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Effects of Melatonin and Putrescine on the Activity of Antioxidant Enzymes of Nectarine Redgold Cultivar under Cold Storage

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ABSTRACT

Under storage conditions, oxidative stress prompts plant cells to enhance their antioxidant system to neutralize reactive oxygen species. Key antioxidant enzymes, including catalase, superoxide dismutase (SOD), and peroxidase, serve as protective mechanisms against oxidative damage, with their activity gradually increasing under cold stress. This study investigated the effects of melatonin and putrescine on enzymatic activity in 'Redgold' nectarines using a factorial storage experiment based on a completely randomized design with three replications. The factors included storage duration (control: first d, 10, 20, and 30 d) and varying concentrations of melatonin (control, 50, 100, and 150 µM) and putrescine (control and 2.5 mM). Throughout different storage stages, researchers assessed the activity of antioxidant enzymes (SOD, ascorbate peroxidase [APX], and phenylalanine ammonia-lyase [PAL]), along with malondialdehyde (MDA) levels, electrolyte leakage, and total soluble solids (TSS). The results revealed that the application of melatonin and putrescine significantly enhanced enzymatic activity in 'Redgold' nectarines compared to the control. Notably, the combination of 150 µM melatonin and 2.5 mM putrescine yielded the most favorable outcomes.

Introduction

Nectarine (*Prunus persica*) belongs to the Rosaceae family and the Prunoideae subfamily. The 'Redgold' cultivar is an exotic and highly popular fruit due to its rich composition of vitamins, phenols, flavonoids, and anthocyanins. However, without proper postharvest management, the fruit deteriorates rapidly, rendering it unusable (Durst and Weaver, 2013; Heydarnajad Giglou et al., 2024). The quality of freshly harvested fruit is influenced by both internal factors—such as morphological, physiological, and biochemical mechanisms, genotype, and fruit type—and external factors, including storage temperature, humidity, handling methods, and chemical treatments (Putnik et al., 2017). Cold storage, while essential for preservation, negatively affects the physiology, biochemistry, and molecular biology of plants. Low temperatures can damage cell membranes, leading to increased malondialdehyde (MDA) levels—an indicator of membrane stability loss—resulting in membrane degradation and disrupted ion homeostasis (Beck et al., 2004; Hou et al., 2010; Heydarnajad Giglou and Torabi Giglou, 2023).

One of the most critical biochemical responses to cold and frost stress is the overproduction of reactive oxygen species (ROS) in chloroplasts and mitochondria. These free radicals form during key processes such as photosynthesis and photorespiration and interact destructively with essential macromolecules and enzymes. This oxidative damage disrupts the plant's metabolism, ultimately leading to cell death (Distelbarth et al., 2012; Heydarnejad et al., 2018). In response to oxidative stress caused by low temperatures, plants activate their antioxidant system to neutralize ROS. Key antioxidant enzymes—including catalase,

superoxide dismutase (SOD), and peroxidase—play a protective role, although their activity increases gradually under cold stress (Ao et al., 2013; Baek and Skinner, 2003; Shi et al., 2015).

Melatonin treatment has been shown to enhance plant resistance to abiotic stress, thereby improving physiological function and increasing crop yield (Posmyker et al., 2009; Shu et al., 2012; Han et al., 2016; Heydarnajad Giglou et al., 2024). As a polar molecule with diverse physiological and cellular functions (Retter et al., 2010, 2013), melatonin was first identified in 1958 as a neurohormone that regulates biological rhythms by being secreted into the bloodstream (Carrillo-Vico et al., 2013). It plays a vital role in modulating sleep cycles, mood, body temperature, appetite, sexual behaviors, and immune responses. Its presence in higher plants was discovered in 1995 (Hatori and Panda, 2010), and recent studies have confirmed that melatonin is widely distributed in plant tissues, including leaves, roots, stems, fruits, and seeds (Retter et al., 2013; Carrillo-Vico et al., 2013).

Polyamines, a class of biodegradable compounds with low molecular weight and aliphatic nitrogen groups, are essential for cell growth and differentiation. Among them, putrescine is one of the most common and is known as an anti-aging agent due to its ability to slow fruit softening and aging by inhibiting ethylene production (Carrión-Antoli et al., 2022). Research has demonstrated that externally applied polyamines help maintain fruit firmness and nutritional properties during storage (Shelp et al., 2012). In particular, spraying putrescine and spermidine on *Redgold* nectarines has been shown to reduce ethylene production, delay tissue softening, preserve titratable acidity, and prevent excessive accumulation of soluble solids (Wunderlichová et al., 2014; Haghshenas et al., 2020).

