



## THE EFFECTS OF PRE-HARVEST MELATONIN APPLICATIONS ON PHYTOCHEMICAL PROPERTIES OF CRIMSON SEEDLESS GRAPE VARIETY (*V. vinifera* L.)

Demir KÖK<sup>1\*</sup>, Erdinç BAL<sup>1</sup>, Ali İzzet TORÇUK<sup>2</sup>, Onur ERGÖNÜL<sup>2</sup>

<sup>1</sup>Tekirdağ Namık Kemal University, Faculty of Agriculture, Department of Horticulture, 59300, Tekirdağ, Türkiye

<sup>2</sup>Tekirdağ Viticulture Research Institute, Republic of Türkiye Ministry of Agriculture and Forestry, Growing Techniques Department, 59300, Tekirdağ, Türkiye

**Abstract:** Foliar melatonin applications are crucial for grape quality as they can enhance skin color development, increase antioxidant capacity and nutritional value of grapes. The effectiveness of preharvest melatonin applications may change depending on fruit species, variety, application time, and dose. In the current study, it was utilized from various doses of melatonin application, including 0, 0.25, 0.50, and 1 mmol l<sup>-1</sup> for improving the phytochemical attributes of Crimson Seedless table grape variety. The results of the principal component analysis showed that different doses of foliar melatonin application had different effects on the yield and biochemical attributes of grape variety. But particularly, 1 mmol l<sup>-1</sup> and 0.50 mmol l<sup>-1</sup> of melatonin doses had significant effects on total phenolic compounds content and antioxidant capacity from the phytochemical properties of Crimson Seedless table grape variety.

**Keywords:** Table grape, N-acetyl-5-methoxytryptamine, Phenolic compounds, Antioxidant capacity, CIELAB color system

\*Corresponding author: Tekirdağ Namık Kemal University, Faculty of Agriculture, Department of Horticulture, 59300, Tekirdağ, Türkiye

E mail: dkok@nku.edu.tr (D. KÖK)

Demir KÖK  <https://orcid.org/0000-0001-5879-8535>

Erdinç BAL  <https://orcid.org/0000-0001-9817-5842>

Ali İzzet TORÇUK  <https://orcid.org/0000-0001-9310-8704>

Onur ERGÖNÜL  <https://orcid.org/0000-0002-2251-426X>

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### 1. Introduction

Crimson Seedless is a widely cultivated late-ripening grape type with firm, crisp berries and exceptional flavor (Martinez-Gil et al., 2013; Amaro et al., 2020). A wide range of phytochemicals and natural metabolites found in grapes such as phenolic acids, ascorbic acids, carotenoids, and flavonoids, significantly contribute to their quality, appearance, and flavor as well as a host of other biological functions and health benefits (Waterhouse, 2002; Cosme et al., 2018; Averilla et al., 2019; Albuquerque et al., 2021; Yakhchi et al., 2023). As a result, one of the main areas of focus in grape production has been on enhancing the phytochemical properties of grapes. Numerous variables, including ecological circumstances, cultivar, rootstock, and various viticultural techniques used in vineyards, have an impact on the phytochemical concentrations and compositions in grapes (Obreque-Slier et al., 2010; Kok, 2022 a, b).

All living things as well as certain plant species like *Rosaceae*, *Poaceae*, *Apiaceae*, *Brassicaceae* and *Vitaceae*, contain the chemical melatonin, which has a low molecular weight and an indole-based structure (Dubbels et al., 1995; Nawaz et al., 2016). The physiological processes of ripening, aging, and defense in fruits and vegetables are all regulated by the endogenous bioactive chemical melatonin (Li et al., 2017). However, the effects

of preharvest melatonin applications on fruit ripening on the plant and quality at harvest have been evaluated in very few studies, and different effects have been reported depending on the fruit types or application doses and times.

Shang et al. (2021) state that melatonin induction triggers the accumulation of total phenolic compounds, flavonoids, and anthocyanins in blueberry fruits and increases the antioxidant capacity of the fruits during the physiological processes of the fruits.

A study conducted by Xia et al. (2021) disclosed that melatonin applications increased soluble solids content in grapes by enhancing sucrose phosphate synthase activity, which is important for the synthesis of anthocyanin.

Vitallini et al. (2011) stated that, as a result of their study, there was a synergistic effect between melatonin and grape polyphenols, resulting in high antiradical activity.

