal of Agronomy and Crop Science. 195, 254-261.
Giannopolitis, C.N, Ries, S.K, 1977. Superoxide dismutase I. Occurrence in higher plants, Plant Physiology, 59, 309-314.
Gomez-Ros, L.V., Gabaldon, C., Nunez-Flores, M.J.L., Gutierrez, J., Herrero, J., Zapata, J.M., Sottomayor. M,.Cuello, J. and Barcelo, A.R. 2012. The promoter region of the Zinnia elegans basic peroxidase isoenzyme gene contains cis-elements responsive to nitric oxide and hydrogen peroxide. Planta, 236(2): 327-342.
Graziano ,M., M.V. Beligni, and L. Lamattina. 2002. Nitric oxide improves internal iron availability in plants.Plant Physiol. 130:1852–1859.
Gratani, L. 1992. A non-destructive method to determine chlorophyll content of leaves. Photosynthetica,26, 469–473.
Hammerschmidt, R.E, Nuckles, E, Kuc, J,1982, Association of enhanced peroxidaseactivity with induced systemic resistance of cucumber to Colletotrichum lagenarium,Physiol. Plant Pathol, 20,73-82.
Hemati, E., Daneshvar, M.H. and Heidari, M. 2019. The roles of sodium nitroprusside, salicylic acid, and methyl jasmonate as hold solutions on vase life of Gerbera jamesonii 'Sun Spot'. Advances in Horticultural Science, 2(33):187-19.
Iyer, S. and A. Caplan. 1998. Products of proline catabolism can induce osmotically regulated genes in rice. Plant Physiol. 116:203–211.
Jahan, M.S., S. Shu, Y. Wang, Z. Chen, M. He, M. Tao, J. Sun, and S. Guo. 2019. Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. BMC Plant Biol. 19:1–16.
Janas, K.M. and Posmyk, M.M., 2013. Melatonin, an underestimated natural substance with great potential for agricultural application. Acta Physiologiae Plantarum, 35(12): 3285-3292.

Jiang, C., Cui, Q., Feng, K., Xu, D., Li, C., Zheng, Q., 2016. Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. Acta Physiol. Plant. 38, 82.
Jamali, B., S. Eshghi, and B. Kholdebarin. 2014. Response of strawberry 'Selva' plants on foliar application of sodium nitroprusside (nitric oxide donor) under saline conditions. J. Hort. Res. 22:139–150.
Kaijv, M., Sheng, L. and Chao, C. (2006) Antioxidation of flavonoids of green rhizome. Journal Food Science 27:110-115.
Kaya, A. and Z.B. Doganlar. 2019. Melatonin improves the multiple stress tolerance in pepper (Capsicum annuum). Sci. Hortic. 256:108509.
Kostopoulou, Z., Therios, I., Roumeliotis, E., Kanellis, A.K., Molassiotis, A., 2015. Melatonin combined with ascorbic acid provides salt adaptation in Citrus aurantium L. seedlings. Plant Physiol. Biochem. 86, 155–165
Lal, P.N., T. Mitchell, P. Aldunce, H. Auld, R. Mechler, A. Miyan, L.E. Romano, and S. Zakaria. 2012. National systems for managing the risks from climate extremes and disasters. In: Managing the risks of extreme events and disasters to Advance climate change adaptation (Field, C.B., V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (eds.). A special report of working groups I and II of the intergovernmental panel on climate change (IPCC). Cambridge University Press, Cambridge, UK, and New York, NY, USA, pp. 339-392.
Lamattina, L., C. García-Mata, M. Graziano, G. Pagnussat. 2003. Nitric oxide: The versatility of an extensive signal molecule. Annu. Rev. Plant Biol. 54:109–136.
Li, C., Tan, D.X., Liang, D., Chang, C., Jia, D. and Ma, F., 2014. Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two Malus species under drought stress. Journal of Experimental and Botany66: 680-669.
Loreto, F. and V. Velikova. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 127:1781–1787.
Maxwell K., and Johnson G.N. 2000. Chlorophyll fluorescence a practical guide. J. Expt. Bot. 51: 659-668.
Marvasi, M. 2017. Potential use and perspectives of nitric oxide donors in agriculture. Journal of the Science of Food and Agriculture, 97(4): 1065–1072.
McCready, R. M., Guggolz, J., SilvieraV. & Owens, H. S 1950. Determination of starch and amylose in vegetables. Analytical Chemistry, 22 (9).
McDonald S, Prenzler P D, Autolovich M and Robards K (2001) Phenolic content and antioxidant activity of olive extracts. Food Chemistry.73:73-84.
McNellie, J.P, J. Chen, X. Li, and J.Yu. 2018. Genetic mapping of foliar and tassel heat stress tolerance in maize. Crop Sci. 58:2484–2493.
Miliauskas G, Venskutonis P R and Van Beek T A (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 85: 231-237.
Oloumi, H., Nasibi, H. & Mozaffari, H. (2018). Investigation of the growth rate and secondary metabolites content of Lepidium sativum under exogenous melatonin treatment. Journal of Nova Biologica Reperta, 5(2), 144-154
Parankusam, S., S.S. Adimulam, P. Bhatnagar-Mathur, and K.K. Sharma. 2017. Nitric oxide (NO) in plant heat stress tolerance: Current knowledge and perspectives. Front. Plant Sci. 8:1–18.
Parankusam, S., S.S. Adimulam, P. Bhatnagar-Mathur, and K.K. Sharma. 2017. Nitric oxide (NO) in plant heat stress tolerance: Current knowledge and perspectives. Front. Plant Sci. 8:1–18.