Extensive research has provided valuable insights into the presence of melatonin and putrescine in plants, their physiological functions in plant cells, and methods for their extraction and accumulation. In plants, melatonin is referred to as phytomelatonin. Recent studies have identified its role as a biological recovery agent, aiding in the chemical detoxification of plants in stressful soils, making it a promising candidate for plant remediation processes. Additionally, melatonin has been proven to play a protective role against both biotic and abiotic stresses, regulate plant rooting and growth, influence various morphological traits, and align with biological rhythms and gene expression (Wilhelmsen et al., 2013).

One of melatonin's most notable properties is its function as a potent antioxidant. Its ability to scavenge reactive free radicals—including hydroxyl radicals, superoxide anions, singlet oxygen, hydrogen peroxide, hydrochloric acid, nitric oxide, peroxynitrite anions, and fatty peroxyl radicals—has been widely documented under both in vitro and in vivo conditions. This antioxidant capacity allows melatonin to regulate redox enzymes in animal tissues and enhance the activity of key antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase, as well as enzymes like nitric oxide synthase and lipoxygenase. These functions contribute to melatonin's anti-inflammatory, anti-stress, and other protective roles (Arnao et al., 2015). Melatonin serves as a crucial defense against cold stress by enhancing the activity of antioxidant enzymes such as catalase, superoxide dismutase, peroxidase, and polyphenol oxidase. By boosting these enzymatic activities, melatonin increases cellular tolerance to low-temperature stress, helping plants endure conditions below their usual tolerance threshold (Mukherjee et al., 2014; Giglou et al., 2023).

Material and methods

Plant materials and experimental design

In this study, fresh *Redgold* nectarine fruits were harvested at the commercial maturity stage from orchards in Mahabad City (Longitude: 45°43′ E, Latitude: 36°46′ N). Fruits of uniform size and color, free from mechanical damage, were selected for the experiment.

The study followed a factorial experiment based on a completely randomized design with three replications. Experimental factors included varying concentrations of melatonin (0, 50, 100, and 150 µM), putrescine (0 and 2.5 mM), and different storage durations (0, 10, 20, and 30 d). The selected fruits were immersed in the designated treatments for five min, then packaged (15 fruits package^{-1}) and stored in a cold room at 4 °C with 90% relative humidity for up to 30 d.

TSS

A handheld refractometer (model MT-098P8A) was used for measuring the amount of total soluble solids (TSS) of 'Redgold' nectarine at 25 °C and expressed as °Brix.

Total carbohydrate content (TC)

The total carbohydrate (TC) content in dry plant tissue was determined using the phenol–sulfuric acid method. First, 0.1 g of dried tissue was added to 10 mL of 70% ethanol and stored at 4 °C for one week, with daily stirring to facilitate the separation of dissolved carbohydrates from the remaining tissue. After one week, 1 mL of the supernatant from each sample was taken and diluted with distilled water to a final volume of 2 mL. Then, 1 mL of 5% phenol and 5 mL of 48% sulfuric acid (H_2SO_4) were added, and the mixture was vortexed thoroughly. The test tubes were then placed in a hot water bath at 65 °C for 20 min before being cooled to room temperature

to halt the reaction. Absorbance was measured using a spectrophotometer at a wavelength of 485 nm. A standard curve was established using glucose solutions with concentrations ranging from 0 to 10 mg 100 mL^{-1}. The TC content in the samples was calculated in mg g^{-1} dry weight (Irigoyen et al., 1992), considering the dry weight of the tissue. Statistical analyses were performed to interpret the obtained data.

Electrolyte leakage

Electrolyte leakage was measured using the method of Yang and Wu (2011). Four g of '*Redgold'* nectarine slices were first rinsed in deionized water for 2–3 min. Then, 15 slices were placed in 50 mL of deionized water and shaken at 100 rpm for 30 min. The initial electrical conductivity was recorded using a conductivity meter. To determine total conductivity, the same samples were placed in a boiling water bath for 10 min. After cooling to ambient temperature, electrolyte leakage was measured again. Relative electrolyte leakage was expressed as a percentage of the total conductivity.

