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MELATONIN PRE-HARVEST FOLIAR APPLICATION IMPROVES PEPPER FRUIT YIELD AND POSTHARVEST FRUIT QUALITY

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ABSTRACT

In this study, the effect of pre-harvest melatonin foliar spray on yield and the contents of both nutritive and bioactive compounds in pepper (Capsicum annuum L, cv. Jalapeño) was investigated at harvest-time and after 45 days of storage in 4°C. Treatments were applied at 15-day intervals beginning with the development of the first floral bunch (MT 50, and 100 µM) and untreated plants (control). Pepper fruits were harvested at the commercial ripening stage throughout the growth cycle (September to January), in both seasons. Melatonin treatment increased total yield by 17.44% due to significant increases in single fruit weight and fruit weight per plant. Furthermore, weight loss, firmness, thickness, and diameter of Jalapeño fruit were all significantly affected by Pre-harvest melatonin spraying. In addition, melatonin pre-harvest treatments increased the content of nutritive (reducing and non-reducing sugar) and bioactive compounds (vitamin C and total phenolics), and antioxidant activity at harvest and after 45 days of cold storage. These findings suggested that melatonin treatment could be a useful technique for enhancing crop yield as well as nutritional value and antioxidant properties of peppers at harvest and after fruit storage. Additionally, maintain and extending postharvest shelf life of Jalapeño fruit.

Keywords: Antioxidant activity; Fruit yield; Jalapeño**;** Melatonin**,** postharvest**.**

1. INTRODUCTION

Hot pepper (Capsicum annum L.) is a member of the Solanaceae family and has a high economic value. It is also very popular among the general public due to its nutritional and medicinal properties; additionally, hot pepper extract is used in a variety of pharmaceutical products. Also, contributes significantly to the global diet. Peppers are widely grown in several regions of Egypt, and the hot pepper fruits are used as fresh, dried, and processed products, as well as spices or condiments, just like vegetables. Hot pepper provides the body with vitamins (A and C), proteins, and a variety of mineral nutrients (Helaly et al., 2020; Bose et al., 1993).

In 2020, Egypt's total cultivated area of pepper (chilies and green) was 42,559 Hectare, yielding 790,525 tonnes with an average yield of 18.59 tonnes/ha. (Ahmed et al., 2022). Egypt ranked seventh global among the pepper-producing countries followed China, Mexico, Turkey, Indonesia, Spain, and USA (Bosland et al., 2012), also is has 1st rank among African countries.

A Jalapeño pepper is a type of Capsicum pod fruit. When compared to other Chilli peppers, it is a medium-sized hot pepper, measuring an average of 2-3.5 inches in length but growing up to 6 inches long or longer. While it originated in Mexico, it is now grown throughout the world for its popular flavor and mild heat level, which averages around 5,000 Scoville Heat Units. That is hot, but not overly so. Fresh chillies are highly perishable and consumed fresh, and the quality and shelf life of the fruit are important factors in its commercial value. Furthermore, fresh chilli loses water quickly after harvest and begins to shrivel and turn color within a few days (Lowands et al., 1994; Díaz-Pérez et al., 2007; Bayoumi, 2008).

Melatonin's chemical name is N -acetyl-5-methoxy-tryptamine, and it is a hormone secreted by the brain's "pineal gland," also known as pineal hormone. Melatonin is a molecule that has been conserved throughout the evolution of animals, plants, and microorganisms. Melatonin was discovered in plants for the first time in 1995 (Dubbels et al., 1995). According to UHPLC-QqQ-MS/MS analysis, the melatonin content of pepper is 31.0-93.4 ng/g (Riga et al., 2014). While, was differing (9.1-42.1 ng/g) for Sena cv. and (7.3–31.2 ng/g) for Mert cv. as determined by HPLC-FD method. (Korkmaz et al., 2014). This could be resulted in variations in maturity phase, cultivars, and detection methods. Furthermore, presence levels of MT synthesis-associated with genes were much higher at midnight than at noon (Wei et al., 2016).

