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Melatonin Treatment of Pomegranate Trees Increases Crop Yield and Quality Parameters at Harvest and during Storage

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Abstract: With the aim to study the effect of melatonin treatment of pomegranate trees on crop yield and fruit quality at harvest and during storage, two experiments were carried out in two consecutive years: 2017 and 2018. In the first year, trees were treated with melatonin (at 0.1 and 1 mM) along the developmental growth cycle and fruit quality parameters were evaluated at harvest and during storage at 10 °C for 90 days. Treatments with melatonin led to an increase of crop yield (number of fruits per tree and kg per tree), as well as higher fruit quality attributes, such as fruit size (diameter and weight), color, total soluble solids (TSS), and total acidity (TA), especially with the 0.1 mM dose. Then, in the second year, melatonin at 0.1 mM was selected for repeating the pre-harvest treatments with similar results in terms of crop yield and fruit quality parameters. During storage, pomegranate fruit treated with 0.1 mM melatonin maintained higher quality attributes than controls, such as TSS, TA, and firmness and lower weight losses were observed in fruit from treated trees, in both trials. In addition, the content of the major sugars (glucose and fructose) and organic acids (malic, succinic and ascorbic acid) were higher in melatonin-treated than in non-treated fruit. These results suggest that pre-harvest melatonin treatment could be a useful tool to increase pomegranate crop yield as well as fruit quality parameters at harvest and their maintenance during storage due to an effect of melatonin on reducing the postharvest ripening process.

Keywords: *Punica granatum*; firmness; color; sugars; organic acids; ripening

Citation: Medina-Santamarina, J.; Serrano, M.; Lorente-Mento, J.M.; García-Pastor, M.E.; Zapata, P.J.; Valero, D.; Guillén, F. Melatonin Treatment of Pomegranate Trees Increases Crop Yield and Quality Parameters at Harvest and during Storage. *Agronomy* **2021**, *11*, 861. https://doi.org/10.3390/agronomy11050861

Academic Editor: Marios Kyriacou

Received: 9 April 2021
Accepted: 26 April 2021
Published: 28 April 2021

1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a natural-occurring compound derivative of tryptamine, and plays important roles in plant growth, such as delay of senescence process and increased tolerance to both biotic and abiotic stress [1]. These effects have been attributed to the melatonin action as a natural antioxidant molecule scavenging free radicals either in vitro as in vivo studies using different vascular plants [2]. The melatonin content in edible fruit has been associated with health-benefits, since high levels of this compound in foods are beneficial for consumers. Thus, the consumption of a Jerte Valley cherry product enhances mood and increases 5-hydroxyindoleacetic acid but reduces cortisol levels in urine, which may protect against stress and act as a mood enhancer by increasing serotonin availability in the organism, particularly with advancing age [3].

As postharvest treatments, melatonin delayed physiological deterioration of cassava roots and reduced the accumulation of H_2O_2 while increasing the activity of antioxidant enzymes, and thus maintaining homeostasis of cellular reactive oxygen species (ROS) through increasing the endogenous melatonin levels [4]. In tomato fruit, postharvest treatment with melatonin at 50 µM stimulated the content of anthocyanins, and might be positively related to fruit ripening but negatively related to fruit senescence, since the proteins related

with senescence were downregulated while an increase of catalase and peroxidase was observed in treated fruit [5]. In peaches, postharvest application of melatonin at 0.1 mM reduced weight loss, decay incidence, and respiration rate as well as maintained firmness, total soluble solids and ascorbic acid contents during seven days of storage at 25–28 °C, with a concomitant increase in antioxidant enzymes and maintenance of membrane integrity, which might be a part of the mechanism implicated on delaying senescence in peach fruit [6].

As pre-harvest treatment, very little evidence exists about the role of melatonin on fruit growth and ripening, and different effects have been reported depending on fruit species, concentration, or time of application. Thus, melatonin was naturally found in grape tissues (skin, flesh and seed), and, during ripening, melatonin content decreased in skin, while it increased in both seed and flesh tissues [7]. Melatonin at 100 mg L^{-1} (0.43 mM), applied once or twice to grapevines at pre-veraison stage, significantly increased (6.6%) the grape berry size, which was correlated with increased concentration of endogenous melatonin [8]. In two cherry cultivars ('Hondeng' and 'Rainier'), melatonin was evaluated during the growth cycle, and a maximum peak at stage 2 was found, which coincided with endocarp lignification in both cherry cultivars [9] and in 'Prime Giant' sweet cherry, melatonin 0.1 and 0.01 mM applied at pit hardening inhibited fruit ripening [10]. However, irrigation of tomato plants with 0.1 mM melatonin increased sugar and lycopene concentration in fruits, showing a positive effect on fruit ripening [11], while, in apricots, foliar spray melatonin treatment increased yield and fruit weight, although no effect on on-tree ripening was observed [12].