Rayan, J. R., Estefan, G. and Rashid, A. (2001) Soil and Plant Analysis Laboratory Manual. 2nd Ed. ICARDA, Syria.
Ritchie, S. W., and Nguyen, H. T. 1990. Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Science, 30: 105-111.
Reiter, R. J., Tan, D. X. and Galano, A. (2014) Melatonin reduces lipid peroxidation and membrane viscosity. Frontier in Physiology 5: 1-4.
Saunders, J.A. and Mcclure, J.W. 1974.The suitability of a quantitive spectrophotometric assay forphenylalanine ammonia lyase activity in barely, buckwheat and pea seedlings.Journal of Plant Physiology, 54: 412-413.
Shafeiee ,M. and P. Ehsanzadeh. 2019. Physiological and biochemical mechanisms of alinity tolerance in several fennel genotypes: Existence of clearly-expressed genotypic variations. Ind. Crops Prod. 132:311–318.
Siddiqui, M.H., M.H. Al-Whaibi, and M.O. Basalah. 2011. Role of nitric oxide in tolerance of plants to abiotic stress. Protoplasma, 248:447–455.
Sarropoulou, V.N., Dimassi-Theriou, K., Therios, I. and Koukourikou-Petridou, M., 2012. Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (Prunus avium×Prunus cerasus). Plant Physiology and Biochemistry, 61: 162-168.
Shiferaw, B., and D.A. Baker. 1996. An evaluation of drought screening techniques for Eragrostis tef. Tropical Science. 36: 74-85.
Siddiqui, M.H., M.H. Al-Whaibi, and M.O. Basalah. 2011. Role of nitric oxide in tolerance of plants to abiotic stress. Protoplasma, 248:447–455.

Tian, X. R. and Lei, Y. B. (2007) Physiological responses of wheat seedling to drought and UV-B Radiation. Effect of exogenous sodium nitroprusside application. Russian Journal of Plant Physiology 54: 763-769.
Turk, H., Erdal, S., Genisel, M., Atici, O., Demir, Y. and Yanmis, D., 2014. The regulatory effect of melatonin on physiological, biochemical and molecular parameters in cold-stressed wheat seedlings. Plant Growth Regulation, 74(2): 139-152.
Uchida, A., A.T. Jagendorf, T. Hibino, T. Takabe, and T. Takabe. 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci. 163:515–523.
Yin, H., Q. Chen, and M.Yi. 2008. Effects of short-term heat stress on oxidative damage and responses of antioxidant system in Lilium longiflorum. Plant Growth Regul. 54:45–54.
Wang, P., Sun, X., Xie, Y., Li, M., Chen, W., Zhang, S., Liang, D. and Ma, F., 2014. Melatonin regulates proteomic changes during leaf senescence in Malus hupehensis. Journal of Pineal Research, 57: (3) 291-307.
Wei, W., Li, Q. T., Chu, Y. N., Reiter, R. J., Yu, X. M., Zhu, D. H., Zhang, W. K., Ma, B., Lin, Q& .Zhang, J. S. (2015). Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. Journal of Expression and Biotechnology, 66, 695–707.
White, R. E. (1976) Studies on the mineral ion absorption by plant, The interaction of aluminium phosphate and pH on the growth of Medicago sativa. Journal of Plant and Soil 46: 195-208.
Wu, X., W. Zhu, H. Zhang, H. Ding, and H.J. Zhang. 2011. Exogenous nitric oxide protects against salt-induced oxidative stress in the leaves from two genotypes of tomato (Lycopersicom esculentum Mill.). Acta Physiol. Plant. 33:1199–1209.
Xu, J.; Zhang, M.; Liu, X.; Lu, G.; Chi, J.; Sun, L. Extraction and antioxidation of anthocyanin of black soybean seed coat. Trans. Chin. Soc. Agric. Eng. 2005, 21, 161–164.
Xu,W., S.Y. Cai, Y. Zhang, Y. Wang, G.J. Ahammed, X-J. Xia, K. Shi, Y-H. Zhou, J-Q. Yu, R.J. Reiter, and J. Zhou. 2016. Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. J. Pineal Res. 61:457–469.
Yang. W., Y. Sun, S. Chen, J. Jiang, F. Chen, W. Fang, and Z. Liu. 2011. The effect of exogenously applied nitric oxide on photosynthesis and antioxidant activity in heat stressed chrysanthemum. Biol. Plant. 55:737–740.
Yin, H., Q. Chen, and M.Yi. 2008. Effects of short-term heat stress on oxidative damage and responses of antioxidant system in Lilium longiflorum. Plant Growth Regul. 54:45–54.
Zahedi, S. M., Hosseini, M. S., Abadía, J. and M.Marjani (2020). Melatonin foliar sprays elicit salinity stress tolerance and enhance fruit yield and quality in strawberry (Fragaria× ananassaDuch.). Plant Physiology and Biochemistry, 149: 313-323.
Zhang, J., Y. Shi, X. Zhang, H. Du, B. Xu, and B. Huang. 2017. Melatonin suppression of heat-induced leaf senescence involves changes in abscisic acid and cytokinin biosynthesis and signaling pathways in perennial ryegrass (Lolium perenne L.). Environ. Exp. Bot. 138:36–45.
Zhang, N., Sun, Q., Zhang, H., Cao, Y., Weeda, S ,.Ren, S.H. and Guo, Y.D., 2015. Roles of melatonin in abiotic stress resistance in plants. Journal of Experimental of Botany, 66: 647-56.
Zhou. Z., K. Guo, A. Elbaz, and Z. Yang. 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of Medicago sativa. Environ. Exp. Bot. 65:27–34.