This study primarily aimed to determine the impacts of various doses of pre-harvest melatonin application on the yield and biochemical properties of Crimson Seedless table grape variety.



**2. Materials and Methods**

The study was performed in an experimental vineyard located in Tekirdağ Viticulture Research Institute, Türkiye (40°58'18.56" N; 27°28" E) in the 2019 vegetation period. In the current research, 5-year-old Crimson Seedless grapevines grafted onto Kober 5 BB (*V. berlandieri* x *V. ruspetris*) rootstock were employed as plant materials. Grapevines were trained to shoot vertically on a trellis (VSP). During the conduct of research, different cultural practices such as fertilization, winter pruning, and green pruning practices were carried out in the vineyard. A standard control program was also used against diseases and pests during the growing period. The climate feature of the research region is a Mediterranean-like warm-temperate climate, and the annual average temperature, daily sunshine duration, relative humidity and total precipitation were recorded as 15.60°C, 4.34 hours, 70.49%, and 334.6 mm, respectively for the 2019 year.

With the aim of improving the phytochemical characteristics of Crimson Seedless table grape variety, powder melatonin (N-acetyl-5-methoxytryptamine) (Sigma-Aldrich, >98%) was used in this research. For this purpose, aqueous solutions of melatonin were prepared in 4 different doses, including 0, 0.25, 0.50, and 1.00 mmol l<sup>-1</sup>.

In the vineyards, uniform grapevines were identified before the research started. In the study, foliar melatonin applications were implemented twice at 10-day intervals, including 10 days before the véraison period (first time) and at the véraison period (second time) by means of a back pump. As the surfactant, tween 20 (0.1 ml l<sup>-1</sup>) was added to all spray solutions of melatonin to increase its effectiveness. Later, various doses of foliar melatonin were sprayed on all grapevines until run-off in the early morning period between 6:00 AM and 9:30 AM, when the temperature was not yet effective.

Berry weight (g), berry length (mm), berry width (mm), bunch weight (g), bunch length (cm), bunch width (cm), and berry firmness (N) were measured as yield variables in the research. In addition, total soluble solids (TSS) (%), titratable acidity (TA) g l<sup>-1</sup>, maturity index, must pH, chromatic parameters like a\*, b\*, c\*, L\*, hue angle (h°), total phenolic compounds (mg GAE 100 g<sup>-1</sup> fw) and antioxidant capacity (µmol TE 100 g<sup>-1</sup> fw) were also found as biochemical variables.

The grapes on control grapevines of the Crimson

Seedless type were harvested in 2019 during the vegetative period when the berries on the control grapevines reached about 16% TSS. After harvest, measurements and analyses for yield and biochemical properties were hastily conducted. Initially, 250 berries were used to determine TSS, TA, and pH. For chromatic measurements and analysis of total phenolic compounds and total antioxidant capacity, samples consisting of 350 berries were separated and stored at -25°C until analysis. The maturity index was calculated as the ratio of TSS (%) to TA content (%) (Perralta-Ruiz et al., 2020).

Grape skin colors were measured as CIE L\*, a\*, b\* color system by using a Hunter-Lab Colorimeter (Hunter Lab DP-9000 Color, USA) (McGuire, 1992).

The total phenolic content of berries was determined using a calorimetric assay and Folin-Ciocalteu reagent (Sigma) through the reagent method (Slinkard and Singleton 1977). Total phenolic compound content was calculated using the spectrophotometer at 765 nm and the results were explained in mg of gallic acid equivalent (mg GAE 100 g<sup>-1</sup> fw).

Antioxidant capacity was assessed via the 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method as described by Brand-Williams et al. (1995) with some modifications. A dose-response curve was generated using Trolox as a standard and the antioxidant capacity was expressed as Trolox equivalent (µmol TE 100 g<sup>-1</sup> fw).

**2.1. Statistical Analysis**

The research was carried out in a completely randomized block design with four replicates. For all data, SPSS statistical software (version 20.0) was used and significant differences were assessed using one-way analysis of variance (ANOVA). Statistical differences for average comparisons were evaluated using the LSD multiple range test at the 5% level.

**3. Results and Discussion**

**3.1. Yield Properties**

The physical properties of fruit species are one of the most important factors that affect fruit quality (Minas et al., 2018). Overall, doses of melatonin application exhibited the same pattern of increase in berry characteristics in the current study, (Table 1). Diverse doses of foliar melatonin applications have no statistical effects on berry length (P<0.05) and the means of berry length varied from 2.12 mm (0.25 mmol l<sup>-1</sup>) to 2.21 mm (0 mmol l<sup>-1</sup>).

