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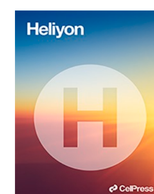

Research article

Effect of melatonin on the contents of fatty acids and antioxidants of saffron

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ABSTRACT

Early leaf senescence at the end of the growing season poses a significant challenge in saffron cultivation. While changes in leaf composition during senescence have been extensively documented in various plants, similar studies on saffron remain unexplored. Furthermore, there has been no investigation into the potential role of melatonin in delaying leaf senescence in saffron. This study aimed to examine the changes in saffron leaf composition and evaluate the effects of melatonin foliar application during the late growth stage. The research was conducted over two consecutive cropping years (2020–2021 and 2021–2022). In the first experiment, five concentrations of melatonin (0, 50, 100, 150, and 200 μM) were applied as foliar sprays to assess their effects on fatty acid composition and plant greenness. The second experiment involved varying melatonin concentrations and two application timings (124 and 131 days after germination) to study their impact on antioxidant enzyme activity. Both experiments were designed as factorial trials within a completely randomized block design with three replicates. The results demonstrated that treatment with 100 μM melatonin significantly increased the production of fatty acids, including C8:0 (67.60 %), C10:0 (98.66 %), C12:0 (40.73 %), and C18:0 (35.32 %) compared to the untreated control. Also, the highest activities of ascorbate peroxidase and catalase enzymes were observed with 100 μM melatonin applied 124 days after germination. On the same day, the highest total protein content was recorded with 50 μM melatonin, although it was not significantly different from the 100 μM treatment. In conclusion, the 100 μM melatonin treatment was found to be the most effective in enhancing plant greenness, modifying fatty acid composition, boosting antioxidant enzyme activity, and increasing total protein content. However, the timing of melatonin application emerged as a critical factor warranting careful consideration. These findings highlight the promising role of melatonin in improving the physiological and biochemical attributes of saffron plants.

1. Introduction

Saffron (*Crocus sativus* L.), a member of the *Iridaceae* family, is a highly valued medicinal plant. Numerous studies have confirmed

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its medicinal properties, including anxiolytic, anti-tumor, anticoagulant, *anti*-DNA damage, antidepressant, anti-diabetic, and anti-oxidant activities [1].

A significant challenge in saffron cultivation is the low yield per hectare, often attributed to early leaf senescence at the end of the growing season [2]. Leaf area index (LAI) decreases progressively during spring, culminating in leaf senescence between late June and late October [3]. According to Elahi Gharabaghlou et al. [4], saffron plants enter senescence approximately 170 days after flowering. While senescence, the final stage of leaf development, has been widely studied in other plant species, limited research exists on this process in saffron. Understanding the dynamics of saffron leaf senescence is critical to preventing sharp declines in photosynthesis and improving crop yield. While much of the previous research has focused on spectral responses in various species, studies specifically addressing saffron leaf composition during different aging stages remain scarce [5].

Leaf senescence is a genetically regulated and environmentally influenced process [6]. One of its hallmark symptoms is membrane degradation [7], typically associated with a decrease in membrane lipids and an accumulation of free fatty acids, driven by excessive reactive oxygen species (ROS). This ROS-induced lipid peroxidation damages unsaturated fatty acids (USFAs) in membrane lipids, compromising membrane integrity [8]. Cell membranes, including those of organelles such as mitochondria and peroxisomes, are rich in USFAs, making their protection against ROS essential for cell function and survival [9]. Plants have evolved sophisticated anti-oxidant systems to scavenge ROS, including enzymes such as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), gluta-thione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDAR).

Although antioxidant compounds and fatty acids have been documented in saffron tissues during dormancy, sprouting, and flowering stages [10–12], little is known about their presence and function during the final stages of saffron growth. USFAs, Partic-ularly, polyunsaturated fatty acids (PUFAs) are critical for preserving membrane fluidity and are recognized as key elements in plant defense against biotic and abiotic stresses [13,14]. The most common PUFAs in plants are C18 species: 18:1 (oleic), 18:2 (linoleic), and 18:3 (alpha-linolenic), where “m:n" denotes an FA with *m* carbon atoms and *n* cis-double bonds [15].

The antioxidant properties of *C. sativus* stigma are attributed to its phenolic content and active compounds such as safranal, cro-cetin, and carotenoids, all of which exhibit potent antioxidant activity [16,17]. Fatty acids and antioxidant enzymes are crucial bioactive substances in plants during late growth stages, coinciding with leaf senescence. For example, in tobacco (*Nicotiana tabacum*), fatty acid content declines from 37 mg. g^{-1} dry weight at week 10–15 mg. g^{-1} dry weight at week 14, with total fatty acid content per leaf reducing from 245 mg on day 37–135 mg on day 77 [18]. Similarly, Yang and Ohlrogge observed a significant decline (over 80 %) in fatty acid levels during leaf aging in species like *Arabidopsis*, *Brachypodium*, and *Switchgrass* [19]. Zhang et al. [20] reported that ROS generation during leaf senescence reduces antioxidant enzyme activity (e.g., CAT, SOD, and POD), leading to imbalances in ROS metabolism.

Melatonin (MEL; N-acetyl-5-methoxytryptamine) is a multifunctional endogenous molecule with potent antioxidant properties [18,21]. It acts as a direct antioxidant by stabilizing biological membranes, enhancing membrane fluidity, and counteracting lipid peroxidation under oxidative stress [22]. Additionally, melatonin indirectly boosts the activity of ROS-scavenging enzymes (e.g., SOD, CAT, APX, and POD) and maintains higher levels of other antioxidants, such as glutathione and ascorbic acid, compared to controls [23,24]. Researchers have also suggested that melatonin can improve fatty acid biosynthesis and mitigate oxidative damage during leaf aging [20,21,24]. Foliar application of melatonin has been shown to increase phospholipid and USFA content, stabilizing cell membranes [25]. Furthermore, melatonin regulates the transcription of enzymes, receptors, and transcription factors involved in plant hormone biosynthesis and catabolism [26]. Melatonin's anti-aging effects have been demonstrated in various crops, including apple [24,27], cucumber, maize [20], and rice [28]. It is known to delay leaf senescence by downregulating key senescence-related genes and enzymes involved in chlorophyll degradation [26,29]. This effect has been observed in Brassica vegetables such as broccoli (*Brassica oleracea* L.) [30]. Additionally, melatonin promotes secondary metabolism, enhancing the biosynthesis of glucosinolates and phenylpropanoids such as flavonoids and lignin [31]. Optimal melatonin concentrations vary depending on plant species, develop-mental stage, and treatment conditions [32].

Despite its proven benefits in other species, the impact of melatonin on delaying leaf senescence in saffron has yet to be investi-gated. This study seeks to explore the effects of melatonin foliar application during the late growth season on saffron leaf fatty acid composition and antioxidant enzyme activity.

2. Materials and methods

2.1. *Experimental site characteristics*

This experiment was conducted at the research field of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (longitude 51°19′ E, latitude 35°41′ N, and altitude 1352 m above sea level) during the cropping years 2020–21 and 2021-22. The soil analysis of the field was conducted before planting the saffron bulbs, and the soil characteristics are presented in Table 1S.