In other cases, the foliar application of melatonin preventing postharvest deterioration of the crops. Furthermore, postharvest quality of horticultural produce is primarily determined by preharvest factors because it cannot be increased after harvesting but can only be maintained. In line with this, tomato seeds infused with melatonin not only increased yield but also maintained postharvest quality by increasing vitamin C, lycopene, and calcium levels. The treated plants also had higher levels of soluble solids and P than the control plants. (Arah et al., 2015; Liu et al., 2016; Sun et al., 2016; Hu et al., 2018).

In this regard, very few studies have estimated the impact of melatonin used as a preharvest treatment on crop yield and quality traits, with varying effects depending on fruit species, concentration, or application time. As a result, the goal of this study was to evaluate the effect of pre-harvest melatonin spraying on Capsicum annuum (cv. Jalapeño), the ability and quality of postharvest fruits, and to provide a theory for selecting an appropriate melatonin concentration to improve chilli pepper fruit yield and quality in actual planting. In the future, this research could lead to the use of melatonin in at harvest and postharvest for yield, ripening and quality improvement of chilli pepper fruit, as well as other horticultural productions.

2. MATERIALS AND METHODS

2.1. Experimental Site and Soil

Two experiments were conducted at a private farm in Dandara, Qena Governorate through both horticulture and Food Sciences Technology Departments, Faculty of Agriculture, South Valley University, Egypt. The site is at (latitude 26° 11' 22.2" N to Longitude 32° 44' 25.5" E), and 81 m above sea level. Pepper, Jalapeño cv., seedlings were similar in growth and 12 -14 cm in length and transplanted to open field on 5th and 7th September during both 2020-21 and 2021-22 seasons, respectively. All agricultural practices were performed as recommended by Ministry of Agriculture and Land Reclamation. The experimental soil was clay soil having pH 7.45, EC 1.24 dSm^{-1}, total N 10.55 g. kg^{-1}, available P 11.21 g. kg^{-1} and exchangeable K 22 g. kg^{-1}.

2.2. The experiment treatments

The experiment included three treatments with three replicates which were arranged in a randomized complete blocks design, i.e., Melatonin solutions of 50, and 100 µM as well as control (distilled water), were sprayed two times using atomizer to completely cover the plant foliage at 15 and 30 days after flowering along with triton B as a wetting agent at 0.1% to improve adherence of the spray to the plant foliage for increasing absorption by the plants.

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Experimental plot consisted of four rows; each was 4 m in length and 0.75 m in width with an area 12 m^2. Each plot received about 1.5-$liter^{-1}$ solution of each concentration according to the age of the plant. The untreated plants (control) were sprayed with distilled water and spreading agent. One line was left between each two experimental plots without spraying as a guard row to avoid the overlapping of spraying solution. Fruits of hot pepper Jalapeño cv. were harvested every 3 days intervals, upon reaching 2-3.5-inch length. Melatonin was obtained from (Tokyo chemical industry Co., LTD Japan). Extracts Preparing: Melatonin solutions of 50, and 100 μM were prepared by dissolving it in distilled water and then foliar applied to the plants as an aqueous solution at both different concentrations.

2.3. Sampling and Collecting Data

Fruits of five plants from each replicate were randomly taken at harvest time and the following characteristics were recorded:

1. The yield and its components expressed as fruit length, fruit diameter fruit weight per plant (g), fruit yield per plant (kg), and total fruit yield per hectare were measured.
2. In the Lab. Firstly, the fruits were saved for 12 hrs. at room temperature to dissipate the field heat, then fruit were soaked for 2 minutes in 3% Sodium hypochlorite solution , after washed three times with distilled water, and dried. Then, 500 g fruits from each treatment were taken to a cold storage at 4°C with a relative humidity of 85%. After 45-days of storage, 10 fruits from each treatment were taken out from the chamber for further analysis.

1. Determination of weight loss

Each treatment contained 3 replicates with 20 fruit per replicate, and the experiment was repeated 3 times. Weight of each fruit was measured following the treatment at 0-day and at the end of the storage period 45-day. Weight loss% was expressed as the percentage loss of the initial weight. And the percentage weight loss was calculated according following formula:

$$\text{Weight loss\%} = (\text{Intial weight} - \text{Final weight})/(\text{Intial weight}) \times 100$$

2. Determination of Firmness.

The fruit firmness (N) was measured using a Handheld Texture Analyzer (Axis, FB200, and Poland). The firmness values of each pod were determined at three different points. The mean of these values was used to express the firmness.

