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The effect of melatonin on nutritional value and antioxidant activity of Pansy (*Viola* × *wittrockiana*) flowers

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ABSTRACT

Edible flowers are a rich source of nutritional, phytochemical, and antioxidant compounds, leading to their increasing popularity among consumers. The pansy plant, as an edible flower, possesses medicinal properties and is widely used in various foods and beverages. Recent studies have indicated that the application of melatonin may have positive effects on plant quality. This study aimed to investigate the effect of melatonin on the nutritional characteristics of pansy in a completely randomized design with three replications in a greenhouse. Treatments included melatonin at concentrations of 0, 50, 100, 150 and 200 mg l^{-1}, applied in two stages. The first application was two weeks after transplanting, and the second occurred ten days later. One week after the last spraying, the flowers were harvested to evaluate the desired traits. The results showed that melatonin treatment had a significant effect on the evaluated traits, the highest cell membrane stability index (87.56%), carotenoid (5.58 mg g^{-1}), total chlorophyll content (5.38 mg g^{-1}), total antioxidant activity (6.53%), vitamin C (49.25 mg $100g^{-1}$), thiamine (3.17 µg g^{-1}), riboflavin (0.134 µg g^{-1}), niacin (131.24 µg g^{-1}), and phenol content (19.85 mg g^{-1}) were observed in melatonin 150 mg l^{-1} treatment. The highest folic acid content (0.53 µg g^{-1}), carbohydrate (82.35 mg g^{-1}), and protein content (14.76 µg g^{-1}) were obtained in melatonin 200 mg l^{-1} treatment. While the lowest trait values were observed in the control group, the results indicated that melatonin application had a positive effect on the nutritional value of *viola* × *wittrockiana* flowers.

1. Introduction

In recent years, edible flowers have gained increasing recognition as a valuable source of nutrients and phytochemicals in the human diet. Their positive health effects are primarily attributed to bioactive compounds, including anthocyanins, carotenoids, polyphenols, vitamins, and minerals [1], which are often present in higher concentrations in flowers compared to common fruits and vegetables. Moreover, edible flowers are widely utilized in the preparation of salads, desserts, sauces, and beverages, providing novel colors, textures, and diversity to culinary products [2].

Pansy (*Viola* × *wittrockiana*), a member of the Violaceae family, is an ornamental plant characterized by its attractive and cold-tolerant flowers [3]. Native to Europe, pansy is known for its medicinal properties, including sedative, laxative, expectorant, anti-inflammatory, diuretic, and antiseptic effects. It is frequently incorporated into appetizers, soups, beverages, salads, and food decorations [4, 5]. The nutritional composition of fresh pansy flowers includes carbohydrates (53.6%), proteins (11.2%), and lipids (0.44%) [6], with fully opened flowers containing the highest levels of carbohydrates and proteins [5]. Additionally, pansy flowers are rich in essential elements, including zinc, strontium, sulfur, phosphorus, sodium, manganese, magnesium, potassium, iron, copper, and calcium [7]. The predominant phytochemical constituents are anthocyanins (delphinidin, cyanidin, petunidin, and malvidin) and flavones (apigenin) [8].

Melatonin, a bioactive molecule derived from the amino acid tryptophan, functions as a plant growth regulator with roles similar to indole-3-acetic acid (IAA), promoting cell expansion and accelerating plant growth [9]. It also contributes to the regulation of circadian rhythms, mitigates photo-oxidative damage in photosynthetic systems, and, at moderate concentrations, protects chlorophyll during senescence [10]. Beyond its potent antioxidant activity under stress conditions, melatonin plays a crucial role in various physiological processes, including seed germination, root and shoot development, growth induction, leaf area expansion, delayed leaf senescence, and ultimately, the enhancement of chlorophyll and carotenoid contents, as well as photosynthesis and carboxylation. These effects collectively improve both the quality and yield of crops [11]. Previous studies have reported that the application of 0.1 mM melatonin enhanced carbohydrate, protein, total phenolic content, peroxidase activity, and vase life in gerbera (*Gerbera jamesonii* cv. Terra Kalina) [12]. Similarly, treatment with 150 µM melatonin increased carotenoid, phenolic, flavonoid, and protein contents in marigold (*Calendula officinalis* L.) [13]. More recently, Wang et al. (2024) demonstrated that application of 50 µM melatonin improved total sugar and protein levels in peony (*Paeonia lactiflora* Pall.) flowers [14].