Meteorological data for two years, including average temperature and precipitation, were obtained from the National Meteoro-logical Organization. The average temperature and precipitation over the two years were 13.56 °C and 142.77 mm, respectively) Fig. 1S(. The precipitation from September 1, 2020, to April 20, 2021, was 104.75 mm, with an average temperature of 13.31 °C. On the other hand, the precipitation from September 1, 2021, to April 13, 2022, was 180.8 mm, with an average temperature of 13.79 °C. The daily maximum, minimum, and average temperatures, as well as daily rainfall during the saffron growth seasons in the years 2020–21 and 2021-22, are illustrated in Figs. 1 and 2, showing the monthly mean daily temperature (maximum and minimum) and daily rainfall during the saffron growth and planting periods in 2020–21 and 2021-22)Fig. 2S).

Table 1
Analysis of variance (mean square) of the effect of different concentrations of melatonin on the percentage of fatty acids in saffron leaves in 2021.

SOV	df	C8: 0	C10: 0	C12: 0	C14: 0	C16: 0	C16:1	C18:0	C18: 1	C18: 2	C18: 3	C20: 4	C22: 0	PUFA/MUFA	PUFA/SFA	UFA/SFA
Block	2	36.082**	11.484**	3.674^{ns}	0.431^{ns}	0.029^{ns}	0.002^{ns}	0.137^{ns}	0.352^{ns}	29.395^{ns}	62.026^{ns}	3.060^{ns}	0.148^{ns}	2.120^{ns}	0.731^{ns}	0.896^{ns}
Melatonin	4	13.831*	5.813**	19.470**	0.075^{ns}	13.958^{ns}	1.185^{ns}	1.221*	2.007^{ns}	3.547^{ns}	74.727*	3.640^{ns}	1.778*	1.305^{ns}	0.369^{ns}	0.494^{ns}
Error	8	2.681	0.632	1.547	0.409	5.806	0.424	0.306	3.147	8.456	17.995	1.121	0.479	1.567	0.133	0.163
CV (%)		32.013	27.698	28.146	21.357	13.877	19.239	21.763	29.487	13.981	15.281	25.784	25.844	21.848	24.734	22.987

ns, *, ** indicate non-significance and significance at 5 % and 1 % levels, respectively.
SOV: Source of variation, df: Degree of freedom, CV: Coefficient of variation
PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono-unsaturated Fatty Acid, USFA: Unsaturated Fatty Acid and SFA: Saturated Fatty Acid

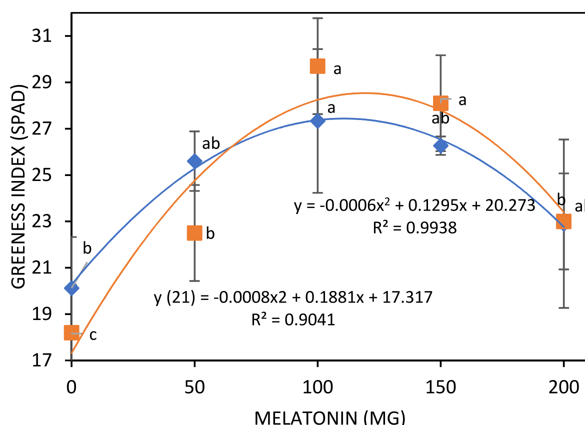

Fig. 1. Effects of melatonin on the greenness index of saffron. Significant differences at $P \leq 0.05$ have been indicated with different letters.

2.2. Experimental design

The study consisted of two experiments. The first experiment (in 2021) was conducted using a completely randomized block design with three replications. Plots measuring 150 × 100 cm were prepared, with a planting depth of 20 cm for the corms, a 5-cm spacing between corms, and a 50 cm spacing between rows. The experimental treatments included five levels of melatonin foliar spray: 0 (control), 50, 100, 150, and 200 μM. The effects of these treatments on fatty acids and plant greenness were examined. In the second experiment (in 2022), the experimental treatments included different concentrations of melatonin (0 (control), 50, 100, 150, and 200 μM) and two application timings (124 and 131 days after germination). The effects of these treatments on antioxidant enzymes and total protein were investigated. Treatments were arranged in a completely random factorial design with three replications.

2.3. Spray application procedure and sampling time

Corms weighing 8–10 g (from Torbat Heydariyeh, Iran) were utilized in this study. Before planting, the corms were soaked in a 65 % Carbendazim Aria fungicide solution for 5 min. Foliar spraying was carried out on a single day for each application. The foliar application dates were as follows: the first application on February 13, the second on February 19, and the third on February 27, for both years of the experiment. The spraying for the three stages occurred 84, 90, and 98 days after flowering, respectively. Following the foliar spray applications with different melatonin concentrations (0, 50, 100, 150, and 200 μM) in 2021 and 2022, samples were collected and stored at −80 °C in a freezer. Subsequently, measurements were taken for total protein content, catalase and peroxidase enzymes, ascorbate peroxidase, and saturated and unsaturated fatty acids.

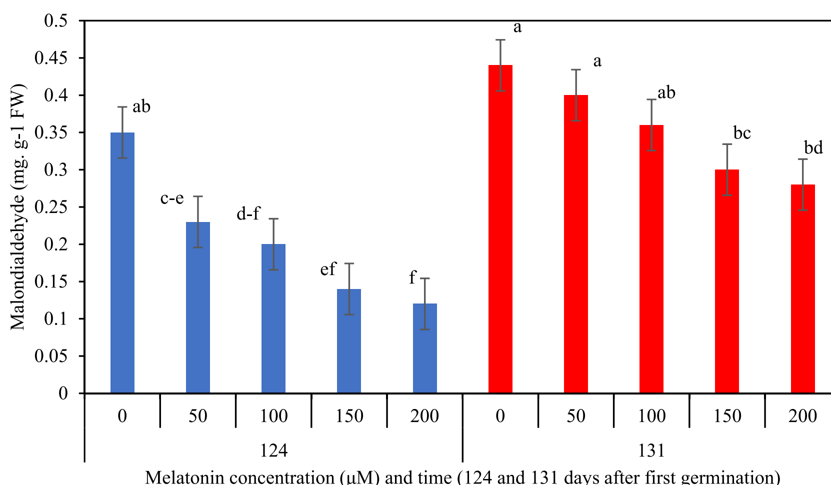

Fig. 2. The two-way interaction between spraying times and different concentrations of melatonin on malondialdehyde. Different letters denoted above the columns indicate statistically significant differences at a significance level of $P \leq 0.05$.

2.4. Extraction of fatty acids from saffron leaves

An appropriate amount of the sample was weighed in a beaker, and 200 mL of hexane were added to the sample container to prepare fatty acid methyl esters (FAMEs). After 1 h, the solvent was evaporated using a rotary evaporator. The remaining extracted oil was esterified by adding 7 mL of heptane and 2 mL of a KOH/MeOH mixture at 40–50 °C for 15 min. The resulting liquid was collected and injected into the gas chromatography device.

Gas chromatographic analysis was conducted using an Agilent instrument, model 7890A, equipped with a flame ionization detector, a split/splitless injector, and a fused silica column BPX-70 (120 m, 0.25 mm i.d., and 0.25 μm stationary phase). Operational parameters were set as follows: The column temperature was maintained at 198 °C (isothermal). The injector and detector temperatures were maintained at 250 and 300 °C. The gas flow rates were as follows: 1 mL/min carrier gas (N_2), 15 mL/min makeup gas (N_2), and 30 mL/min and 300 mL/min for flame gases (H_2 and synthetic air, respectively). The sample split ratio was 1:100. Injections were duplicated with an injection volume of 1 μL. Data collection and parameter calculations were performed using Chemstation software [33].