Malondialdehyde (MDA)

Malondialdehyde (MDA) content was measured using the method of Zhang et al. (2014). First, 4 g of fruit tissue was homogenized in 0.1% trichloroacetic acid (TCA) and then centrifuged at 12,000 rpm for 10 min. The supernatant was collected, and 2 mL of the extract was mixed with 4 mL of 20% TCA containing 0.5 g of thiobarbituric acid (TBA). The mixture was heated in a bain-marie at 100 °C for 20 min, then immediately cooled on ice. After 10 min of centrifugation at 3,000 rpm, absorbance was measured at 532 nm, with the non-specific absorbance at 600 nm subtracted. The MDA content was calculated using the following formula:

$$MDA\ (mmol\ kg^{-1}\ FW) = \frac{6.45 \times (OD_{532}532 - OD_{600}) - 0.56 \times OD_{450}] \times Vt \times Vr}{Vs \times m}$$

Vt: is the total volume of supernatant (mL)

Vr: volume of the reaction mixture
Vs: volume of the sample
m: mass of the sample

Measuring the activity of ascorbate peroxidase (APX)

The reaction mixture for ascorbate peroxidase (APX) activity measurement contained 1500 µL of 50 mM phosphate buffer (pH 7), 600 µL of 0.1 mM EDTA, 400 µL of 0.5 mM ascorbic acid, 400 µL of 30% H_2O_2, and 100 µL of enzyme extract. In this reaction, ascorbate peroxidase catalyzes the oxidation of ascorbic acid by H_2O_2, producing dehydroascorbate. The reaction progress was monitored over time by measuring absorbance at 290 nm. An increase in absorbance indicates higher enzyme activity. APX enzyme activity was determined following the method of Ranieri et al. (2003), where one unit of APX was defined as the oxidation of 1 mM ascorbate min^{-1} mg^{-1} protein. The specific activity of the APX enzyme was reported in mM of enzyme (units) mg^{-1} protein.

Measurement of superoxide dismutase (SOD) enzyme activity

Superoxide dismutase (SOD) activity was assessed by measuring the inhibition of nitroblue tetrazolium (NBT) photoreduction by the SOD enzyme, following the method of Zhang et al. (2014). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin, and 100 µL of crude extract, making up a final volume of 3.0 mL. A control reaction was performed without the crude extract. The reaction was initiated by exposing the mixture to white light for 15 min at room temperature. After incubation, absorbance was measured at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit 50% of the photochemical reduction of NBT (Zhang et al., 2014).

Measuring the activity of phenylalanine ammonialyase enzyme

Phenylalanine ammonia-lyase (PAL) catalyzes the conversion of phenylalanine to cinnamic acid. To assess PAL activity, 300 mg of fresh tissue was homogenized in 6.5 mL of 50 mM Tris-HCl buffer (pH 7.8) containing 15 mM mercaptoethanol, using a chilled mortar. The resulting solution was immediately centrifuged at 9500 rpm at 4 °C. Enzyme activity was estimated based on the amount of cinnamic acid produced, using phenylalanine as the enzyme precursor. The assay involved measuring the inhibition of superoxide formation by nitroblue tetrazolium (NBT), which is affected by PAL activity.

To measure PAL activity, 1 mL of extraction buffer was mixed with 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of double-distilled water, and 0.1 mL of enzyme extract. The mixture was incubated at 37 °C for 1 h. To stop the reaction, 0.5 mL of 6 M hydrochloric acid was added.

The reaction product was then extracted using 15 mL of ethyl acetate, after which the ethyl acetate was evaporated. The remaining solid residue was dissolved in 3 mL of 0.5 mM sodium hydroxide. The absorbance of the resulting solution was measured at 290 nm using a spectrophotometer. One unit of enzyme activity was defined as the production of one micromole of cinnamic acid h^{-1} mg^{-1} protein.

A standard curve of cinnamic acid was generated by preparing concentrations ranging from 0 to 100 µM and measuring their absorbance at 290 nm. The cinnamic acid content in the samples was then calculated using the linear equation derived from the standard curve (Wang et al., 1996).

Statistical analysis

The experimental data were analyzed using MSTAT-C and SAS software, with mean comparisons conducted using the LSD test at a 1% probability level. Graphical representations and certain calculations were performed using Microsoft Excel.

Results

TSS

The data on the effects of putrescine and melatonin (Fig. 1) on the total soluble solids (TSS) of red gold nectarine fruits revealed a significant difference in the results. While the amount of soluble solids decreased overall, the decrease was more pronounced in the control samples. The highest TSS level was observed in the samples treated with melatonin at 150 µM and putrescine at 2.5 mM (Fig. 1).