Given the nutritional value of edible flowers and the beneficial effects of melatonin as a natural bioactive compound, this study was conducted to evaluate the impact of melatonin on the nutritional quality and antioxidant activity of pansy (*Viola* × *wittrockiana*). The simultaneous assessment of these traits, combined with the use of melatonin as a biostimulant, is expected to provide novel insights into enhancing the overall quality of edible flowers.

2. Materials and Methods

2.1. Sample Preparation

This experiment was conducted in a greenhouse with an average daily temperature ranging from 17 to 22 °C and a relative humidity of 65–75%. Pansy (*Viola* × *wittrockiana*) seeds were purchased and sown in plastic pots (15 cm diameter) filled with a mixture of cocopeat and perlite. Foliar application of melatonin was performed at five concentrations (0, 50, 100, 150, and 200 mg L^{-1}) at the 4–6 and 6–8 leaf stages. Sampling and trait evaluation were carried out at the flowering stage of the plants.

2.2. Cell Membrane Stability Index

To determine the cell membrane stability index, petal samples of pansy were placed in a water bath at 30 °C for 1 hour, and the initial electrical conductivity (EC_1) was measured using a conductivity meter (Model 7310, Maham Azma Co.). The samples were then autoclaved at 121 °C for 20 min to obtain the final conductivity (EC_2). The membrane stability index was calculated according to Equation (1) [15].

$$MSI = [\,1 - (EC1/EC2)\,] \times 100 \quad (1)$$

2.3. Total Chlorophyll

Leaf total chlorophyll content was extracted using 80% acetone. Absorbance was recorded at 645 and 663 nm using a UV-Visible spectrophotometer (Spectro Flex 6600). The total chlorophyll content was calculated using Equation (2) and expressed as mg g^{-1} fresh weight [16].

Total Chlorophyll = 20.2 (A645nm) + 8.02 (A663nm) × Volume/ (1000× Sample weight) (2)

2.4. Carotenoids

Petal carotenoids were extracted following the method described by Shabanifard et al. (2024) and quantified spectrophotometrically at 480 and 510 nm. Carotenoid content was calculated using Equation (3) and expressed as mg g^{-1} fresh weight [16].

Carotenoid = 7.6 ($A_{480\ nm}$)- 1.49 ($A_{510\ nm}$) + Volume/ (1000×Sample weight) (3)

2.5. Total Antioxidant Activity (DPPH Assay)

To assess the total antioxidant capacity, 5 mL of the plant extract was mixed with 1 mL of 1 mM DPPH methanolic solution. The mixture was incubated for 30 min at room temperature in the dark, after which absorbance was measured at 517 nm. Antioxidant activity was calculated as a percentage using Equation (4) [17].

DPPH radical-scavenging activity % = $[(A_{control} - A_{sample}) / A_{control})] \times 100$ (4)

2.6. Vitamin C

Vitamin C content was determined using a two-step redox titration method. Ascorbic acid in the samples was first oxidized, followed by titration with a standard solution. Results were expressed as mg ascorbic acid per 100 g fresh weight of petals [18].

2.7. B-group Vitamins (Thiamine, Riboflavin, Niacin, and Folic Acid)

Plant samples were dried, ground, and mixed with distilled water, then incubated at 60°C for 1 hour. To enhance extraction, 0.1 M HCl was added, and the extracts were subsequently filtered. Quantification of thiamine, riboflavin, niacin, and folic acid was performed using high-performance liquid chromatography (HPLC; Merck Hitachi, Lachrom Pump 7100) equipped with a C18 column and UV-Vis or fluorescence detectors. Thiamine (vitamin B1) and riboflavin (vitamin B2) were identified using fluorescence detection, whereas niacin (vitamin B3) and folic acid (vitamin B9) were determined using UV detection. Excitation and emission wavelengths were 365/435 nm for thiamine, 440/530 nm for riboflavin [19], 322/380 nm for niacin [20], and 290/360 nm for folic acid [21].