2.5. Preparation of saffron leaf extract for enzyme measurement

The enzymatic extract of saffron was prepared according to Panda [34] to determine the activity of antioxidant enzymes. In this method, 5 g of saffron leaves were mixed with a 50 mM potassium phosphate buffer (pH 7.0) and 1 % polyvinyl pyrrolidone powder. The mixture was placed in a centrifuge for 30 min at 4 °C and 12,000 rpm. Subsequently, the resulting liquid extract, for enzyme activity assessment, was collected and stored in a refrigerator at −20 °C.

2.6. Antioxidant enzyme activity (catalase, peroxidase, ascorbate peroxidase, and glutathione reductase)

The activity of the ascorbate peroxidase enzyme was measured using Yamaguchi et al. [35] method. For the assessment of ascorbate peroxidase enzyme activity, 994 μl of 50 mM potassium phosphate buffer (pH 7.0) and 100 mM ascorbic acid solution were mixed with 0.2 μl of 100 mM H_2O_2 solution and 5 μl of the enzyme extract. The initial absorbance was immediately read at 290 nm using a spectrophotometer, and after 2 min, the secondary absorbance was also measured (molar absorptivity: 2.8 mM^{-1} cm^{-1}).

Catalase enzyme activity was determined using the Panda method [34]. In this method, a reaction mixture was prepared by adding 915 μl of 50 mM potassium phosphate buffer (pH 7.0), 60 μl of 1 % H_2O_2, and 25 μl of the plant extract. The initial absorbance was immediately recorded using a spectrophotometer at a 240 nm wavelength, and after 2 min, the final absorbance was measured (molar absorptivity: 39.4 mM^{-1} cm^{-1}). The maximum absorption of hydrogen peroxide occurs at a wavelength of 240 nm. Therefore, as the catalase enzyme initiates the reaction, the amount of hydrogen peroxide in the reaction mixture gradually decreases, reducing absorption at the 240 nm wavelength [36].

The peroxidase enzyme activity was determined using the Panda [34] method. In this method, a reaction mixture was prepared by adding 2.913 μl of potassium phosphate buffer solution (pH 7.0), 1.8 μl of guaiacol solution, 25 μl of the extracted enzyme, and 60 μl of hydrogen peroxide solution. All samples were read at a wavelength of 470 nm using a Nanodrop instrument for 2 min. The extinction coefficient used was 36.32 mM^{-1} cm^{-1}.

Glutathione reductase (GR) activity was measured at 412 nm based on the reduction of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) in the presence of reduced glutathione. For this, a buffer solution of 50 mM phosphate (pH 7.8), 1 mM oxidized glutathione (GSSG), 0.1 M NADPH, 0.75 mM DTNB, and 0.5 mM EDTA was prepared. Enzyme activity was calculated using the extinction coefficient ($\varepsilon =$ 14.15 M^{-1} cm^{-1}). One unit of GR enzyme activity was defined as the formation of 1 nM of TNB per minute at 25 °C [37].

2.7. Measurement of malondialdehyde

Lipid peroxidation was determined by measuring malondialdehyde content following the method of Heath and Packer [38]. For this, 100 mg of fresh leaves were homogenized in 1000 μl of trichloroacetic acid (TCA). After centrifugation at 10,000 rpm for 5 min at 4 °C, 200 μl of the supernatant was collected. The extract was then mixed with 800 μl of 20 % TCA containing 0.5 % thiobarbituric acid. After incubating in a water bath for 30 min, absorbance readings were taken at 600 and 532 nm, and the difference was used to calculate malondialdehyde content (extinction coefficient: 1.55×10^5 M^{-1} cm^{-1}).

2.8. Measurement of electrolyte leakage

For the membrane stability index (MSI), the youngest fully developed leaves were sampled. The samples were placed in vials containing 10 mL of double-distilled water and incubated under laboratory conditions for 24 h. Electrolyte leakage was then measured using a Jenway EC meter (model Jenway) (EC1). To determine total electrolyte leakage after cell death, the samples were autoclaved at 110 °C and 1.2 atm for 30 min. After another 24-h incubation, electrical conductivity was measured again (EC2). The percentage of electrolyte leakage (EL) was calculated, and the membrane stability index (MSI) was determined using equation (1) [39]:

$$MSI = 100 - EL\% \qquad \text{Equation (1)}$$

2.9. Measurement of photosynthesis

Photosynthesis measurements were performed using a portable gas exchange system (Li-Cor 6400, Li-Cor Inc., Lincoln, NE, USA). The measurements were performed on the leaf of three randomly selected plants in each plot. Measurements were taken between 10:00 a.m. and 13:00 p.m. Each measurement lasted 1–3 min to allow for stabilization of gas concentrations.

2.10. The total protein concentration was determined at 595 nm

A volume of 10 μl of the enzyme extract was combined with 1 mL of Bradford reagent. After thorough mixing, the solution was allowed to stand for 10 min until the solution homogenized. Subsequently, 300 μl of this solution was transferred to a cuvette, and the absorbance of the samples was measured at 595 nm using a Nanodrop instrument. The standard protein concentration curve was used to calculate the protein concentration [40].

2.11. Determining the amount of leaf greenness

The SPAD-502 chlorophyll meter (SPAD-502, Minolta, Japan) was utilized to determine the leaf greenness of saffron plants. The leaves were randomly selected and measured. Since the melatonin spray was applied at the end of the growth season, leaf greenness was measured using the SPAD meter after 156 days.

2.12. Statistical analysis

In this experiment, the data were analyzed using SAS software version 9.1. The UNIVARIATE procedure was used to assess residual normality. The means of the data were compared using the least significant difference (LSD) test at a significance level of 0.05 ($P \leq 0.05$). Additionally, Excel was utilized to plot the graphs based on the obtained data. The Pearson's correlations ($p \leq 0.05$) were computed and a heatmap was generated using the corrplot package in R (ver. 4.2.1).

3. Results

3.1. The effect of different concentrations of melatonin on the greenness index

Fig. 1 shows a significant difference between the experimental treatments, which can be seen in the first year of the experiment (2021-2020). In the second year of the experiment (2021–2022), there was no difference between melatonin concentrations in terms of the greenness index; however, the regression analysis showed that the optimal rate of melatonin consumption for the greenness of the plant is about 118 μg (μM converted to microgram). Also, regression analysis showed that the highest greenness index could be achieved by using about 108 μg of melatonin. The melatonin value of 108 μg was calculated using the derivative of the quadratic equation presented in Fig. 1. However, according to the data from the mean comparison table, the 100 μM treatment had the greatest effect. Considering that 118 μg and 108 μg are close to 100 μM melatonin treatment, 100 μM treatment positively affected greenness.

Table 2
Effect of different concentrations of melatonin on the percentage of fatty acids in saffron leaves in 2021.