Pomegranate is one of the oldest known edible fruits which has gained popularity and scientific interest in the last several years due to its nutritional value and health benefits, since it is very rich in bioactive compounds with antioxidant activity [13,14]. The quality parameters for pomegranate are based on external attributes such as size, shape, and color of the skin, as well as on internal ones such as aril color, sugar and acid content, and the presence of small and soft seeds [15,16]. The color of the pomegranate increases in both skin and arils during ripening, due to the accumulation of anthocyanins, the major in the sweet varieties being cyanidin 3-glucoside, followed by delphinidin 3,5-diglucoside and pelargonidin 3-glucoside, as well as sugar content, while decreases occur on acid content and fruit firmness [16–19]. However, during postharvest storage, pomegranate exhibits important quality losses due to several physiological and enzymatic disorders, the major ones being weight loss, together with loss of firmness, aril color, and acidity, which lead to a reduction of consumers' acceptability in terms of freshness, juiciness, and taste [15,16,19–21].

However, no literature is available about the effect of pre-harvest melatonin treatment on pomegranate growth and ripening on-tree, or on quality attributes at harvest and during postharvest storage, which has been the main objective of this paper. In addition, the effect of melatonin treatment on fruit yield was evaluated.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Pomegranate (*Punica granatum* L. cv. Mollar de Elche) trees (10 years-old) were used for this study. The experiment was carried out during the developmental cycle of the 2017 and 2018 spring−summer periods, in a commercial plot located at Elche (Alicante, Spain), and full blossom (FB) was established on 30 April and 9 May, in the 2017 and 2018 experiments, respectively. During 2017, 5 trees were selected for each treatment: control (distilled water) and melatonin (purchased from Sigma, Sigma-Aldrich, Madrid, Spain) at 0.1 mM or 1 mM concentrations. Freshly prepared solutions (containing 0.05% Tween 20) were foliar sprayed with a mechanical mist sprayer and repeated at five dates of the growth cycle (30, 60, 90, 105, and 120 days after full blossom: T1, T2, T3, T4, and T5). Five fruits were labeled around the equatorial perimeter of each tree, in which fruit growth was followed by measuring cheek diameter. Fruits were harvested according to commercial

criteria, when external color and size characteristic of this cultivar were acquired, and ca. 15° Brix. However, the ripening process of pomegranate fruit is heterogeneous in the tree and, therefore, some fruit reaches their commercial ripening stage before others. Thus, two harvests (first and second harvest) were carried out ten days apart, in which yield (kg tree^{-1} and number of fruit tree^{-1}), and fruit weight were determined. Immediately after harvest, pomegranate fruit of the first harvest (about 200 fruit from each treatment) were transferred to the laboratory, sorted, and 120 homogeneous fruit were grouped in lots of 10 pieces of fruit. Fruit was stored at 10 °C (a non-chilling temperature for pomegranate fruit) and 85% relative humidity in cardboard boxes covered with perforated films. Three lots of 10 pieces of fruit were taken at random for each treatment, and sampling date (0, 30, 60 and 90 days) in which fruit firmness, weight loss, total soluble solids (TSS), total acidity (TA), the ratio between TSS/TA (ripening index) and color (external and internal) were determined. During 2018, and considering the best results obtained, the 0.1 mM concentration was chosen for repeating the experiment by choosing 5 different trees for each treatment in the same commercial plot. As previously mentioned, in the 2017 experiment, two different harvests were performed and, for each yield (kg tree^{-1} and number of fruit tree^{-1}), fruit weight was again determined. Fruit from the second harvest date was transferred to the laboratory for storage at 10 °C, as performed in the 2017 experiment. After 0, 30, 60, and 90 days of storage, three lots from each treatment were taken at random, in which the above-mentioned parameters were evaluated. In addition, the composition of individual sugars and organic acids was analyzed in control and melatonin-treated pomegranates at harvest time.

2.2. Fruit Growth and Crop Yield

The evolution of fruit growth was determined in the labeled fruit from T1 treatment until the first harvest date by measuring the fruit diameter (mm) at 7–10 day intervals by using a Vernier digital calliper. For each harvest date, crop yield was expressed as kg tree^{-1} and number of fruit tree^{-1}. The total number of fruit and total kg tree^{-1} were used to calculate fruit weight at the two harvest dates. Results were expressed as the mean ± SE.