2.8. Carbohydrates

Soluble carbohydrates were quantified using the anthrone method. Briefly, 100 µL of the extract was mixed with 3 mL of anthrone reagent and heated in a boiling water bath for 100 min. Absorbance was read at 630 nm, and results were expressed as mg g^{-1} fresh weight of petals [22].

2.9. Total Phenols

Total phenolic content was determined using the Folin–Ciocalteu reagent. A 10 µL aliquot of methanolic extract was mixed with 50 µL Folin–Ciocalteu reagent and 580 µL distilled water. After incubation for 5–8 min, 150 µL of 1 M sodium carbonate solution was added. Absorbance was measured at 765 nm using a spectrophotometer, and results were expressed as mg g^{-1} fresh weight [23].

2.10. Proteins

Protein content was determined according to the Bradford (1976) method. A 0.1 mL aliquot of protein extract from each sample was mixed with 5 mL of Bradford reagent, vortexed for 20 min, and absorbance was measured at 595 nm. Protein content was expressed as µg mg^{-1} fresh weight of petals [24].

2.11. Statistical Analysis

The experiment was arranged in a completely randomized design (CRD) with three replications. Data were analyzed using SPSS software (version 23). Mean comparisons were performed using Duncan's multiple range test at the 1% and 5% probability levels.

3. Results and Discussion

The analysis of variance (ANOVA) results indicated that the effect of melatonin treatment was significant at the 1% probability level for carotenoid content, total chlorophyll, carbohydrate content, phenolic compounds, and antioxidant capacity. In addition, significant effects were observed at the 5% probability level for cell membrane stability index, vitamin C, niacin, riboflavin, thiamine, folic acid, and protein content (Table 1).

Table 1. Analysis of variance of the effect of melatonin on nutritional value of Pansy (*Viola* × *wittrockiana*)
* and ** are significant at the 5% and 1% levels, Respective

Source of variation	DF	Mean square											
		Cell membrane stability index	Total chlorophyll	Carotenoid	Vitamin C	Thiamin	Riboflavin	Niacin	Acid folic	Carbohydrate	Phenol	Protein	Total antioxidant
Treatment	4	14.36*	8.58**	8.67**	71.45*	4.37*	0.218*	185.41*	0.86*	101.17**	24.36**	19.78*	9.45**
Error	10	1.651	0.598	0.635	1.238	0.158	0.011	1.975	0.024	1.235	0.019	0.968	0.712
CV (%)	---	10.71	8.32	9.37	9.47	9.85	8.79	10.35	10.12	9.65	9.73	9.45	10.29

3.1. Cell Membrane Stability Index

The results showed that the application of melatonin significantly increased the cell membrane stability index. The highest value (87.56%) was obtained with 150 mg L^{-1} melatonin treatment, while the lowest value (66.45%) was recorded in the control (Figure 1). Melatonin, as a potent antioxidant, reduces the level of reactive oxygen species (ROS), thereby preventing lipid peroxidation in the cell membrane and maintaining membrane integrity. Additionally, melatonin enhances the activity of antioxidant enzymes, which helps maintain redox balance within cells and prevents potential membrane damage [25]. Moreover, by regulating membrane lipid metabolism and promoting the synthesis of phospholipids, melatonin improves membrane flexibility and fluidity [26]. Consistent with the present findings, melatonin application has also been reported to increase the cell membrane stability index in Gerbera (*Gerbera jamesonii*) [27].

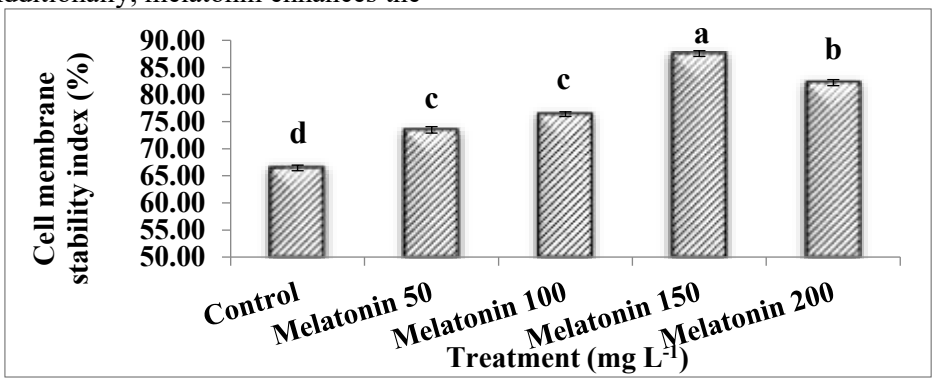

Fig 1 Effect of melatonin on cell membrane stability index of Pansy (*Viola* × *wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