Fatty acids	Melatonin (μM)				
	0	50	100	150	200
C8: 0	$3.41^{b} \pm 1.29$	$5.34^{b} \pm 2.55$	$8.67^{a} \pm 0.88$	$3.48^{b} \pm 2.26$	$4.67^{b} \pm 1.25$
C10: 0	$1.73^{b} \pm 0.68$	$2.66^{b} \pm 1.17$	$5.24^{a} \pm 0.95$	$2.00^{b} \pm 1.00$	$2.72^{b} \pm 0.97$
C12: 0	$2.04^{b} \pm 0.52$	$6.64^{a} \pm 1.21$	$7.67^{a} \pm 0.67$	$3.10^{b} \pm 0.91$	$2.69^{b} \pm 0.53$
C14: 0	$2.82^{a} \pm 0.47$	$2.85^{a} \pm 0.39$	$3.13^{a} \pm 0.23$	$3.00^{a} \pm 0.49$	$3.17^{a} \pm 0.14$
C16: 0	$19.08^{a} \pm 1.02$	$13.74^{b} \pm 1.40$	$18.33^{a} \pm 0.88$	$17.08^{ab} \pm 0.93$	$18.60^{a} \pm 1.76$
C16:1	$3.56^{a} \pm 0.30$	$3.93^{a} \pm 0.06$	$2.30^{b} \pm 0.32$	$3.61^{a} \pm 0.50$	$3.53^{a} \pm 0.36$
C18:0	$2.30^{bc} \pm 0.35$	$1.65^{c} \pm 0.02$	$3.40^{a} \pm 0.31$	$2.75^{ab} \pm 0.18$	$2.62^{abc} \pm 0.45$
C18: 1	$5.37^{a} \pm 0.77$	$5.86^{a} \pm 0.87$	$6.19^{a} \pm 0.67$	$7.33^{a} \pm 0.88$	$5.33^{a} \pm 1.31$
C18: 2	$20.89^{a} \pm 1.20$	$22.33^{a} \pm 2.33$	$19.33^{a} \pm 1.45$	$21.06^{a} \pm 1.93$	$20.41^{a} \pm 2.89$
C18: 3	$31.27^{a} \pm 2.49$	$31.65^{a} \pm 3.18$	$19.30^{b} \pm 1.42$	$28.30^{a} \pm 4.40$	$28.28^{a} \pm 2.64$
C20: 4	$3.98^{ab} \pm 1.02$	$2.33^{b} \pm 0.44$	$4.17^{ab} \pm 0.93$	$5.00^{a} \pm 0.57$	5.05a ±0.30
C22: 0	$3.46^{a} \pm 0.29$	$1.40^{b} \pm 0.40$	$2.67^{ab} \pm 0.33$	$2.92^{a} \pm 0.46$	$2.95^{a} \pm 0.34$
PUFA/MUFA	$6.44^{a} \pm 0.86$	$5.91^{a} \pm 0.90$	$5.06^{a} \pm 0.28$	$5.02^{a} \pm 0.50$	$6.22^{a} \pm 0.97$
PUFA/SFA	$1.65^{a} \pm 0.23$	$1.70^{a} \pm 0.28$	$0.88^{b} \pm 0.08$	$1.71^{a} \pm 0.38$	$1.53^{ab} \pm 0.37$
USFA/SFA	$1.90^{a} \pm 0.24$	$1.99^{a} \pm 0.29$	$1.05^{b} \pm 0.09$	$2.05^{a} \pm 0.46$	$1.78^{ab} \pm 0.40$

The common letters in each row indicate non-significance at a 5 % probability level.
PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono-unsaturated Fatty Acid, USFA: Unsaturated Fatty Acid and SFA: Saturated Fatty Acid

3.2. The effect of different melatonin concentrations on fatty acids

Based on the results of the analysis of variance presented in Tables 1 and it was evident that different concentrations of melatonin had a significant effect on fatty acids C8:0, C18:0, C18:3, and C22:0 at a 5 % level and fatty acids C10:0 and C12:0 at a 1 % level in 2021 (Table 1). The comparison of mean traits revealed that the application of 100 μM melatonin led to an increase in the production of the fatty acids C8:0 (67.60 %), C10:0 (98.66 %), C12:0 (40.73 %), and C18:0 (35.32 %) compared to the control without melatonin application (Table 2). Furthermore, the levels of fatty acids C16:1 (47.41 %) and C18:3 (2.39 %) increased with the application of 50 μM melatonin compared to 100 μM (which had the lowest content of these fatty acids). The highest content of fatty acids, C14:0 (4.11 % compared to the control without melatonin) and C20:4 (86.53 % compared to 50 μM), was observed at 200 μM melatonin. Additionally, the highest amount of fatty acid C18:1 (74.24 % compared to the control without melatonin) was achieved with 150 μM melatonin. Under melatonin non-application (control) conditions, the highest level of fatty acid C22:0 was obtained, which did not significantly differ from the application of 100, 150, and 200 μM melatonin. Furthermore, the highest ratios of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) and unsaturated fatty acids to saturated fatty acids (UFA/SFA) were observed under the application of 150 μM melatonin, with no statistically significant difference compared to non-application, 50, and 200 μM melatonin (Table 2).

The analysis of the variance table for the effect of melatonin on various fatty acids in saffron leaves showed that different levels of melatonin did not have a significant effect on the levels of various fatty acids in the leaves, and this remained non-significant in 2022 (Table 3).

3.3. The effect of different concentrations of melatonin on antioxidant enzymes and total protein

Based on the analysis of variance presented in Table 5 and 6, the effects of different melatonin concentrations on malondialdehyde content, electrolyte leakage, and glutathione reductase were statistically significant at the 5 % level, while the effects on photosynthetic efficiency, catalase enzyme activity, and total protein content were significant at the 1 % level. However, there was no significant effect on ascorbate peroxidase and peroxidase activities. Additionally, the timing of melatonin foliar application significantly influenced malondialdehyde content and electrolyte leakage at the 5 % level, photosynthetic efficiency, all examined enzymes, and total protein content at the 1 % significance level. The interaction between melatonin concentration and application timing also significantly affected malondialdehyde content, electrolyte leakage, photosynthetic efficiency, protein content, and ascorbate peroxidase activity at the 1 % level, as well as glutathione reductase and peroxidase activities at the 5 % level (Table 4).

3.3.1. Malondialdehyde

The effect of melatonin foliar application on ascorbate peroxidase in saffron leaves was different at two application times (124 and 131 days after sprouting). Specifically, at 124 days after sprouting, the foliar application of 100 μM melatonin resulted in the highest level of ascorbate peroxidase enzyme, which was not statistically different from other melatonin concentrations at 131 days after sprouting (Fig. 2).

3.3.2. Electrolyte leakage

At different application times, electrolyte leakage decreased with increasing melatonin concentrations. However, the electrolyte leakage in the control (without melatonin) at 131 days after germination was lower than at 124 days, although this difference was not statistically significant. At other melatonin concentrations, the electrolyte leakage at 131 days after germination was significantly higher compared to 124 days (Fig. 3).

3.3.3. Photosynthesis efficiency

The effects of different treatments on the photosynthetic efficiency of saffron leaves showed that at 124 days after germination, photosynthetic efficiency was at its lowest without melatonin application, while the highest efficiency was observed with the application of 200 μM melatonin. Furthermore, across all melatonin levels, photosynthetic efficiency at 124 days after germination was higher compared to 131 days after germination (Fig. 4).

Table 3
Variance analysis of the effect of different concentrations of melatonin on the percentage of fatty acids in saffron leaves in 2022.

SOV	df	C14: 0	C16: 0	C16: 1	C18: 0	C18: 1	C18: 2	C18: 3	PUFA/MUFA	PUFA/SFA	USFA/SFA
Block	1	0.14ns	1.31ns	0.00ns	0.64ns	3.13ns	0.01ns	9.98ns	2.46ns	0.36ns	0.30ns
Melatonin	4	0.02 ns	0.91 ns	0.15 ns	0.53 ns	3.70 ns	1.10ns	7.42 ns	2.68ns	0.31ns	0.30ns
Error	4	0.09	0.29	0.21	0.36	0.89	0.95	2.78	0.82	0.15	0.16
CV (%)		13.43	4.24	22.18	28.10	12.35	3.68	3.56	11.67	8.92	8.18

ns, *, ** indicate non-significance and significance at 5 % and 1 % levels, respectively.
SOV: Source of variation, df: Degree of freedom, CV: Coefficient of variation
PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono-unsaturated Fatty Acid, USFA: Unsaturated Fatty Acid and SFA: Saturated Fatty Acid

Table 4
Analysis of variance of the effect of different concentrations of melatonin on malondialdehyde (MDA), electrolyte leakage, photosynthesis efficiency, total protein and antioxidant enzymes in saffron leaves in 2022.