2.3. Fruit Quality Parameters

Fruit quality parameters were measured according to Mirdehghan et al. [22] and García-Pastor et al. [20]. Weight loss was measured for each individual lot by recording the fruit weight at harvest (0 day) and at the different sampling dates during storage. Cumulative weight losses were expressed as a percentage with respect to fruit weight at day 0. External color was determined in three equidistant points along the equatorial perimeter of 10 fruits from each replicate, by using a Minolta colorimeter (CRC200, Minolta Camera Co., Tokyo, Japan), and the CIELab coordinates and color were expressed as Hue angle (h°). The pomegranate was cut into 2 halves and again 3 readings were performed in the arils from each fruit to measured internal color [20]. Fruit firmness was determined independently in the 10 fruits of each replicate using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the force–deformation ratio (N mm^{-1}). The arils from 10 fruit of each replicate were combined to obtain a homogeneous sample for each replicate. TSS were determined in duplicate in the juice obtained from 50 g of each sample with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as g 100 g^{-1}. Titratable acidity (TA) was determined in duplicated in the same juice by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O, and results expressed as g 100 g^{-1} malic acid equivalent. The ratio of TSS/TA (ripening index) was then calculated.

2.4. Sugars and Organic Acids

For organic acid and sugar determinations, 5 g of arils from each replicate were extracted with 10 mL phosphate buffer (50 mmol L^{-1}, pH = 7.8) and then centrifuged at 15,000× g for 15 min at 4 °C. The supernatant was used for sugars and organic acids quantification in duplicate as previously described by Mirdehghan et al. [22]. One mL of the extract was filtered through a 0.45 µm Millipore filter and then injected into a Hewlett-Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1}. Organic acids were eluted through a Supelco column (Supelcogel C-610H, 30 cm 7.8 mm, Supelco Park, Bellefonte, PA, USA) and detected by absorbance at 210 nm. A standard curve of pure organic acids (L-ascorbic, malic, citric, oxalic, and succinic acids) purchased from Sigma (Madrid, Spain) was used for quantification. Results were expressed as g 100 g^{-1}. For sugar concentrations, the same HPLC, elution system, flow rate, and column were used and they were detected by using a refractive index detector. A standard curve of pure sugars (glucose, fructose, and sucrose) purchased from Sigma was used for quantification. Results were expressed as g 100 g^{-1}.

2.5. Statistical Analyses

All data are represented as means ± standard error of the mean (S.E.M.). Statistical analyses were performed using SPSS software, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were subjected to an analysis of variance (ANOVA), the means were compared using Student's *t*-tests, and the differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of Pre-Harvest Melatonin Treatment on Crop Yield

Fruit diameter was recorded during on-tree fruit growth and ripening and results are shown in Figure 1, in which a simple-sigmoid growth curve from full blossom (FB) to harvest (155 d after full blossom, DAFB) can be observed. In addition, results showed that melatonin treatment at 0.1 mM concentration stimulated fruit growth from the second application (T2), leading to pomegranates with significantly higher size ($p < 0.05$) in 0.1 mM melatonin-treated trees (≈84 mm) than in 1 mM or control ones (≈79 and 77 mm, respectively) at the first harvest date. The increase of fruit size as a consequence of 0.1 mM melatonin treatment was not due to an increased peel width but to an increase in the portion of the arils, as can be observed in the photograph of cut pomegranate fruit detailed below.

With respect to yield, the results of the 2017 experiment are presented in Table 1, showing that both the number of fruit harvested by tree and kg of fruit harvested by tree were significantly higher ($p < 0.05$) in 0.1 mM melatonin-treated trees than in controls, although these increases were not significant in 1 mM melatonin treated trees. In addition, fruit ripening was delayed in treated trees, since yield at the first harvest date was significantly ($p < 0.05$) higher in control trees (28.65 ± 3.16 kg $tree^{-1}$) than in those treated with 0.1 and 1 mM melatonin doses (ca. 21 kg $tree^{-1}$), as well as the number of fruit per tree (78.0 ± 7.9, 61.40 ± 4.01 and 65.00 ± 3.83 for control and 0.1 and 1 mM melatonin treated trees, respectively). The average fruit weight, taking into account data from the two harvest dates, was significantly higher ($p < 0.05$) in fruit from 0.1 mM melatonin treated than from controls, although no significant effect was observed with 1 mM melatonin treatment. However, since total yield per tree was only significantly increased in 0.1 mM melatonin treated trees, this concentration was used in the next year, in which these effects were confirmed. Thus, higher values of kg $tree^{-1}$, the number of fruits per tree and average fruit weight were obtained in 0.1 mM-melatonin treated trees compared with control ones (Table 2).