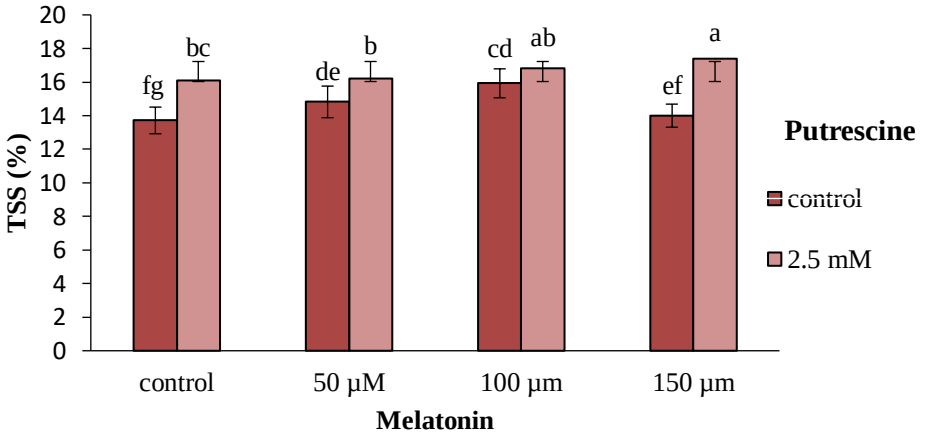

Fig. 1. Effects of melatonin and putrescine on the amount of total soluble solids (%) in red gold nectarine fruits under storage conditions.

Firmness and total carbohydrate (TC)

In examining the effects of the treatments, it was found that both putrescine and melatonin had a noticeable impact on tissue firmness and total carbohydrate content in the fruits. Specifically, with putrescine, the highest levels of tissue firmness (50.086 N cm^{-2}) and total carbohydrates (6.24 mg g^{-1}) were observed at a concentration of 2.5 mM. Additionally, under the influence of melatonin, the highest levels of tissue firmness and total carbohydrates were observed at concentrations of 150 and 100 µM, respectively. These values represented a 5.77% increase in tissue firmness and an 11.56% increase in total carbohydrates compared to the control samples (Table 1).

Table 1. Changes in firmness and total carbohydrates under the influence of putrescine and melatonin during storage.

Treat		Firmness (N m^2)	TC (mg g^{-1} DW)
Pt (µM)	Control	59.32^{b}	5.99^{c}
	2.5	58.086^{b}	6.24^{ab}
Melatonin (µM)	Control	57.36^{c}	5.88^{cd}
	50	57.74^{c}	6.12^{b}
	100	59.04^{b}	6.56^{a}
	150	60.67^{ab}	5.91^{c}
Storage times (d)	0	65.058^{a}	5.42^{d}
	10	60.96^{b}	6.042^{c}
	20	55.59^{c}	6.66^{a}
	30	53.199^{d}	6.33^{ab}

Regarding storage time, the study revealed that tissue firmness decreased during storage, showing a 22.29% reduction from the first d up to 30 d. In contrast, the total carbohydrate content increased during storage, with the highest level observed on d 20, which was 22.87% higher than on the first d of storage (Table 1).

Electrolyte leakage

The results indicated that putrescine at a concentration of 2.5 mM alone had the greatest effect (Fig. 2) on reducing electrolyte leakage at zero time. Melatonin, on the other hand, was most effective at high concentrations (150 µM) during storage. The combined application of putrescine and melatonin at concentrations of 2.5 mM and 50 µM, respectively, significantly controlled the reduction of alcohol leakage in the fruit (Fig. 2b). Overall, as storage time increased, the control samples showed an increase in the electrolyte leakage index.

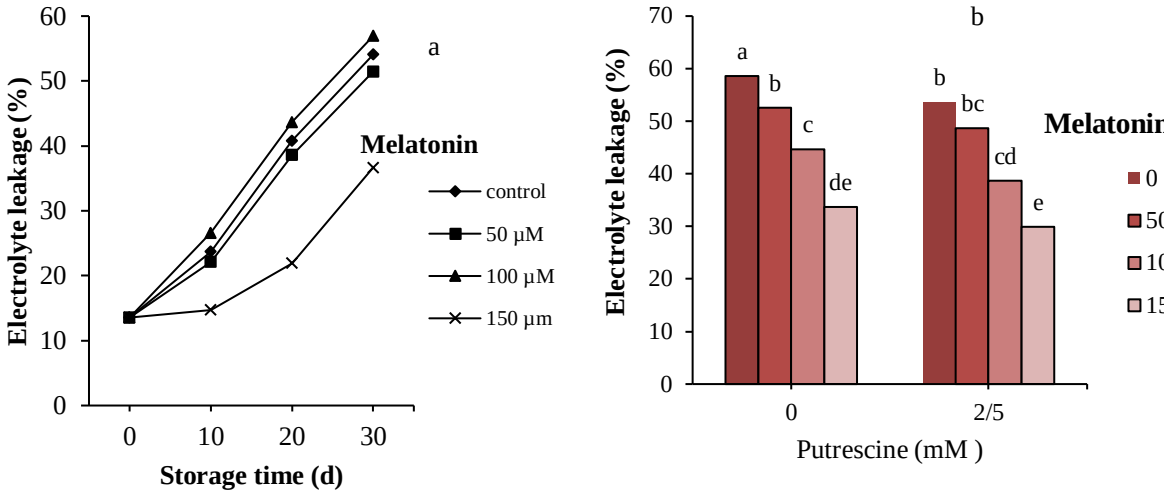

Fig. 2. Effects of (**a**) melatonin; (**b**) and putrescine on the amount of electrolyte leakage (%) in red gold nectarine fruits under storage conditions.