SOV	df	MDA	Electrolyte leakage	Photosynthesis efficiency	Total protein	Ascorbate peroxidase	Catalase	Peroxidase	Glutathione reductase
Block	1	0.007^{ns}	87.6^{ns}	2.23^{ns}	0.01^{ns}	0.0001^{ns}	0.00013**	2.19**	0.00007^{ns}
Melatonin	4	0.020*	138*	89.2**	0.57**	0.7446^{ns}	0.00009**	0.08^{ns}	0.0006*
Main error	4	0.005	75.1	10.6	0.01	0.4961	0.00001	0.01	0.0002
Time	1	0.003*	108*	93.6**	2.89**	5.8985**	0.00040**	0.60**	0.004**
Time × Melatonin	4	0.587**	415**	161**	0.61**	1.4661**	0.00001^{ns}	0.58*	0.0004*
Sub error	5	0.004	56.0	9.15	0.02	0.2629	0.00001	0.14	0.00034
Total error	40	0.002	36.8	7.70	0.14	0.3603	0.00001	0.05	0.00008
CV (%)		16.9	21.7	11.8	28.68	45.43	34.87	39.11	8.73

The common letters in each row indicate non-significance at a 5 % probability level.
SOV: Source of variation, df: Degree of freedom, CV: Coefficient of variation

Table 5
The two-way interaction between spraying times and different concentrations of melatonin on ascorbate peroxidase, peroxidase, catalase and total protein.

Time (days after sprouting)	Melatonin (μM)	Ascorbate peroxidase (U mg^{-1})	Peroxidase (U mg^{-1} protein)	Catalase (U mg-1 protein)	Total protein (mg g^{-1}FW)
124	0	1.32^{a}±0.37	0.5^{a}±0.12	0.0066^{b} ± 0.0012	1.21^{b} ± 0.26
131		1.05^{b} ± 0.12	0.62^{b} ± 0.15	0.003^{c}±0.0005	1.41^{a}±0.12
124	50	0.58^{c}±0.1	0.12^{a}±0.07	0.006^{b} ± 0.0012	1.92^{a}±0.14
131		1.93^{a}±0.36	0.46^{b} ± 0.1	0.0113^{ab} ± 0.0017	1.06^{c}±0.11
124	100	2.37^{a}±0.3	0.94^{a}±0.19	0.0147^{a}±0.0018	1.86^{a}±0.19
131		1.27^{a}±0.3	0.46^{b} ± 0.1	0.0138^{ab} ± 0.0016	1.23^{b} ± 0.09
124	150	1.04^{b} ± 0.21	0.49^{a}±0.05	0.0096^{a}±0.0011	1.44^{c}±0.1
131		1.526^{a}±0.21	0.7^{ab} ± 0.15	0.0058^{b} ± 0.0012	0.66^{d} ± 0.13
124	200	0.84^{bc}±0.1	0.05^{a}±0.09	0.0094^{a}±0.0012	1.19^{c}±0.06
131		1.27^{a}±0.35	0.5^{b} ± 0.09	0.0133^{ab} ± 0.0024	1.05^{c}±0.04

The common letters in each row indicate non-significance at a 5 % probability level.

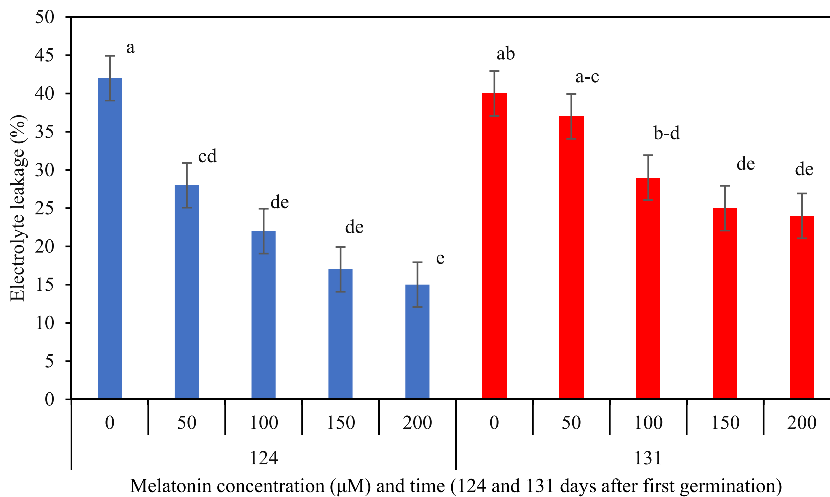

Fig. 3. The two-way interaction between spraying times and different concentrations of melatonin on electrolyte leakage. Different letters denoted above the columns indicate statistically significant differences at a significance level of $P \leq 0.05$.

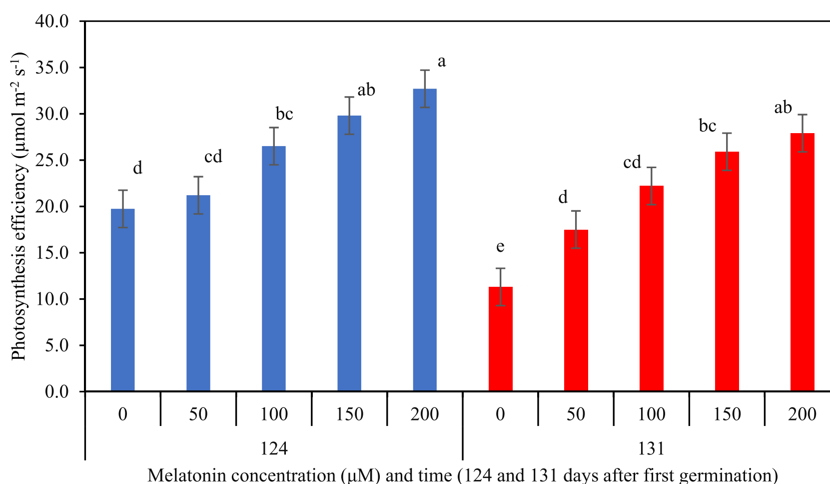

Fig. 4. The two-way interaction between spraying times and different concentrations of melatonin on photosynthesis efficiency. Different letters denoted above the columns indicate statistically significant differences at a significance level of $P \leq 0.05$.

3.3.4. Total protein

The impact of various treatments on the total protein content varied with different application times and melatonin concentrations. The highest total protein level was associated with 50 μM melatonin at 124 days after sprouting, which was not significantly different from 100 μM melatonin (Table 5).

3.3.5. Peroxidase

Based on the mean comparison results in Table 4, melatonin foliar application at 124 days after sprouting with 100 μM concentration had the most significant effect on peroxidase enzyme levels.

3.3.6. Catalase

The effect of different treatments on catalase enzyme levels showed that at 124 days after sprouting, catalase enzyme levels were minimal without melatonin application, while the highest level of catalase enzyme was produced with the application of 100 μM melatonin, which was not significantly different from melatonin concentrations of 50, 100, and 200 μM (Table 5).

3.3.7. Glutathione reductase

The effect of melatonin foliar application on glutathione reductase in saffron leaves varied between the two application times (124 and 131 days after germination). Specifically, at 124 days after germination, the application of 200 μM melatonin resulted in the highest glutathione reductase activity. Statistically, this was not significantly different from the activity levels observed at 100, 150, and 200 μM melatonin concentrations at 131 days after germination (Fig. 5).