**Table 1.** Influences of diverse doses of foliar melatonin applications on yield parameters of Crimson Seedless grape variety

Applications (mmol l <sup>-1</sup> )	Berry Length (mm)	Berry Width (mm)	Berry Weight (g)	Berry firmness (N)	Bunch Length (cm)	Bunch width (cm)	Bunch weight (g)
M 0.00	2.21	1.69	4.18	5.91 <sup>b</sup>	18.60	14.53	521.33
M 0.25	2.12	1.61	3.69	5.69 <sup>b</sup>	18.20	15.93	603.13
M 0.50	2.15	1.64	3.89	6.12 <sup>ab</sup>	18.53	15.26	586.40
M 1.00	2.19	1.65	3.84	6.89 <sup>a</sup>	18.80	14.13	490.66
LSD <sub>5%</sub>	N.S.	N.S.	N.S.	1.33	N.S.	N.S.	N.S.

Different letters stand for significant differences in each column at 5% level in accordance with the LSD multiple range test. M 0.00: 0 mmol l<sup>-1</sup>, M 0.25: 0.25 mmol l<sup>-1</sup>, M 0.50: 0.50 mmol l<sup>-1</sup>, M 1.00: 1.00 mmol l<sup>-1</sup> N.S.: Non-significant

Concerning berry width (Table 1), there are no significant differences among the diverse doses of melatonin application (P<0.05). In the study, melatonin-applied grapevines had lower berry width means, including 1.61 mm (0.25 mmol l<sup>-1</sup>), 1.64 mm (0.50 mmol l<sup>-1</sup>), and 1.65 mm (1.00 mmol l<sup>-1</sup>) compared to 0 mmol l<sup>-1</sup> (1.69 mm).

As far as berry weight is concerned shown in Table 1, berry weight is not significantly influenced by diverse doses of foliar melatonin application (P<0.05). In the research, the highest berry weight mean was recorded for 0 mmol l<sup>-1</sup> (4.18 g), followed by 0.50 mmol l<sup>-1</sup> (3.89 g), 1.00 mmol l<sup>-1</sup> (3.84 g) and 0.25 mmol l<sup>-1</sup> (3.69 g).

As shown in Table 1, berry firmness is significantly influenced by the diverse doses of foliar melatonin application (P<0.05). In the study, the lowest berry firmness means were respectively 5.69 and 5.91 N for 0.25 mmol l<sup>-1</sup> and 0 mmol l<sup>-1</sup>, whereas the highest berry firmness mean was 6.89 N for 1.00 mmol l<sup>-1</sup>.

With regard to bunch length (Table 1), diverse doses of foliar melatonin application have no significant effects on bunch length (P<0.05) and the lowest bunch length mean was 18.20 cm for 0.25 mmol l<sup>-1</sup>, whereas the highest bunch length mean was 18.80 cm for 1.00 mmol l<sup>-1</sup>.

As regards bunch width, it is seen in Table 1 that diverse doses of foliar melatonin application do not significantly affect bunch width (P<0.05). While the highest bunch width mean was 15.93 cm for 0.25 mmol l<sup>-1</sup> in the study, the lowest bunch width mean was 14.13 cm for 1.00 mmol l<sup>-1</sup>.

In view of the bunch weight, it is apparent in Table 1 that diverse doses of foliar melatonin applications have no statistical effects on bunch weight (P<0.05). In the present study, bunch weight means altered from 490.66 g (1.00 mmol l<sup>-1</sup>) to 603.13 g (0.25 mmol l<sup>-1</sup>).