Ascorbate peroxidase enzyme (APX)

The investigation of APX activity revealed that melatonin at 150 µM and putrescine at 2.5 mM had a noticeable effect during the storage period of red gold nectarines from d zero to d 30 (Fig. 3a). Additionally, the application of putrescine at these two concentrations showed a significant effect during the early storage period (up to 10 d). However, in general, as storage time increased to 20 and 30 d, the enzyme activity decreased (Fig. 3b).

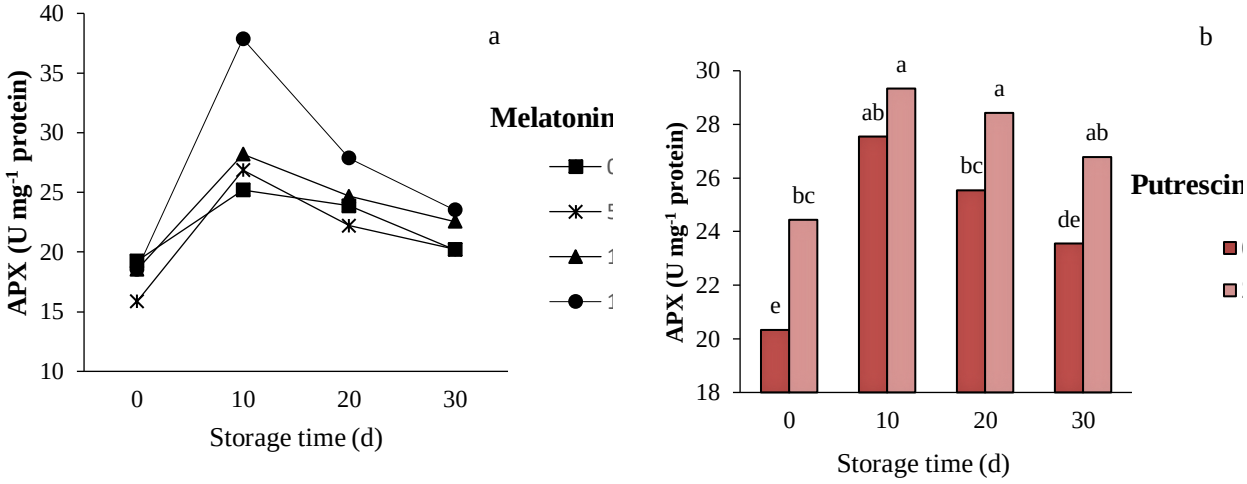

Fig. 3. Effects of (**a**) melatonin; (**b**) and putrescine on the amount of APX enzyme (U mg^{-1} protein) in red gold nectarine fruits under storage conditions.

Phenylalanine ammonialyase enzyme

The results indicated that the simultaneous application of melatonin at 150 µM and putrescine at 2.5 mM had a significant effect on the phenylalanine ammonia-lyase enzyme activity, leading to a decrease in this enzyme during storage. The highest enzyme activity, with a value of 1.132, was observed in the control treatment (Fig. 4).

Superoxide dismutase enzyme (SOD)

The results from the SOD analysis showed a combined effect of melatonin and putrescine, with the most notable effect observed at the third concentration of melatonin (150 µM) and the second concentration of putrescine (2.5 mM) (Fig. 5). From d zero to d 30 of storage, an increasing trend in SOD activity was observed (Fig. 5).