3.4. Correlation between investigated traits

The analysis of trait correlations in the first year (2020–2021) of the experiment revealed that fatty acid C18:2 showed a positive correlation with fatty acids C16:1, C18:3, PU/S, US/S, and with the peroxidase enzyme (Fig. 6). However, this fatty acid exhibited a negative correlation with fatty acids C22:0, C20:4, C16:0, C14:0, C18:0, C10:0, and C8:0. Consistent with the observed trait correlations, C18:2 displayed the highest positive correlation with the peroxidase enzyme, and among the fatty acids, it showed the highest positive correlation with fatty acid C16:1. Moreover, the correlation results indicated that fatty acid C16:1 had a positive correlation with fatty acids C18:2, C18:3, PU/S, US/S, and PU/MU, as well as with the peroxidase enzyme. Conversely, it displayed a negative correlation with fatty acids C16:0, C14:0, C18:0, C10:0, C8:0, and C12:0, as well as with total protein. Its highest positive correlation was observed with fatty acid PU/S among the fatty acids. PU/MU demonstrated positive correlations with fatty acids C18:2, C16:1, C18:3, PU/S, and US/S while showing negative correlations with fatty acids C14:0, C18:0, C18:1, C10:0, C8:0, C12:0, as well as with catalase and ascorbate peroxidase enzymes. Among the fatty acids, it exhibited the highest positive correlation with C18:3. C22:0 showed a positive correlation with fatty acids C20:4, C16:0, and C18:0 while displaying negative correlations with fatty acids C18:2, C12:0, the peroxidase enzyme, and total protein. C14:0 exhibited positive correlations with fatty acids C16:0, C20:4, C10:0, C8:0, and C18:0, as well as with the catalase enzyme, while displaying negative correlations with fatty acids C18:2, C16:1, C18:3, PU/S, US/S, PU/MU, and with the peroxidase enzyme. C8:0 showed positive correlations with fatty acids C10:0, C12:0, and total protein, while displaying negative correlations with fatty acids C18:2, C16:1, C18:3, PU/S, US/S, PU/MU, and with the peroxidase enzyme. Among the fatty acids, it had the highest positive correlation with C10:0. C12:0 exhibited positive correlations with fatty acids C8:0, C10:0, and total protein, while displaying negative correlations with fatty acids C16:1, C18:3, PU/S, US/S, PU/MU, C22:0, C20:4, and C16:0 with the peroxidase enzyme (Fig. 6).

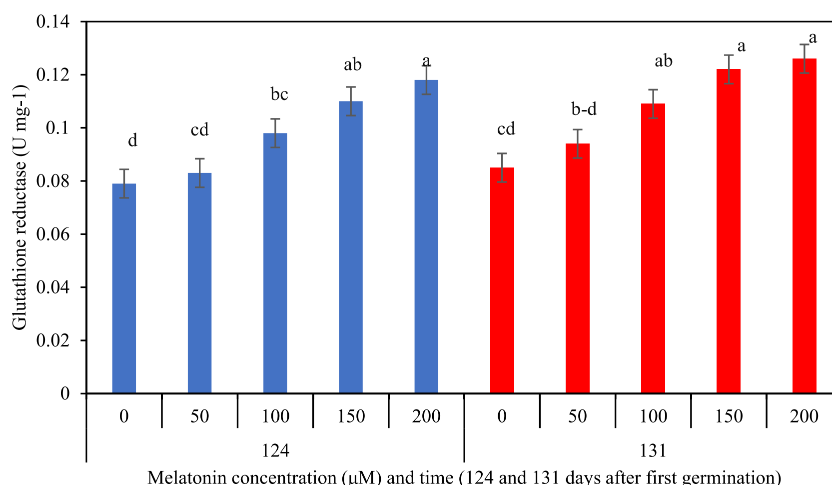

Fig. 5. The two-way interaction between spraying times and different concentrations of melatonin on glutathione reductase. Different letters denoted above the columns indicate statistically significant differences at a significance level of $P \leq 0.05$.

Fig. 6. Correlation among fatty acids, enzymes, and total protein in 2020–2021.
PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono-unsaturated Fatty Acid, USFA: Unsaturated Fatty Acid and SFA: Saturated Fatty Acid, Asc: Ascorbate peroxidase, Cat: Catalase, Per: Peroxidase, Tpr: Total protein.

In the second year (2021–2022) of the experiment, the results of trait correlations demonstrated that fatty acid C16:0 exhibited positive correlations with fatty acids C16:1, C18:2, and PU/MU, as well as with the enzyme catalase (Fig. 7). However, this fatty acid negatively correlated with PU/S, US/S, C18:3, C18:1, C14:0, total protein, peroxidase, and ascorbate enzymes. According to the observed trait correlations, C16:0 showed the highest positive correlation among enzymes with catalase and fatty acids with fatty acid C16:1. Fatty acid C18:2 demonstrated positive correlations with fatty acids C16:0, C16:1, and PU/MU, as well as with the enzyme catalase. On the other hand, it showed negative correlations with fatty acids C18:0, C14:0, C18:1, and total protein. It had the highest positive correlation with fatty acid PU/MU among the fatty acids. PU/MU exhibited positive correlations with fatty acids C16:0, C18:2, C16:1, PU/S, US/S, and with the enzyme catalase. Conversely, it showed negative correlations with fatty acids C18:1, C14:0, C18:0, and total protein. Fatty acid C14:0 positively correlated with fatty acids C18:1, C18:0, total protein, and the ascorbate enzyme. Conversely, it had a negative correlation with fatty acids PU/MU, C18:2, C16:0, and C16:1. Among fatty acids, it showed the highest positive correlation with fatty acid C18:1. Fatty acid C18:0 showed a positive correlation with fatty acids C14:0, C18:1, and total protein. On the other hand, it negatively correlated with fatty acids C18:3, US/S, PU/S, PU/MU, and C18:2, as well as with the peroxidase enzyme. Among fatty acids, it exhibited the highest positive correlation with fatty acid C14:0 (Fig. 7).

4. Discussion

4.1. The effect of melatonin on the amount of greenness of the plant

Chlorophyll undergoes substantial degradation during leaf senescence through the activity of various enzymes, ultimately resulting in a significant reduction in leaf chlorophyll content [41]. In this study, experimental results highlighted the significant positive effect of melatonin on the plant's greenness index. Regression analysis indicated that the maximum chlorophyll index was achieved with an application of approximately 108 μg of melatonin, which was statistically comparable to the 100-μg treatment. Melatonin application effectively delayed the leaf senescence process. A study by Mohammadi et al. [42] on soybean leaves exposed to melatonin treatments demonstrated that the greenness index remained constant or increased for about 110 days after planting, followed by a decline. The