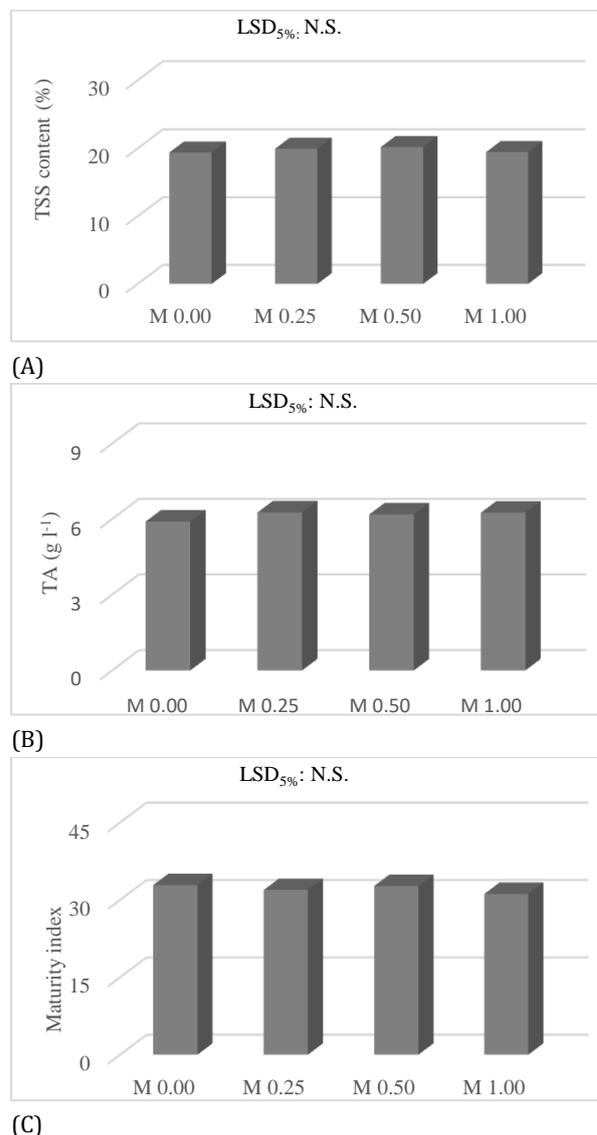
### 3.2. Biochemical Properties

Sugar is the main soluble solid in grape musts and sweet wines and the quantity of soluble solids is a leading indicator of grape maturity (Jackisch, 1985). With regard to TSS content demonstrated in Figure 1A, there are no differences among the diverse doses of foliar melatonin application (P<0.05). In the current study, the lowest TSS content mean was 19.40% for 0 mmol l<sup>-1</sup>, whereas the highest TSS content mean was 20.20% for 0.50 mmol l<sup>-1</sup>.

There are numerous organic acids such as malic acid, tartaric acid, and citric acid in grapes and these organic acids greatly contribute to the organoleptic quality of grapes and wines (Silva et al., 2015). In connection with TA pointed out in Figure 1B, TA is insignificantly affected by diverse doses of foliar melatonin application (p< 0.05) and TA means changed from 5.90 g l<sup>-1</sup> (0 mmol l<sup>-1</sup>) to 6.26 g l<sup>-1</sup> (0.25 mmol l<sup>-1</sup>). While melatonin treatments were not clearly altering titratable, tartaric, and malic acids in

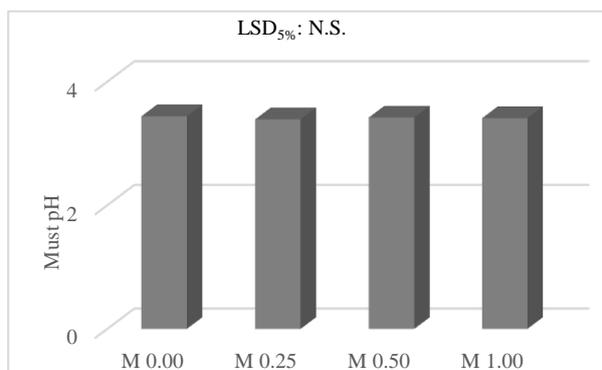
a study led by Xu et al. (2017), the contents of titratable acids in another study led by Meng et al. (2019) were increased by melatonin treatments. But melatonin treatments had no significant effects on TSS content.

The TSS/TA ratio is considered the maturity index employed to establish the ideal harvest time for grapes (Liang et al., 2005). On the subject of the maturity index represented in Figure 1C, it is noteworthy that diverse doses of foliar melatonin application have no effects on the maturity index (p <0.05). While the highest maturity index mean was 32.88 for 0 mmol l<sup>-1</sup>, the lowest maturity index mean was 31.16 for 1.00 mmol l<sup>-1</sup> in the study.



**Figure 1.** Influences of diverse doses of foliar melatonin applications on TSS (A), TA (B) content and maturity index (C).