3.2. Total Chlorophyll

The results indicated that the highest total chlorophyll content (5.38 mg g^{-1} FW) was observed in plants treated with 150 mg L^{-1} melatonin. In comparison, the lowest value (3.05 mg g^{-1} FW) was recorded in the control (Figure 2). The positive effect of melatonin on chlorophyll accumulation may be attributed to its role in enhancing the antioxidant capacity of plants [28]. Melatonin also promotes chlorophyll biosynthesis by increasing the concentration of magnesium ions, carotenoids, anthocyanins, and flavonoids, and protects chlorophyll molecules from reactive oxygen species [29]. Previous studies have demonstrated that melatonin downregulates the expression of pheophorbide a oxygenase (a key gene in chlorophyll degradation), the senescence-associated gene (SAG12), and chlorophyllase, thereby contributing to higher chlorophyll retention [30]. Consistent with the present findings, melatonin application was also reported to increase total chlorophyll content in giant hyssop (*Agastache foeniculum* [Pursh] Kuntze) [31].

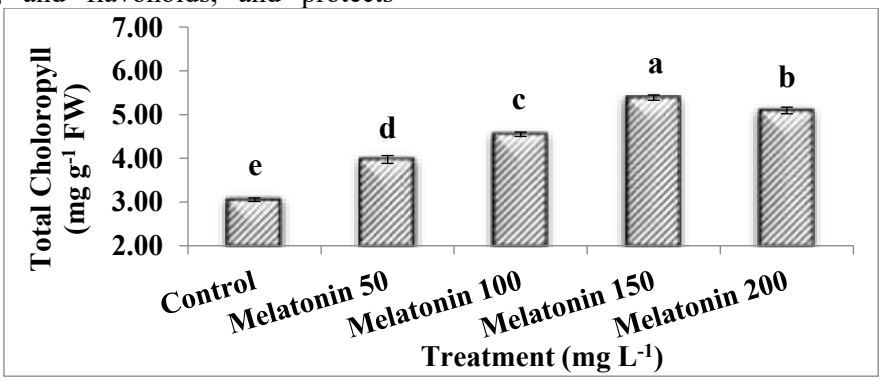

Fig 2 Effect of melatonin on total chlorophyll content of Pansy (*Viola* × *wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

3.3. Carotenoids

The comparison of mean values showed that the highest carotenoid content (5.58 mg g^{-1} FW) was obtained with 150 mg L^{-1} melatonin treatment. In contrast, the lowest value (3.48 mg g^{-1} FW) was recorded in the control. Previous studies have reported that melatonin enhances photosynthesis, which in turn improves both chlorophyll and carotenoid contents [32]. Additionally, melatonin regulates the expression of key genes in the carotenoid biosynthetic pathway, including those encoding phytoene synthase and lycopene cyclase, thereby promoting carotenoid synthesis [26]. Similarly, Karimi et al. (2024) also reported that melatonin application increased carotenoid content in marigold (*Calendula officinalis* L.) [13].

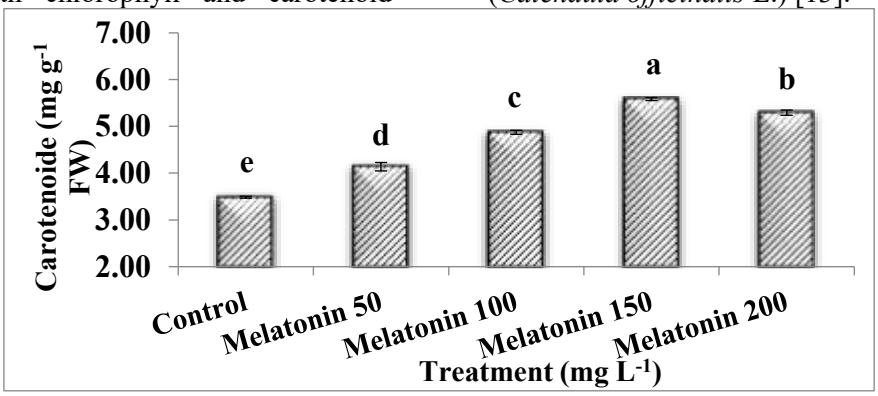

Fig 3 Effect of melatonin on carotenoid content of Pansy (*Viola × wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