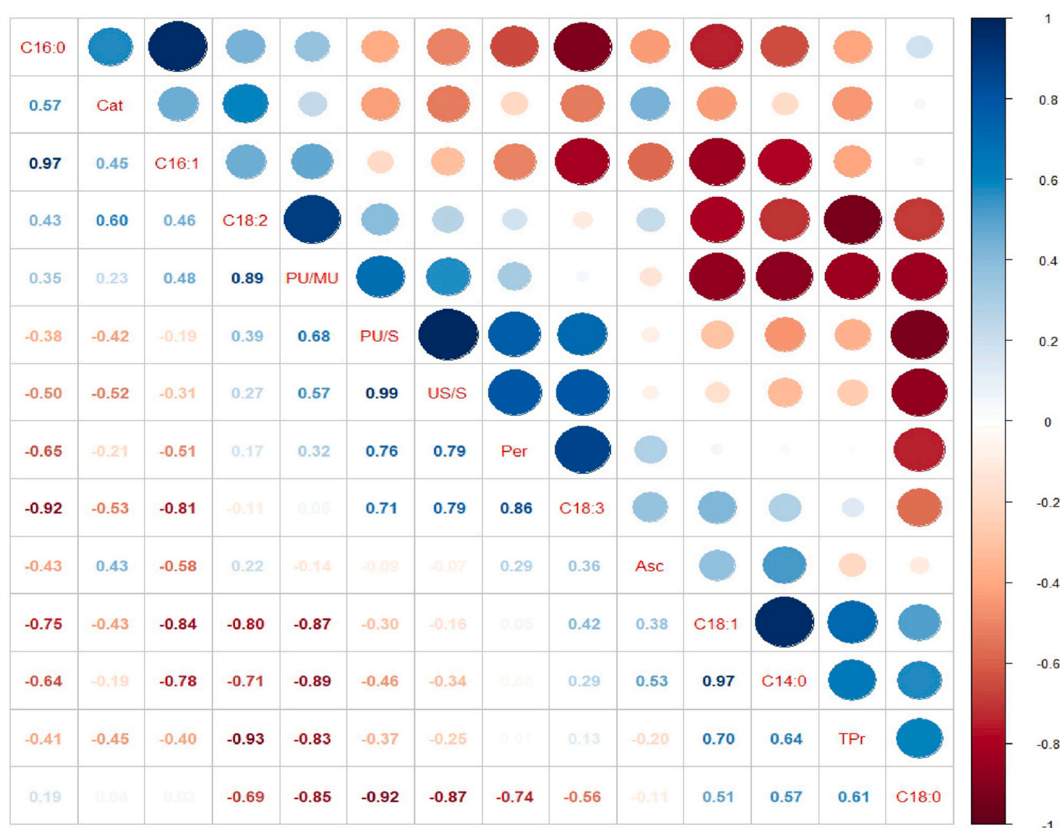

Fig. 7. Correlation among fatty acids, enzymes, and total protein in 2021–2022.
PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono-unsaturated Fatty Acid, USFA: Unsaturated Fatty Acid and SFA: Saturated Fatty Acid, Asc: Ascorbate peroxidase, Cat: Catalase, Per: Peroxidase, Tpr: Total protein.

highest leaf greenness was observed in the middle leaves when treated with 0 and 0.1 μM melatonin, and in the lower leaves with 0.2 μM melatonin. After 100 days, middle leaves exhibited the highest greenness levels, attributed to the role of melatonin in slowing chlorophyll degradation. Higher concentrations of melatonin were associated with a more gradual reduction in leaf greenness, whereas untreated plants experienced a more rapid decline. Similar findings have been reported in other crops, including barley and apple leaves, where melatonin application increased chlorophyll content [24] and delayed leaf senescence [28,43]. For example, single mutations in chlorophyll degradation genes (CDGs) such as *sgr1*, *myc1*, *pph*, and *nol1* inhibited chlorophyll breakdown, resulting in a persistent green phenotype in Arabidopsis leaves [44]. Exogenous melatonin treatments influenced CDG expression and mitigated senescence. In broccoli, melatonin delayed yellowing by reducing the activity of chlorophyll-degrading enzymes, including chlorophyllase (CLH), pheophytinase (PPH), pheophorbide a oxygenase (PAO), and red chlorophyll catabolite reductase (RCCR), while suppressing the expression of genes such as *BoNYC1*, *BoNOL*, *BoPPH*, *BoPAO*, *BoRCCR*, and *BoSGR1* [30]. Furthermore, Wang et al. [25] demonstrated that melatonin application delayed darkness-induced senescence in isolated *Malus* leaves by inhibiting the expression of PAO and *SAG12*, preserving chlorophyll content and Fv/Fm values. The researchers also observed that melatonin suppressed the expression of the *PetF* gene, a producer of reactive oxygen species (ROS). Reduced *PetF* expression was proposed to mitigate ROS accumulation, which could otherwise exacerbate salt stress effects such as growth retardation and H_2O_2 buildup [45]. Ferredoxin, regulated by *PetF*, plays a critical role in ascorbate reduction and chlorophyll protection against degradation [46]. Long-term soil application of 100 μM melatonin in apple trees also resulted in increased chlorophyll content compared to untreated control trees [27].

4.2. Effect of melatonin on the efficiency of photosynthesis

A stable supply of chlorophyll and photosynthetic proteins is crucial for the successful assembly of photosystems and the normal progression of photosynthesis [47]. Senescing leaves exhibit a decline in photosynthetic efficiency, largely due to reduced PSII activity, which disrupts the balance between electron generation and transmission. This imbalance triggers increased photochemical and nonphotochemical energy dissipation, ultimately leading to a reactive oxygen species (ROS) burst [47]. Melatonin has been shown to maintain high levels of photosynthetic carbon reduction and photorespiratory carbon oxidation while suppressing the rise of O2-dependent alternative electron flux, a major source of ROS under stress conditions [48]. In stress-induced senescing leaves,

melatonin significantly mitigated the decline in Fv/Fm and Y(II) values, indicators of photosynthetic efficiency [49]. Furthermore, leaves treated with exogenous melatonin demonstrated enhanced net photosynthetic rates, higher CO_2 assimilation rates, improved stomatal conductance, and greater water use efficiency [50]. Melatonin application also counteracted the reduction in the expression of key genes involved in electron transport (*PetF*), ATP synthesis (*ATPF1A*), and the Calvin cycle (*rbcS*, *GAPC1*, and *GAPCP-2*) [51]. Consistent findings across multiple crops, including maize [52], rapeseed [53], and common bean [54], have reported increased photosynthetic pigment concentrations and photosynthetic rates under abiotic stress conditions following melatonin application.

4.3. Effect of melatonin on electrolyte leakage and peroxidation of membrane lipids

Cell membrane integrity during senescence can be effectively assessed through electrolyte leakage and lipid peroxidation, both of which are critical indicators of membrane stability under stress or aging conditions [6]. Electrolyte leakage, a hallmark of membrane disruption, reflects the release of solutes from leaves, especially under drought stress or during senescence [55,56]. Our findings demonstrate that melatonin treatment significantly reduces electrolyte leakage in aging plants, aligning with previous studies that showed melatonin's ability to mitigate stress-induced membrane damage. For instance, Li et al. [57] reported that melatonin-pretreated plants exhibited reduced H_2O_2 accumulation and electrolyte leakage, likely due to enhanced antioxidant enzyme activity. Similarly, in soybean and bermudagrass, melatonin application alleviated ROS accumulation and oxidative damage, reinforcing its role in protecting membrane integrity [58].

The observed reduction in H_2O_2 levels in melatonin-treated plants further underscores its antioxidant properties, consistent with studies where melatonin scavenged ROS and maintained ROS homeostasis [47]. This protective effect was particularly evident under drought conditions, as melatonin-treated plants demonstrated lower oxidative damage and improved membrane stability [59]. These findings align with our results, where melatonin effectively minimized membrane disruption during senescence.

Lipid peroxidation, another reliable indicator of membrane deterioration, results from the oxidative degradation of polyunsaturated fatty acids, producing markers like malondialdehyde (MDA) [60,61]. In our study, melatonin treatments significantly reduced MDA levels, suggesting a protective mechanism against lipid oxidation. This finding supports earlier research indicating that melatonin mitigates oxidative stress by scavenging ROS and preserving membrane integrity [62]. Li et al. [63] also found that 100 μM melatonin reduced MDA levels in tea plants while enhancing photosynthetic efficiency and antioxidant activity, further corroborating our results.