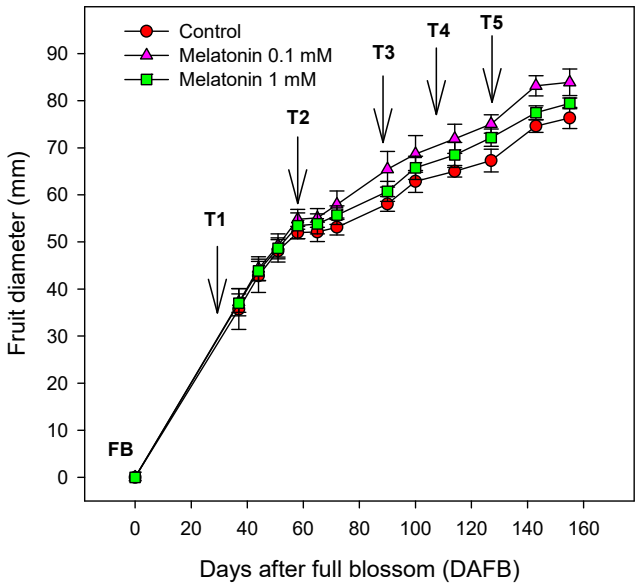

Figure 1. Evolution of fruit size (diameter) during pomegranate development from full blossom (FB) to harvest, in fruit from control and 0.1 or 1 mM melatonin-treated trees. T1–T5 represent the day of treatment. Data are the mean ± SE.

Table 1. Data for yield (kg tree^{-1} and number of fruit tree^{-1}), and fruit weight in control and melatonin-treated pomegranate trees (0.1 and 1 mM) at two harvest dates (1 and 2). Year 2017.

Parameter	Control	Melatonin 0.1 mM	Melatonin 1 mM
Yield (kg tree^{-1})			
Harvest 1	28.65 ± 3.16 a	21.68 ± 2.02 b	20.66 ± 2.84 b
Harvest 2	9.10 ± 0.98 a	20.36 ± 3.60 b	18.05 ± 2.29 b
Total	37.75 ± 3.28 a	42.04 ± 1.06 b	38.71 ± 2.49 a
Yield (number of fruit tree^{-1})			
Harvest 1	78.0 ± 7.9 a	61.40 ± 4.01 b	65.00 ± 3.83 b
Harvest 2	29.8 ± 3.21 a	58.00 ± 3.98 c	46.60 ± 5.65 b
Total	107.8 ± 5.16 a	119.40 ± 3.96 b	111.60 ± 4.15 ab
Fruit weight (g)			
Harvest 1	367.31 ± 11.29 a	353.09 ± 9.14 a	317.84 ± 10.21 b
Harvest 2	305.37 ± 7.62 a	351.03 ± 9.95 b	387.33 ± 8.45 c
Average	350.18 ± 6.19 a	352.03 ± 12.97 a	346.86 ± 9.15 b

Data are the mean ± SE. For each parameter and harvest date, different letters within a row show significant differences at $p < 0.05$.

Table 2. Data for yield (kg tree^{-1} and number of fruit tree^{-1}), total number of fruits in 5 trees and fruit weight in control and 0.1 melatonin-treated pomegranate trees at two harvest dates (1 and 2). Year 2018.

Parameter	Control	Melatonin 0.1 mM
Yield (kg tree^{-1})		
Harvest 1	26.73 ± 1.72 a	22.91 ± 2.34 b
Harvest 2	10.37 ± 1.37 a	20.34 ± 3.19 b
Total	37.10 ± 1.56 a	43.25 ± 1.57 b
Yield (number of fruit tree^{-1})		
Harvest 1	72.40 ± 3.64 a	60.90 ± 3.85 b
Harvest 2	36.75 ± 4.02 a	60.40 ± 5.92 b
Total	109.15 ± 3.92 a	121.30 ± 4.96 b
Fruit weight (g)		
Harvest 1	369.20 ± 9.55 a	376.19 ± 7.45 a
Harvest 2	282.17 ± 6.46 a	336.75 ± 6.17 b
Average	339.90 ± 5.15 a	357.43 ± 8.07 b

Data are the mean ± SE. For each parameter and harvest date, different letters within a row show significant differences at $p < 0.05$.

3.2. Effect of Melatonin on Fruit Quality Parameters

Weight losses increased during storage in pomegranate fruit from control and treated trees, although these increases were significantly reduced by melatonin treatment, with significant differences ($p < 0.05$) after 60 days of storage (Table 3). With respect to TSS, levels at harvest in the 2017 experiment were 15.17 ± 0.26 g 100 g^{-1} in fruit from control trees and significantly higher ($p < 0.05$) in those from 0.1 mM treated trees, although no significant differences were observed due to 1 mM melatonin treatment (Table 3). TSS content increased during storage in fruit from control and treated trees and these increases occurred later in fruit from melatonin treated trees with respect to those from controls (Table 3). Acidity levels (TA) at harvest were also significantly increased at harvest by melatonin treatments and they decreased during storage, but the decrease was much lower in melatonin-treated fruit (both 0.1 and 1 mM) than in controls (Table 3). The ratio between TSS/TA, also known as ripening index (RI), increased during storage, but it was reduced by the pre-harvest application of melatonin (Table 3). Melatonin treatments also affected pomegranate skin and aril color, since significantly lower ($p < 0.05$) values of Hue angle were recorded in fruit from melatonin treated trees than in controls and those differences were maintained during the whole storage period, although Hue angle of skin and arils decreased in all pomegranate fruit (Table 3).