3.4. Vitamin C

As shown in Figure 4, the highest vitamin C content (49.25 mg 100 g^{-1} FW) was recorded in plants treated with 150 mg L^{-1} melatonin, while the lowest value (34.62 mg 100 g^{-1} FW) was observed in the control. Melatonin enhances the activity of enzymes involved in vitamin C biosynthesis, thereby increasing the accumulation of vitamin C in the plant. Acting as a metabolic regulator, melatonin improves plant growth and development processes, which indirectly contributes to higher vitamin C production. Furthermore, as a potent antioxidant, melatonin strengthens the plant's antioxidant systems and helps maintain elevated vitamin C levels [11]. Consistent with these findings, melatonin application has also been reported to preserve vitamin C content in broccoli (*Brassica oleracea*) [33].

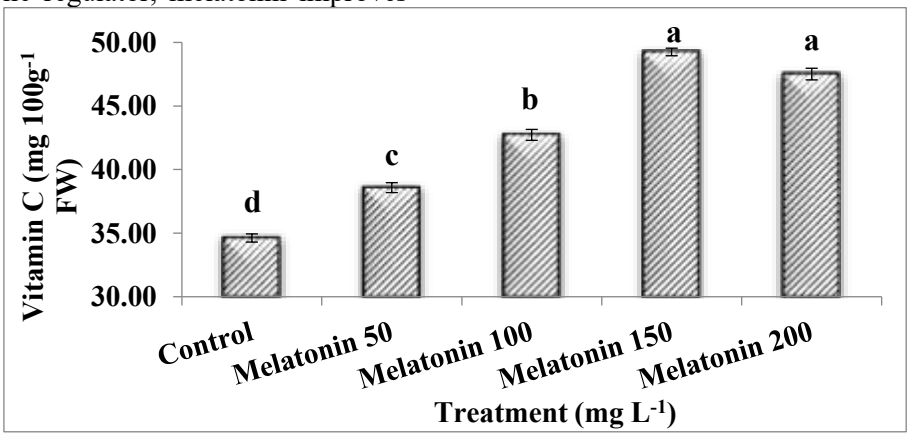

Fig 4 Effect of melatonin on vitamin C of Pansy (*Viola × wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

3.5. B-Group Vitamins (Thiamine, Riboflavin, Niacin, and Folic Acid)

The results indicated that the highest thiamine (B1) content (3.17 µg g^{-1} DW) was observed in plants treated with 150 mg L^{-1} melatonin, while the lowest value (2.24 µg g^{-1} DW) was recorded in the control. Similarly, the highest riboflavin (B2) content (0.134 µg g^{-1} DW) was obtained

with 150 mg L^{-1} melatonin, whereas the control showed the lowest level (0.095 µg g^{-1} DW). Niacin (B3) content was highest (131.24 µg g^{-1} DW) in the 150 mg L^{-1} melatonin treatment and lowest (93.48 µg g^{-1} DW) in the control. Folic acid (B9) content reached its maximum (0.53 µg g^{-1} DW) under 200 mg L^{-1} melatonin treatment and minimum (0.38 µg g^{-1} DW) in the control (Table 2).

Studies suggest that melatonin can act as a key regulator in the biosynthesis and maintenance of these vitamins. Although direct evidence of melatonin's effect on thiamine, riboflavin, niacin, and folic acid levels in plants is currently limited, multiple reports indicate that melatonin, by promoting growth and development, enhancing antioxidant systems, and stimulating enzymes associated with vitamin biosynthesis, can indirectly increase their accumulation in plants [32].