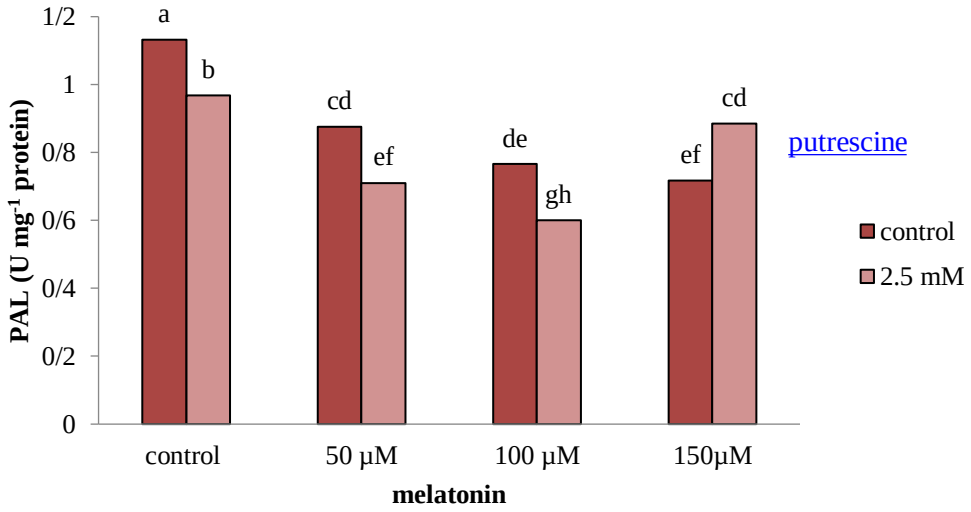

Fig. 4. Effects of melatonin and putrescine on soluble PAL enzyme (U mg^{-1} protein) in red gold nectarine fruits.

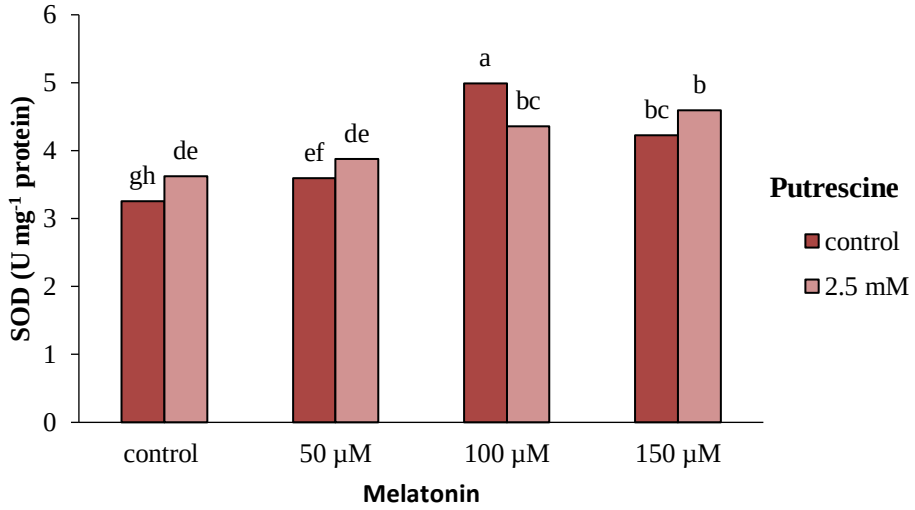

Fig. 5. Effects of melatonin and putrescine on soluble SOD (U mg^{-1} protein) enzyme in red gold nectarine fruits.

Malondialdehyde (MDA)

The results of this experiment showed that the greatest effect of the treatments on the amount of MDA during the 30 d storage of red gold nectarines occurred with the application of 2.5 mM putrescine, where MDA levels increased with storage time. Additionally, melatonin reduced MDA levels as its concentration increased. The simultaneous use of putrescine and melatonin, particularly at lower concentrations, had a more significant effect on the peroxidation of unsaturated fatty acids in phospholipids during storage. However, at higher concentrations, the amount of MDA decreased (Fig. 6).

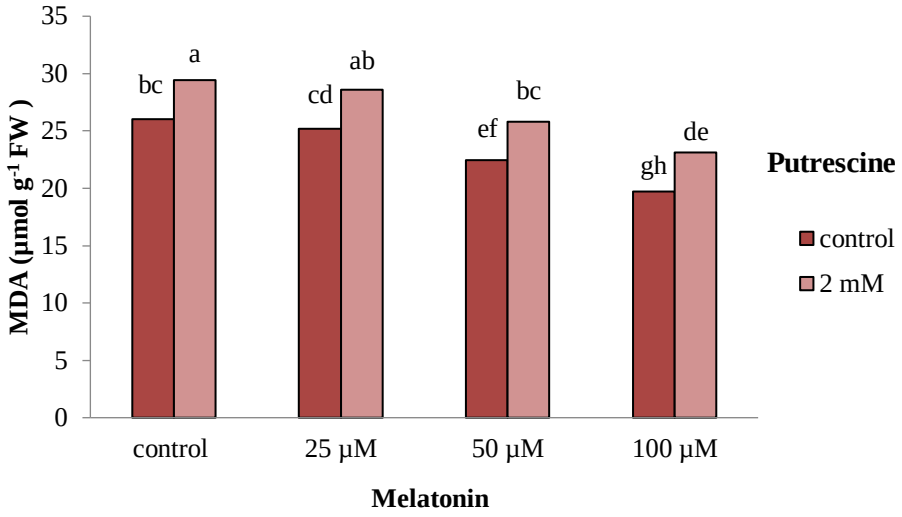

Fig. 6. Effects of melatonin and putrescine on soluble MDA (µmol g^{-1} FW**)** in red gold nectarine fruits during storage.