Table 3. Physiological and biochemical parameters in pomegranate fruits from control and melatonin-treated trees (0.1 and 1 mM) at harvest (day 0) and during 90 days of storage at 10 °C. Year 2017.

Parameter	Days	Control	Melatonin 0.1 mM	Melatonin 1 mM
Weight loss (%)	0	-	-	-
	30	3.94 ± 0.19 aA	3.67 ± 0.15 aA	3.75 ± 0.20 aA
	60	4.82 ± 0.49 bA	3.83 ± 0.48 aB	4.38 ± 0.11 bB
	90	6.87 ± 1.09 cA	4.86 ± 0.26 bC	5.59 ± 0.17 cB
TSS (g 100 g^{-1})	0	15.17 ± 0.26 aA	16.37 ± 0.22 aB	15.38 ± 0.35 aA
	30	16.68 ± 0.12 bA	16.38 ± 0.10 aA	16.40 ± 0.12 aA
	60	16.93 ± 0.23 bcA	16.48 ± 0.16 aA	17.18 ± 0.26 a,bA
	90	17.42 ± 0.15 cA	17.25 ± 0.19 bA	17.32 ± 0.12 bA
TA (g 100 g^{-1})	0	0.45 ± 0.03 aA	0.56 ± 0.01 aA	0.56 ± 0.05 aA
	30	0.38 ± 0.01 bA	0.51 ± 0.02 bB	0.57 ± 0.03 aB
	60	0.32 ± 0.02 bcA	0.46 ± 0.01 bB	0.45 ± 0.02 bB
	90	0.21 ± 0.01 cA	0.38 ± 0.02 cB	0.30 ± 0.01 cB
RI (TSS/TA)	0	35.04 ± 0.51 aA	29.23 ± 0.52 aB	27.46 ± 0.54 aB
	30	43.88 ± 0.73 bA	32.01 ± 0.54 bB	28.77 ± 0.38 aB
	60	52.91 ± 0.84 cA	35.82 ± 0.74 cB	38.18 ± 0.56 bB
	90	82.95 ± 1.22 dA	45.39 ± 0.41 dB	57.73 ± 0.62 cC
External Color Hue	0	68.30 ± 1.01 aA	58.84 ± 1.47 aC	62.69 ± 1.12 aB
	30	64.71 ± 1.07 abA	55.89 ± 1.88 abB	57.71 ± 1.03 abB
	60	61.53 ± 1.09 bcA	53.66 ± 1.88 bcB	55.80 ± 1.68 bB
	90	59.93 ± 1.57 cB	52.26 ± 1.89 cB	54.48 ± 1.42 bB
Internal Color Hue	0	34.13 ± 1.71 aA	30.34 ± 1.18 aB	31.92 ± 1.29 aAB
	30	31.78 ± 1.04 abA	28.34 ± 0.73 abB	28.77 ± 1.22 abB
	60	28.91 ± 0.53 bA	26.00 ± 0.64 bB	26.93 ± 0.65 bcB
	90	26.91 ± 0.83cA	23.84 ± 0.55 cB	24.74 ± 0.69 cAB

Data are the mean ± SE. For each parameter, different lowercase letters within a column show significant differences at $p < 0.05$ during storage, while capital letters show significant differences at $p < 0.05$ among treatments for each sampling date.

Fruit firmness at harvest, in the 2017 experiment, was significantly higher ($p < 0.05$) in fruit from 0.1 mM melatonin treated trees than in controls (26.52 ± 0.50 and 28.93 ± 0.710 N mm^{-1}, respectively), while no significant effect was observed with 1 mM melatonin treatment. During storage, fruit firmness decreased in pomegranates from control and treated trees, although firmness levels were the highest in 0.1 mM melatonin treated fruit until the last sampling date (Figure 2).