Table 2. The effect of melatonin on B vitamins of Pansy (*Viola* × *wittrockiana*)

Treatments (mg L^{-1})	Thiamin (µg g^{-1} DW)	Riboflavin (µg g^{-1} DW)	Niacin (µg g^{-1} DW)	Folic acid (µg g^{-1} DW)
Control	2.24±0.11^{d}	0.095±0.02^{d}	93.48±1.5^{d}	0.38±0.01^{d}
Melatonin 50	2.49±0.13^{c}	0.106±0.01^{c}	109.31±1.64^{c}	0.41±0.02^{c}
Melatonin 100	2.78±0.10^{b}	0.121±0.01^{b}	118.37±1.43^{b}	0.46±0.01^{b}
Melatonin 150	3.17±0.12^{a}	0.134±0.02^{a}	131.24±1.38^{a}	0.52±0.03^{a}
Melatonin 200	3.05±0.10^{a}	0.130±0.03^{a}	128.45±1.31^{a}	0.53±0.02^{a}

Values marked by different letters are significantly different (P< 0.05).

3.6. Carbohydrates

The results showed that the highest carbohydrate content in pansy petals (65.42 mg g^{-1} FW) was observed in the 200 mg L^{-1} melatonin treatment, while the lowest value (35.82 mg g^{-1} FW) was recorded in the control (Figure 5). The significant increase in carbohydrate content due to melatonin application may be attributed to its positive effect on photosynthesis and the enhancement of carbohydrate biosynthesis. Melatonin, by increasing chlorophyll content, reducing oxidative stress, protecting chloroplast and cellular membranes, and safeguarding macromolecules such as proteins and enzymes, can promote carbohydrate accumulation in plants [34]. Similarly, Alami et al. (2024) reported an increase in carbohydrate content in basil (*Ocimum basilicum* L.) following melatonin treatment [35].

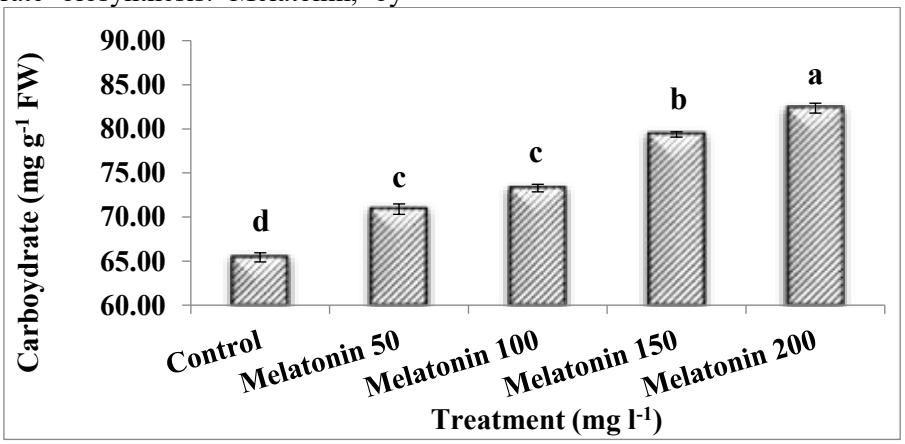

Fig 5 Effect of melatonin on carbohydrate of Pansy (*Viola* × *wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05

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3.7. Phenolic Compounds

The results showed that the highest and lowest phenolic contents in pansy petals were 19.85 and 12.29 mg g^{-1} FW, respectively, observed in the 150 mg L^{-1} melatonin treatment and the control (Figure 6). Melatonin enhances phenolic accumulation by stimulating biosynthetic pathways, including the phenylpropanoid pathway, and increasing the activity of enzymes such as phenylalanine ammonia-lyase [36]. Similarly, melatonin

application has been reported to increase phenolic content in coriander (*Coriandrum sativum* L.) and dill (*Anethum graveolens* L.) [37].