Melatonin's dual hydrophilic and lipophilic properties enable it to localize in cytoplasm and lipid membranes, where it stabilizes lipid bilayers and reduces peroxidation [49]. In our study, the reduction in lipid peroxidation with melatonin application highlights its ability to protect membrane lipids from oxidative damage during aging, aligning with findings in tea and soybean plants [59,63]. Notably, our results also suggest a concentration-dependent organization of melatonin within membranes, consistent with its reported alignment parallel to lipid tails at low concentrations and bilayer insertion at higher levels [49].

Overall, the observed decrease in electrolyte leakage, H_2O_2 accumulation, and MDA levels in our study underscores melatonin's protective role in maintaining membrane integrity during senescence. These findings align with previous studies while contributing new insights into its efficacy in alleviating aging-related membrane deterioration. Future research should explore the concentration-dependent mechanisms of melatonin in enhancing membrane stability and its broader implications for crop resilience.

4.4. Effect of melatonin on fatty acids

Fatty acids, as fundamental components of cell membranes, sphingolipids, and cuticular waxes, play critical roles in plant growth, stress tolerance, and environmental interactions [64]. Specifically, unsaturated fatty acids (UFAs) are pivotal in mitigating abiotic and biotic stresses by maintaining membrane fluidity and regulating plant defense gene expression [13,65,66]. Consistent with previous studies, our results highlight the impact of melatonin on fatty acid profiles in saffron leaves, particularly during different growth stages.

In 2021, melatonin treatments significantly increased the levels of fatty acids, including C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, and C20:4. By 2022, the effect was more pronounced in specific fatty acids, such as C16:0, C18:1, and C18:3, during late growth stages, mirroring findings from studies on Arabidopsis, Brachypodium, and Switchgrass that showed a natural decline in fatty acid levels with aging [19]. These results align with previous observations that melatonin counteracts lipid degradation and enhances fatty acid levels during senescence, likely by reducing lipid peroxidation and preserving membrane fluidity [67,68].

Our findings also corroborate studies on saffron by-products in Morocco, which identified C16:0, C18:2, and C18:3 as predominant fatty acids in saffron leaves [69]. Similarly, Feizy and Reyhani [70] reported comparable fatty acid profiles in saffron petal oil. These results are consistent with melatonin's reported ability to increase C18:1 and C18:3 levels, as shown in crops such as safflower, soybean, and coffee [71,72].

The observed differences between the two study years highlight a temporal variability in melatonin's efficacy, with stronger effects noted in the first year, particularly 124 days after germination. This temporal variation could be attributed to environmental factors or differences in plant physiological conditions between the two years. Additionally, while melatonin enhanced fatty acid levels overall, it had no statistically significant effect on C22:0, suggesting that certain fatty acids are less responsive to melatonin treatments.

These findings align with previous evidence supporting melatonin's protective role against lipid peroxidation and its ability to maintain fatty acid content during aging [67,68]. However, our study also highlights the potential risks of excessive melatonin concentrations, as reported in other studies, where high doses can exhibit phytotoxic effects [73]. This underscores the need for optimizing melatonin concentrations to balance its benefits with potential adverse effects.

By demonstrating melatonin's role in modulating fatty acid profiles across different growth stages, our findings contribute to a growing body of evidence supporting its utility in enhancing crop resilience and quality. Future studies should explore the underlying molecular mechanisms and optimal application strategies to maximize its benefits.

4.5. The effect of melatonin on antioxidant enzymes and total protein levels

As plants age, the decline in endogenous antioxidant defenses and the increase in reactive oxygen species (ROS) production lead to oxidative stress and disrupt the balance between oxidants and antioxidants [74]. The presence of antioxidants is vital for mitigating oxidative damage, not only in plants but also in other organisms, highlighting their universal importance. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) catalytically eliminate oxidants, with their activity often increasing in response to oxidative stress [75]. This experiment demonstrated that melatonin significantly enhanced the activity of antioxidant enzymes, including ascorbate peroxidase, peroxidase, and catalase, at both 124 and 131 days after germination. The effect was more pronounced at 131 days, suggesting melatonin's enhanced efficacy during later growth stages. These results align with previous findings in tomato seedlings, where melatonin application improved the activity of antioxidant compounds, including SOD, CAT, and APX, particularly during late growth stages [76]. Similarly, studies on alfalfa have shown that melatonin increases root antioxidant capacity under both stress and non-stress conditions [77]. The increase in antioxidant enzymes observed in this study is consistent with reports that melatonin acts as a potent antioxidant by scavenging ROS, maintaining cellular redox balance, and activating genes involved in antioxidant defense [78,79]. For instance, melatonin-treated alfalfa seedlings exhibited upregulated expression of genes encoding antioxidant enzymes such as Cu/Zn-SOD and CAT, underscoring its regulatory role in enhancing oxidative stress tolerance [79].

Melatonin's dual hydrophilic and lipophilic properties allow it to traverse cellular compartments and localize within lipid membranes, where it protects cells from oxidative damage [78]. By facilitating the scavenging of ROS and boosting antioxidant enzyme activity, melatonin reduces lipid peroxidation and protein oxidation, preserving cellular integrity [80]. These findings are in line with reports on tomatoes and parsley, where melatonin not only activated antioxidant enzymes but also improved the ASC/DHA and GSH/GSSG ratios, crucial for ascorbate and glutathione regeneration [81,82].

In the present study, melatonin application also significantly increased total protein content in saffron plants at both 124 and 131 days after germination. This result corresponds with findings in African parsley, where melatonin enhanced protein content, suggesting its role in preventing protein oxidation and maintaining protein functionality under stress [20,82]. Similarly, studies on cilantro and dill have demonstrated that melatonin stimulates protein biosynthesis, likely due to its protective effects on photosynthetic processes and metabolic recovery [83,84].

The results of this study collectively demonstrate that melatonin not only enhances antioxidant defense mechanisms but also preserves structural and functional components such as proteins. These findings underscore melatonin's dual role as a direct antioxidant and regulatory molecule, reinforcing plant resilience against oxidative stress and supporting biosynthetic processes critical for growth and development.

5. Conclusion

In this experiment, the positive effect of melatonin treatments on the studied traits of saffron was observed during the late stages of growth. Among the experimental treatments, spraying 100 μM melatonin led to the highest greenness in saffron leaves. Moreover, different melatonin doses positively affected most of the fatty acids found in saffron, but among them, spraying 100 μM melatonin was more effective than the rest of the treatments. Thus, it can be concluded that melatonin is needed during the late growth season of saffron to delay leaf senescence and prolong the plant's lifespan. Of course, the spraying time of exogenous melatonin is also an important issue that should be considered. Future research and recent advancements could pave the way for innovative approaches to enhance plant productivity and strengthen plant defense mechanisms. Additionally, future studies should incorporate RT-PCR or similar molecular techniques to validate the gene expression patterns associated with the observed biochemical and physiological changes.

CRediT authorship contribution statement

Mohammad Mehdi Samim: Writing – original draft, Project administration, Investigation. **Ali Sorooshzadeh:** Supervision. **Ali Mokhtassi-Bidgoli:** Writing – review & editing, Supervision, Software, Investigation, Formal analysis, Data curation. **Mohammad Sadegh Sabet:** Methodology, Investigation.

Data availability

Data can be provided upon reasonable request from the corresponding author.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41766.

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