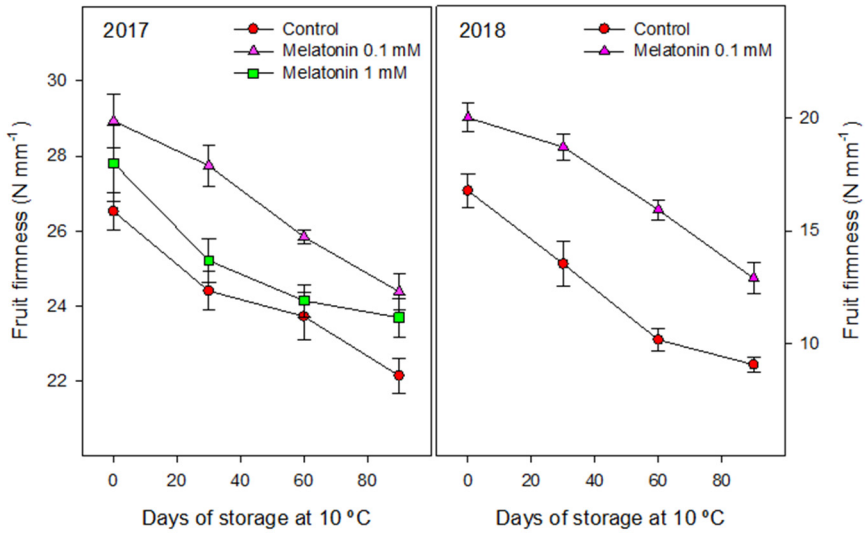

Figure 2. Fruit firmness evolution (Nmm^{-1}) during refrigerated storage in two consecutive production cycles (2017 and 2018), in control fruit and 0.1 or 1mM melatonin-treated trees. Data are the mean ± SE.

Similar results with respect to firmness (Figure 2), weight loss, TSS, TA, and RI were obtained in the experiment assayed in 2018 (Table 4), in which 0.1 mM melatonin concentration was chosen, since better results were obtained compared with 1 mM dose in the 2017 experiment. Accordingly, melatonin treatments did not affect skin color, although aril color was affected by treatment, since Hue angle values were significantly lower in melatonin treated than in control fruit, either at harvest or during storage (Table 4), which indicated a darker red color of the arils. These differences are evident in Figure 3, in which the visual aspect of pomegranates from control and melatonin-treated trees (0.1 mM) at harvest and during storage is shown. Small shrivelling symptoms were observed on pomegranate fruit after 90 days of storage in fruits from control and treated ones without differences attributed to melatonin treatment (data not shown).

Table 4. Quality parameters in control and 0.1 mM melatonin-treated pomegranate fruits at harvest (day 0) and during postharvest storage at 10 °C. Year 2018.

Parameter	Treatment	Day 0	Day 30	Day 60	Day 90
Weight loss (%)	Control	-	7.07 ± 0.49 Aa	8.49 ± 0.48 Ab	9.88 ± 0.54 Ac
	Melatonin		6.03 ± 0.14 Aa	7.18 ± 0.25 Bb	7.98 ± 0.24 Bc
TSS (g 100 g^{-1})	Control	16.05 ± 0.10 Aa	17.03 ± 0.15 Ab	17.52 ± 0.16 Ab	17.41 ± 0.22 Ab
	Melatonin	17.18 ± 0.12 Ba	17.02 ± 0.13 Aa	17.13 ± 0.18 Aa	17.25 ± 0.36 Aa
TA (g 100 g^{-1})	Control	0.35 ± 0.03 Aa	0.31 ± 0.03 Ab	0.21 ± 0.02 Ac	0.19 ± 0.04 Ac
	Melatonin	0.42 ± 0.04 Ba	0.37 ± 0.03 Ba,b	0.34 ± 0.02 Bb,c	0.29 ± 0.01 Bc
Ripening index (TSS/TA)	Control	45.85 ± 1.74 Aa	54.93 ± 2.15 Ab	83.42 ± 1.65 Ac	91.63 ± 1.97 Ad
	Melatonin	40.90 ± 1.03 Ba	48.62 ± 0.78 Bb	50.38 ± 2.18 Bb	59.48 ± 2.18 Bc
External Color Hue	Control	66.41 ± 1.22 Aa	64.20 ± 1.52 Aa	60.02 ± 1.34 Ab	58.38 ± 0.99 Ab
	Melatonin	60.02 ± 0.51 Ba	56.70 ± 1.57 Bbc	54.07 ± 1.24 Bcd	52.49 ± 1.61 Bd
Internal Color Hue	Control	33.13 ± 0.58 Aa	30.75 ± 1.90 Aab	29.23 ± 0.56 Ab	27.65 ± 1.07 Ac
	Melatonin	30.56 ± 1.24 Ba	27.22 ± 1.40 Bab	26.02 ± 1.18 Bb	22.79 ± 1.17 Bc

Data are the mean ± SE. For each parameter, different lowercase letters within a row show significant differences at $p < 0.05$ during storage, while capital letters show significant differences at $p < 0.05$ between treatments for each sampling date.