Discussion

Under stressful conditions, plants tend to increase their melatonin levels, and the external application of melatonin can effectively help plants resist stress since they can absorb and store it (Zhang et al., 2014). Melatonin directly scavenges H_2O_2, a key molecule involved in the aging process, which is associated with increased membrane permeability and the release of proteins and other cellular contents (Liang et al., 2018). Furthermore, melatonin enhances leaf membrane stability by reducing lipid oxidation (Ding et al., 2019) and improving cellular homeostasis (Gao et al., 2016). To mitigate the harmful effects of reactive oxygen species (ROS), such as H_2O_2, plants must balance their production and decomposition. One way plants address this challenge under stress is by utilizing enzymatic and non-enzymatic antioxidant systems to neutralize ROS (Sytar et al., 2017).

Mansouri et al. (2019) demonstrated that pre- and post-harvest treatment of strawberries with 1000 µM melatonin increased the activity of the PAL enzyme during the ripening process. Post-harvest results showed that fruits treated with 100 µM melatonin had better firmness during storage, which was associated with a higher TSS/TA ratio. Additionally, the antioxidant capacity of fruits harvested from melatonin-treated plants was higher compared to the control group during storage. Melatonin has been shown to increase the activity of antioxidant enzymes in plants subjected to various stresses, including salinity (Li et al., 2018), drought (Wang et al., 2014), oxidative stress (Afreen et al., 2006), and cold stress (Zhang et al., 2016). Reports indicate that melatonin treatment increases the activity of superoxide dismutase (SOD) and catalase enzymes in melon seeds exposed to cold stress (Zhang et al., 2016).

Excessive and uncontrolled accumulation of ROS is a primary cause of aging, and their destructive effects are a normal response to aging in plants (Khanna-Chopra, 2012). Melatonin can prevent these harmful effects across different organisms (Zhang et al., 2016). Tawakli et al. (2018) found that melatonin treatment led to the accumulation of phenols, flavonoids, and anthocyanins, thereby increasing the antioxidant capacity of cherry fruit extract. However, they also noted a negative effect of melatonin on soluble solids content. The efficacy of melatonin treatment was concentration-dependent, with concentrations of 150 and 100 mM L^{-1} proving more effective than the 50 mM L^{-1} concentration.

Melatonin's antioxidant activities can be carried out through five mechanisms: direct scavenging of free radicals, stimulation of antioxidant enzymes (peroxidase, superoxide dismutase, catalase), enhancement of other antioxidants (ascorbic acid, soluble sugars, flavonoids), protection of enzymes from oxidative damage, and increasing mitochondrial electron transport chain efficiency, which reduces electron leakage and the production of free radicals (Gong et al., 2016). Ghorbani and Najafzadeh (2019) found that fruits treated with 200 mM melatonin exhibited increased levels of phenolic compounds (25 mg 100 g^{-1} fresh weight of gallic acid), soluble solids (4% Brix), and peroxidase and ascorbate peroxidase enzyme activity (20 units mg^{-1} protein), compared to other treatments and the control. Melatonin is a potent antioxidant, acting as a protective compound to inhibit free radical activity. Polyamines (PAs) are essential stress-regulating biomolecules and low molecular weight nitrogenous compounds that naturally occur in plants in various forms (Jahan et al., 2019). Exogenous polyamine treatments, such as putrescine and spermidine, have been shown to reduce frost damage by enhancing

antioxidant enzyme activity during the storage period in different apricot cultivars (Koushesh Saba et al., 2012; Gonzalez-Aguilar et al., 1998; Ataii et al., 2017). Qadri and Rostgar (2018) found that putrescine, when applied at the appropriate concentration, effectively prevented frost damage. Although putrescine did not significantly affect ion leakage in their experiment, the treated fruits exhibited higher antioxidant levels. Over the storage period, antioxidant levels decreased gradually, with the lowest malondialdehyde levels and frost damage observed in fruits treated with 0.5 mM putrescine.