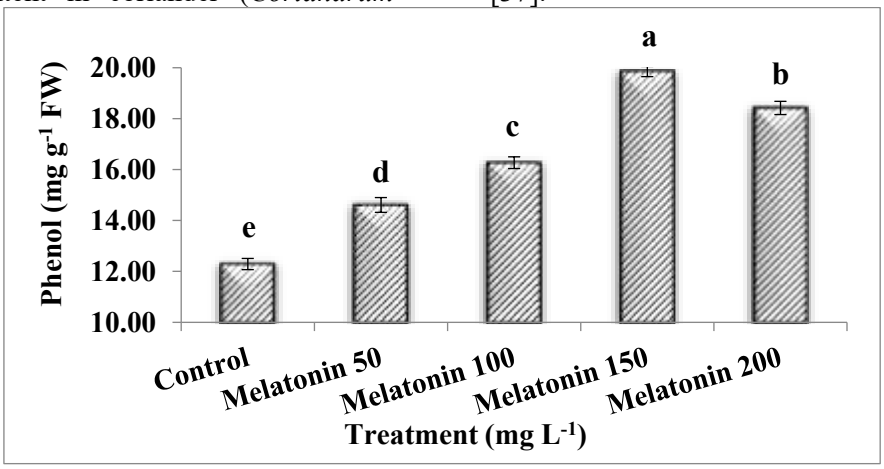

Fig 6 Effect of melatonin on phenol of Pansy (*Viola* × *wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

3.8. Proteins

The results indicated that the highest protein content (14.76 µg mg⁻¹ FW) was obtained with 200 mg L⁻¹ melatonin, while the lowest (11.32 µg mg⁻¹ FW) was recorded in the control (Figure 7). Melatonin can enhance the expression of genes related to growth, development, photosynthesis, and cellular metabolism, and acts as a plant antioxidant to prevent protein oxidation, thereby maintaining protein structure and function [37]. Zare Zeinali et al. (2020) also reported that melatonin increased protein content in marigold (*Tagetes erecta*), and similar effects were observed in marigold (*Calendula officinalis* L.) [13, 38].

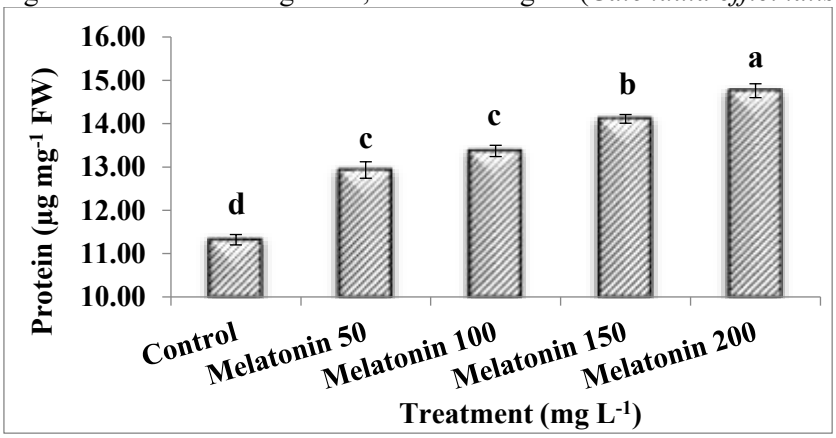

Fig 7 Effect of melatonin on protein content of Pansy (*Viola* × *wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

3.9. Total Antioxidant Activity

The highest total antioxidant activity (53.6%) was observed in the 150 mg L⁻¹ melatonin treatment, while the lowest value (37.1%) was recorded in the control (Figure 8). The increase in antioxidant activity is attributed to melatonin's role as an antioxidant, which enhances both enzymatic and non-enzymatic antioxidant activities [32]. Consistent with these findings, melatonin application increased total antioxidant activity in strawberry (*Fragaria* × *ananassa* cv. Camarosa) [39] and marigold (*Calendula officinalis* L.) [13].

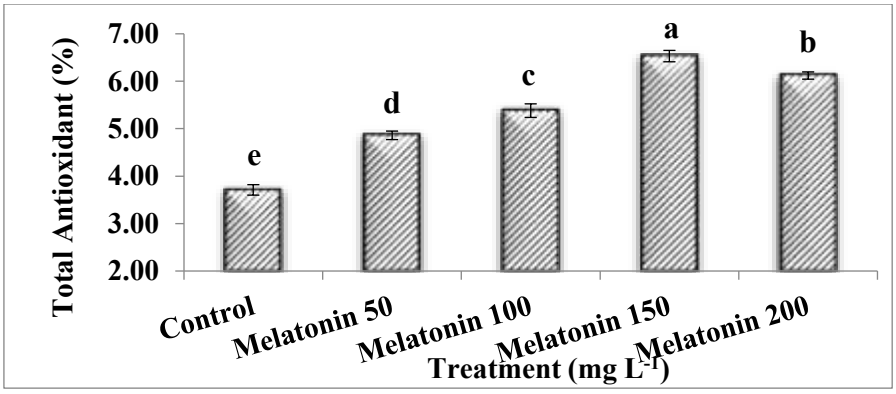

Fig 8 Effect of melatonin on total antioxidant of Pansy (*Viola* × *wittrockiana*)