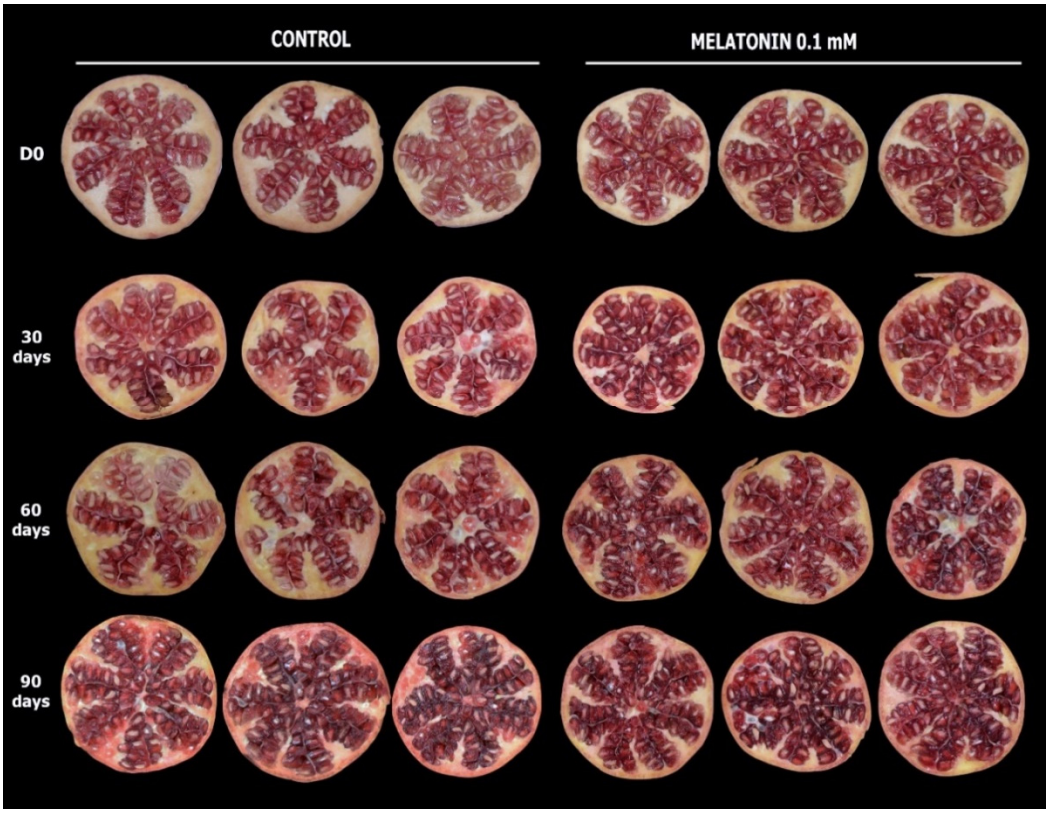

Figure 3. Photography displays the visual aspect of pomegranates from control and 0.1 mM melatonin-treated trees during storage in the 2018 experiment.

Individual sugars are mainly responsible for TSS in pomegranate and their quantification by HPLC showed that the major one was fructose, followed by glucose, while sucrose was found at very low concentrations (Table 5). Interestingly, fruit from melatonin treated trees had significantly higher $p < 0.05$ content of fructose and glucose at harvest than controls. With respect to organic acids, the major one found in 'Mollar de Elche' pomegranate was malic acid, followed by succinic and citric acids, which were also found at higher concentration in fruit from melatonin-treated trees, while no significant differences were observed in the minor ones, citric, fumaric, and oxalic acid. However, it is worth mentioning the effect of pre-harvest melatonin treatment on increasing ascorbic acid content in the arils, with concentrations being 1.5 higher in treated than in control fruit (Table 5).

Table 5. Reducing sugars and organic acids in fruits from control and 0.1 mM melatonin-treated trees at harvest. Year 2018.

Parameter	Control	Melatonin 0.1 mM
Sugars (g 100 g^{-1})		
Sucrose	0.06 ± 0.01 a	0.06 ± 0.02 a
Glucose	4.17 ± 0.10 a	4.81 ± 0.24 b
Fructose	11.61 ± 0.33 a	12.54 ± 0.26 b
Organic acids (g 100 g^{-1})		
Malic acid	0.32 ± 0.03 a	0.36 ± 0.04 b
Succinic acid	0.07 ± 0.01 a	0.12 ± 0.01 b
Citric acid	0.09 ± 0.01 a	0.09 ± 0.01 a
Ascorbic acid	0.04 ± 0.01 a	0.06 ± 0.01 b
Fumaric acid	0.03 ± 0.01 a	0.03 ± 0.01 a
Oxalic acid	0.01 ± 0.01 a	0.01 ± 0.01 a

Data are the mean ± SE. For each parameter, different letters within a row show significant differences at $p < 0.05$.