The pH of grape juice must normally vary between 3.0 and 4.0 depending on metabolic activities (Bisson et al., 2007). In relation to must pH exhibited in Figure 2, there are no significant differences among the diverse doses of foliar melatonin application (p<0.05) and pH means ranged from 3.39 (0.25 mmol l<sup>-1</sup>) to 3.44 (0 mmol l<sup>-1</sup>).



**Figure 2.** Influences of diverse doses of foliar melatonin applications on must pH.

Skin color is remarkable for table grape quality and consumer acceptance and the CIELAB color system is used for color description in various fruit species (Sahin and Sumnu, 2006). In the CIELAB color system, a\* value represents redness (+a) and greenness (-a). When it comes to a\* values of grape skin denoted in Table 2, it is viewed that a\* values are significantly influenced by diverse doses of foliar melatonin application (p<0.05). In the present study, the lowest a\* value mean was recorded for 0.25 mmol l<sup>-1</sup> (4.96), whereas the highest a\* value means were respectively recorded for 1.00 mmol l<sup>-1</sup> (6.25), 0 mmol l<sup>-1</sup> (6.39) and 0.50 mmol l<sup>-1</sup> (6.76).

In the CIELAB color system, the b\* value indicates the yellowness (+b) and blueness (-b) (Sahin and Sumnu, 2006). Regarding b\* values of grape skin manifested in

Table 2, diverse doses of foliar melatonin applications have no statistical effects on b\* values (p <0.05) and b\* value means in the study varied from 2.27 (0.25 mmol l<sup>-1</sup>) to 2.77 (0.50 mmol l<sup>-1</sup>).

Chroma is considered the quantitative property of color and is used to determine the degree of difference between a color tone and a gray color with the same lightness (Pathare et al., 2013). Regarding the c\* values of grape skin illustrated in Table 2; it is notable that diverse doses of foliar melatonin application have significant effects on c\* values (P<0.05). In the study, the highest c\* value means were successively obtained from 0.50 mmol l<sup>-1</sup> (7.44), 0 mmol l<sup>-1</sup> (6.96), and 1.00 mmol l<sup>-1</sup> (6.75) when compared with 0.25 mmol l<sup>-1</sup> (5.67).

In the CIELAB color system, the L value shows lightness from black (0) to white (100) (Sahin and Sumnu, 2006). Concerning L\* values of grape skin demonstrated in Table 2, there are no differences among the diverse doses of foliar melatonin application (P<0.05), and L\* values in the current study differed from 36.19 (1.00 mmol l<sup>-1</sup>) to 37.13 (0.50 mmol l<sup>-1</sup>).

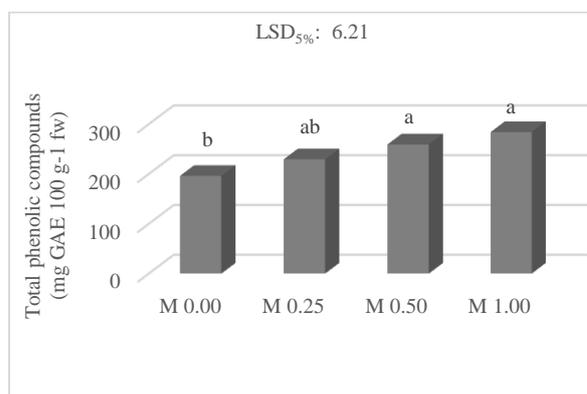
Hue angle is considered the qualitative property of color and is employed to define the difference between a certain color and a gray color with the same lightness (Pathare et al., 2013). In reference to hue angle (h°) attributes of grape skin demonstrated in Table 2, diverse doses of foliar melatonin application have no significant effects on hue angle (P<0.05). The lowest hue angle mean was 21.14 (0.50 mmol l<sup>-1</sup>) in this study, whereas the highest mean was 36.31 (0.25 mmol l<sup>-1</sup>).

**Table 2.** Influences of diverse doses of foliar melatonin applications on chromatic attributes of gape skin

Applications (mmol l <sup>-1</sup> )	a*	b*	c*	L*	h°
M 0.00	6.39 <sup>a</sup>	2.58	6.96 <sup>a</sup>	36.37	24.68
M 0.25	4.96 <sup>b</sup>	2.27	5.67 <sup>b</sup>	36.41	36.31
M 0.50	6.76 <sup>a</sup>	2.77	7.44 <sup>a</sup>	37.13	21.14
M 1.00	6.25 <sup>a</sup>	2.41	6.75 <sup>a</sup>	36.19	24.21
LSD <sub>5%</sub>	1.23	N.S.	1.14	N.S.	N.S.