In studies examining different concentrations of putrescine on the postharvest life of strawberries, apricots, peaches, and cherries, the storage period was significantly extended with the application of putrescine in all fruits. In cherries, putrescine increased soluble solids, whereas it reduced soluble solids in other fruits (Zakaei and Eznaeshari, 2017). Polyamines, such as putrescine, have been reported to improve postharvest life and fruit quality by maintaining tissue firmness, reducing ethylene production and water loss, delaying color changes, and enhancing fruit resistance to frost and mechanical damage (Valero et al., 2002).

Additionally, melatonin treatment at a concentration of 0.3 mM has been shown to increase total soluble solids (TSS) in sweet cherry cultivars, while also affecting the content of bioactive compounds such as total phenols and enhancing antioxidant properties (Carrión-Antoli et al., 2022).

Polyamines, such as putrescine, can act as inactivators of free radicals and protect cell membranes from oxidation, thereby enhancing membrane resistance (Martinez-Tellez et al., 2002). To counter the harmful effects of free radicals, plant cells develop an antioxidant system that includes enzymatic antioxidants like ascorbate peroxidase (APX) and catalase (CAT), as well as non-enzymatic antioxidants such as ascorbic acid, alpha-tocopherol (vitamin E), and others (Spinardi, 2005). In a study on the effect of different concentrations of putrescine at high temperatures on the postharvest life of Hayward kiwi fruit, it was observed that putrescine treatment significantly increased antioxidant capacity but did not affect soluble solids content. The results indicated that putrescine delayed the ripening process and extended the storage life of kiwi (Rohi et al., 2014).

Polyamines can deactivate free radicals and protect cell membranes from oxidation, thereby increasing membrane stability by raising internal polyamine levels. The antioxidant effect of polyamines is primarily attributed to their cationic properties, which enable them to scavenge free radicals and inhibit lipid peroxidation (Liu et al., 2007; Giglou et al., 2023). In gerbera flowers, all putrescine treatments helped maintain flower quality after harvest. The flowers treated with putrescine lasted for 4 to 8 d, and the treatment resulted in higher catalase enzyme activity, soluble solids, and phenylalanine ammonia-lyase enzyme levels, as well as lower ion leakage and malondialdehyde (Mohammadi et al., 2019).

In a study by Atai et al. (2016), treatment with 2 mM putrescine resulted in a reduction in ion leakage, less accumulation of malondialdehyde (MDA), lower H_2O_2 levels, and improvements in catalase and ascorbate peroxidase enzyme activity compared to the control treatment. This indicates that polyamines can act as free radical scavengers and protect cell membranes from oxidation, thus enhancing membrane resistance. Polyamine treatments can also increase the internal polyamine levels, contributing to membrane stability (Liu et al., 2007). An increase in ion leakage and MDA accumulation is one of the hallmarks of aging in plants (Shahri and Tahir, 2011). In Lisianthus flowers treated with 2 mM putrescine, the increasing trends of ion leakage and MDA accumulation, along with lipoxygenase enzyme activity, were significantly slowed, helping maintain membrane integrity (Ataei et al., 2016). Pre-harvest treatment with putrescine has also been reported to reduce ion leakage and MDA accumulation in Lilium cut flowers during their life span (Majidiyan, 2013).

Furthermore, it has been suggested that a decrease in the activity of ascorbate peroxidase is a key factor in initiating cell death (Hossain et al., 2006). The pre-harvest treatment with putrescine seems to positively impact the activity of catalase and ascorbate peroxidase enzymes, thereby delaying the aging process in Lisianthus cut flowers during the post-harvest stage (Ataei et al., 2017; Heydarnajad Giglou et al., 2024).

Melatonin, a potent regulator of cold stress and a free radical scavenger, plays a crucial role in promoting growth, signalling, and antioxidative responses in plants (Zhang et al., 2014). External application of melatonin has been shown to improve the ripening behavior of bananas by modulating ethylene production (Hu et al., 2017). In pear fruit, melatonin reduces chilling injury (CI) by inhibiting ethylene synthesis via nitric oxide regulation (Liu et al., 2019). Moreover, exposure to melatonin has been found to reduce cold damage and enhance antioxidant activity in tomato (Aghdam et al., 2019), peach (Gao et al., 2016), and grape (Xu et al., 2018). Melatonin also delays senescence and minimizes browning in the pericarp tissue of litchi fruit (Zhang et al., 2018).

Conclusion

The results of our research indicated that the effects of different melatonin and putrescine concentrations are influenced by storage time. Antioxidant enzymes such as catalase, superoxide dismutase, and

peroxidase play a crucial role in protecting against oxidative stress, and their activity gradually increased under cold stress. Our findings showed that applying putrescine and melatonin during storage significantly enhanced enzyme activity in Red Gold nectarine fruit compared to the control samples. Notably, the simultaneous use of melatonin and putrescine produced the best results, outperforming the control group.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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