4. Discussion

As far as we know, this is the first report showing the effects of melatonin, applied as pre-harvest treatment, in pomegranate yield and fruit quality properties, although some evidence exists in other plant species. In a recent study performed on Arabidopsis treated with melatonin and grown at 4 °C, melatonin-treated plants had significantly greater fresh weight, primary root length, and shoot height compared with untreated plants, the effect being both time and concentration dependence [23]. However, the yield increase found in the present study was higher with 0.1 mM melatonin treatment than with 1 mM dose, and due to an increase in fruit size (diameter and weight). This effect was due to an increased aril portion but to an increase in peel width as can be observed in the photograph of cut pomegranates in Figure 3. On the other hand, the amount of fruit harvested by tree was also increased by melatonin treatment. Given the fact that treatments were applied when fruit was in its active phase of growth, the higher amount of fruit harvested from treated fruit could be attributed to the effect of melatonin on decreasing the normal fruit abscission that occurs during fruit development due to environmental factors, such as wind or rain. In addition, the effect of melatonin on alleviating biotic and abiotic stress in plants has been reported [23,24]. Thus, giving the semi-arid climate conditions of Southern Spain, the melatonin treatment could increase net photosynthesis rate and productivity throughout enhancement of tree tolerance to heat and drought stresses. Accordingly, grape berries treated with melatonin at pre-veraison exhibited higher endogenous melatonin accumulation and increased berry size and weight [8]. These authors attributed this effect to an increase of the sink strength of the berry, leading the fruit to uptake more sugars and develop a larger size at harvest. On the other hand, melatonin foliar spray treatment increased fruit weight and yield for 'Canino' apricot [12]. In addition, we have found increased crop yield 'Colorado' and 'Mikado' apricot cultivars after melatonin treatment (unpublished data), which were attributed to increases in tree net photosynthesis, due to enhanced total chlorophyll and leaf area.

'Mollar de Elche' pomegranate is very much appreciated by consumers due to the high content of sugars and low acidity, which confer a sweet taste while also being very aromatic [15–18]. In this sense, the higher values of TSS, TA, sugars, and organic acids found at harvest in pomegranates of melatonin treated trees show that they had higher quality attributes than controls. However, this cultivar is characterized by having a pale aril color compared with other cultivars [15,17,25] and thus several research papers have been performed with the aim to increase aril color while enhancing anthocyanin synthesis, by applying water restrictions in summer, during the linear phase of fruit growth [26], as well as treatment with methyl jasmonate or salicylates during on-tree pomegranate fruit development [16,27]. Results of the present research show that melatonin-treated fruit had a deeper red color than controls (lower Hue angle) at harvest and during storage, showing a stimulation of the anthocyanin biosynthesis by melatonin treatment, which is the pigment contributing to the red color of pomegranates [15,16,20,27]. On the other hand, no effects of melatonin treatments on fruit taste and flavour were appreciated either at harvest or during storage, although a proper sensorial analysis would be useful to a scientific validation of this observation.

On the other hand, results show that pre-harvest melatonin treatment delayed the postharvest ripening process during storage, since weight, firmness, and acidity losses were delayed in fruit from melatonin-treated trees with respect to controls, these effects being higher for 0.1 mM dose than for 1 mM. No previous reports are available in the literature regarding the effect of preharvest melatonin treatment on the evolution of fruit quality parameters during storage, although information exists about postharvest treatments. Thus, in banana, exogenous application of melatonin (at 0.05, 0.2 and 0.5 mM) resulted in a delay of postharvest ripening, although in this report the effect was dose dependent [28]. The lower weight losses observed in fruits from melatonin treated trees with respect to those of controls might be attributed to an effect of melatonin on increasing cuticle thickness, as recently proposed for nectarines [29] and mangos [30] after postharvest melatonin

treatment. Accordingly, postharvest 0.1 mM melatonin treatment delayed the postharvest ripening process during cold storage in apples [31], peaches [32], nectarines [29], pears [33], and mangos [34], which was attributed to a inhibition of ethylene production, although similar effects have been reported in non-climacteric fruits such as sweet cherries [35].

5. Conclusions

This is the first report in which melatonin (at 0.1 or 1 mM) has been applied as pre-harvest treatment with a significant effect on increasing crop yield and pomegranate fruit quality at harvest and during storage. The best results were found for the 0.1 mM dose. In addition, the concentration of sugars (glucose and fructose) and ascorbic acid were also higher after melatonin treatment. During postharvest storage, reduced softening and weight and acidity losses were found in melatonin-treated fruit. Overall, melatonin could be a reliable, feasible, and cost-effective tool to be used as plant bio-stimulant in order to increase pomegranate crop yield and fruit quality parameters at harvest and to maintain them during storage. In the future, the possible role of melatonin on bioactive compounds responsible for their beneficial health effects should be investigated.

Author Contributions: M.S. and D.V. conceived and designed the experiment in association with the other authors. P.J.Z. and F.G. performed the field treatments. J.M.-S., J.M.L.-M. and M.E.G.-P. performed most of the analytical determination in collaboration with the other authors. D.V., M.S., and F.G. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been funded by the Spanish Ministry of Science and Innovation through Projects AGL2015-63986R and RTI2018-09966-B-100 and the European Commission with FEDER funds.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors thank Miguel Hernández University for the PhD-scholarships of M.E. García-Pastor and J.M. Lorente-Mento.

Conflicts of Interest: The authors declare no conflict of interest.

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