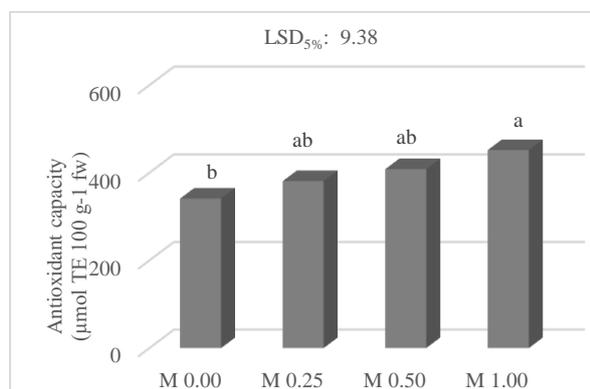
Different letters stand for significant differences in each column at 5% level in accordance with LSD multiple range test. M 0.00= 0 mmol l<sup>-1</sup>, M 0.25= 0.25 mmol l<sup>-1</sup>, M 0.50: 0.50 mmol l<sup>-1</sup>, M 1.00= 1.00 mmol l<sup>-1</sup>. N.S. = non-significant

Phenolic composition in grapes is broadly influenced by different factors, including viticulture practices, ecological conditions, and grape variety (Downey et al., 2006). As for the total phenolic compound contents displayed in Figure 3, it is discerned that diverse doses of foliar melatonin applications significantly influence the total phenolic compound contents (P<0.05). In the existing study, the highest total phenolic compound means were recorded for 1.00 mmol l<sup>-1</sup> (283.96 mg GAE 100 g<sup>-1</sup> fw) and 0.50 mmol l<sup>-1</sup> (259.10 mg GAE 100 g<sup>-1</sup> fw) compared to 0 mmol l<sup>-1</sup> (195.96 mg GAE 100 g<sup>-1</sup> fw). According to results of a study conducted by Meng et al. (2015), it has been concluded that melatonin treatments of pre-veraison grape berries alter wine aroma components of Merlot grape variety.



**Figure 3.** Influences of diverse doses of foliar melatonin applications on total phenolic compounds.

The grape has high levels of antioxidant capacity and is an important fruit species that contributes the most to antioxidant capacity (Gross, 2016). With respect to the antioxidant capacity indicated in Figure 4, it is clear that antioxidant capacity is significantly affected by diverse doses of foliar melatonin applications ( $P < 0.05$ ). While the highest antioxidant capacity mean was 451.96 mol TE 100 g<sup>-1</sup> fw in this study, the lowest mean was 341.26 mol TE 100 g<sup>-1</sup> fw (0 mmol l<sup>-1</sup>). The results of a study conducted by Xu et al. (2017) revealed that the contents of 18 of the 22 detected individual phenolic compounds were enhanced by melatonin treatment; especially, the resveratrol content was largely increased concomitantly with the up-regulation of STS gene expression. In the meantime, melatonin treatments enhanced the antioxidant capacity of berries. Yang et al. (2020) also declared that exogenous melatonin significantly enhanced the biosynthesis of each flavonol and flavanol component of cv. Kyoho, especially catechin, which was almost increased double by 200 μM of melatonin treatment.



**Figure 4.** Influences of diverse doses of foliar melatonin applications on antioxidant capacity.

#### 4. Conclusion

In recent times, there has been great interest in harnessing melatonin applications to boost the quality characteristics of grape varieties. Foliar melatonin applications may greatly enhance the nutritional contents and phytochemical properties of grapes. In the present study, it was found that various concentrations of foliar melatonin significantly and favorably affected the phytochemical characteristics of the table grape variety. Due to the mentioned properties of melatonin, applications of pre-harvest foliar melatonin may be useful for raising grape quality in viticulture. As a consequence of this research, 1.00 mmol l<sup>-1</sup> and 0.50 mmol l<sup>-1</sup> of melatonin concentrations were found to be effective for improving phenolic compounds and antioxidant properties of Crimson Seedless table grape variety.

#### Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	D.K.	E.B.	A.İ.T.	O.E.
C	40	40	10	10
D	50	50		
S	25	25	25	25
DCP	25	25	25	25
DAI	50	50		
L	100			
W	100			
CR	50	50		
SR	50	50		
PM	30	30	20	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

#### Conflict of Interest

The authors declared that there is no conflict of interest.

#### Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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