(Means with the same letter(s) in each column are not significantly different at P≤0.05)

4. Conclusion

Considering that edible flowers possess numerous health-promoting properties, the use of natural compounds, such as melatonin, can enhance their nutritional value. The present study demonstrated that melatonin application enhanced the nutritional traits of pansy (*Viola* × *wittrockiana*). The highest cell membrane stability index, total chlorophyll, carotenoids, total antioxidant capacity, vitamin C, thiamine, riboflavin, niacin, and phenolic content were observed with 150 mg L^{-1} melatonin. In contrast, the highest folic acid, carbohydrate, and protein contents were obtained with 200 mg L^{-1} melatonin. Overall, the application of 150 mg L^{-1} melatonin had a positive effect on the nutritional quality of pansy.

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مقاله علمی_پژوهشی

تاثیر ملاتونین بر ارزش تغذیه‌ای و فعالیت آنتی‌اکسیدانی گل بنفشه Pansy (*Viola* × *wittrockiana*)

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چکیده

گل‌های خوراکی منبع بسیار خوبی از ترکیب‌های تغذیه‌ای، فیتوشیمیایی و آنتی‌اکسیدانی هستند که محبوبیت آن روز به روز در بین مصرف کنندگان آن‌ها در حال افزایش است. گیاه بنفشه نیز به‌عنوان گل خوراکی علاوه بر خواص دارویی آن دارای کاربردهای فراوانی در انواع غذاها و نوشیدنی‌ها می‌باشد. همچنین مطالعات اخیر نشان داده است که کاربرد ملاتونین ممکن است اثرات مثبتی بر کیفیت گیاهان داشته باشد. این پژوهش با هدف بررسی اثر ملاتونین بر خصوصیات تغذیه‌ای گل بنفشه در قالب طرح کاملاً تصادفی با سه تکرار در گلخانه اجرا شد. تیمارها شامل ملاتونین با غلظت‌های صفر، ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی‌گرم در لیتر بود که طی دو مرحله اعمال شد. مرحله اول دو هفته پس از انتقال نشاءها و مرحله دوم ده روز بعد، مجدد تکرار شد و یک هفته پس از آخرین محلول‌پاشی، برداشت گل‌ها برای ارزیابی صفات مورد نظر انجام شد. نتایج نشان داد تیمار ملاتونین تاثیر معنی‌داری بر صفات مورد ارزیابی داشت، بیشترین شاخص ثبات غشاء سلول (۸۷/۵۶ درصد)، محتوای کاروتنوئید (۵/۵۸ میلی‌گرم در گرم)، کلروفیل کل (۵/۳۸ میلی‌گرم در گرم)، فعالیت آنتی‌اکسیدانی کل (۶/۵۳ درصد)، میزان ویتامین‌های ث (۴۹/۲۵ میلی‌گرم در ۱۰۰ گرم وزن تر)، تیامین (۳/۱۷ میکروگرم در گرم)، ریبوفلاوین (۰/۱۳۴ میکروگرم در گرم)، نیاسین (۱۳۱/۲۴ میکروگرم در گرم) و فنل (۱۹/۸۵ میلی‌گرم در گرم) در تیمار ملاتونین ۱۵۰ میلی‌گرم در لیتر و بیشترین میزان اسید فولیک (۰/۵۳ میکروگرم در گرم)، کربوهیدرات (۸۲/۳۵ میلی‌گرم در گرم) و پروتئین (۱۴/۷۶ میکروگرم در گرم) در تیمار ملاتونین ۲۰۰ میلی‌گرم در لیتر بود در حالیکه کمترین میزان صفات در شاهد مشاهده شد. با توجه به نتایج به‌دست آمده کاربرد ملاتونین تاثیر مثبتی در افزایش ارزش تغذیه‌ای گل بنفشه (*Viola* × *wittrockiana*) نشان داد.