3. Determination of fruit length, diameter and thickness.

For each hot pepper fruit group, three linear dimensions were measured by using a digital vernier caliper with an accuracy of ±0.01mm.

4. Determination of Proximate Chemical Composition

Chemical composition (moisture, crude ash; protein and crude fat) of the Jalapeño pepper were determined by the method described by James (1995). The moisture was determined by the air oven by heating in an oven at 70 °C to constant weight; Ash was determined by gravimetric of incinerated sample in a muffle furnace at 550°C for 3 hrs. Crude protein was determined by

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the Kjeldahl method and converted to protein using the factor 6.25. Crude fat was extracted by the Soxhlet using petroleum ether.

5. Determination of Ascorbic acid

Determination of vitamin C content was performed by titration of ascorbic acid with 2.6-dichloroindophenol. This method is based on measurement of the extent to which a 2, 6-dichlorophenol-indophenol solution is decolorized by ascorbic acid in sample extracts .The results were expressed as milligrams of ascorbic acid per 100 g of FW, method given by Ranganna (2011).

6. Determination of total phenol

Total phenolic content was determined colorimetric by using the Folin- Ciocalteu method (Dannehl et al., 2011). The absorbance was recorded at 750 nm using on a spectrophotometer against the blank that consisting of all reagents and solvents without test compounds. The total phenolic concentrations were calculated using a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalents GAE/100 g FW.

7. Determination of antioxidant activity

Antioxidant activity and bioactive compounds were assessed according to methods previously reported by Brand-Williams et al., (1995) .DPPH radical scavenging activity was analysed by the method reported by DPPH solution 0.5 ml of the DPPH solution (about 50 mg/100ml) was diluted in 4.5 ml of methanol and 0.1 ml of methanolic solution of the extract was added at a wavelength of 515nm .The decrease in absorbance was measured at 515 nm against the blank without extract with spectrophotometer. From the calibration curve obtained with different amounts of extract, the IC50 was calculated. The IC50 was that concentration of an antioxidant which was required to quench 50% of the initial DPPH radical under the experimental condition given. Radical scavenging activity (%) was calculated as follow: -Radical scavenging activity (%) = (1-absorbance of sample /absorbance of control).

8. Determination of Chlorophyll

Chlorophyll a and b were determined according to (Katoch**,** 2011). Chlorophyll a and b were extracted in 80% acetone and the absorbance was taken at 663 nm and 645 nm in a spectrophotometer against the solvent (80% acetone) blank, the amount of chlorophyll was calculated. Chlorophyll mg/g fresh weight were calculated by the formula given below:

$$\text{mg/ Chlorophyll a} = 12.7(A663) - 2.9(A645) \times V/1000 \times W$$

$$\text{mg/ Chlorophyll b} = 22.9\,(A645) - 4.68\,(A663) \times V/1000 \times W$$

A663 = Absorbance at 663 nm, A645 = Absorbance at 645 nm, V = Final volume of chlorophyll extract in 80% acetone and W = Weight of sample taken.

Statistical analysis

Data for the analytical determinations were exposed to analysis of variance with sources of variation being treatments (melatonin) and storage (days 0 and 45). The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated and used to detect significant

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differences among treatments at harvest date or along storage time. (Steel et al., 1997). All analyses were performed with using Statistix software package v. 9.0.

3. RESULTS AND DISCUSSION

3.1 Hot pepper fruit yield and its components response to melatonin application at two harvest cycle

The greatest significant goal and desired outcome for which plants are grown is yield. The average fruit weight (g), fruit weight per plant (kg/plant), and total fruit yield (ton/ha) are shown in Table 1.

The average fruit weight (g), as shown in (Table 1.) increased significantly in MT treated plants at the first and last harvests, compared with the control (untreated plants). A 9.33 and 5.94 g increase was recoded with MT_{100} and MT_{50} treatments respectively, compared with the control (37.03 g) at 1st harvest. There were no significant changes between MT_{50} and MT_{100} at the last harvest time. Furthermore, fruit weight decreased throughout the harvest cycle, with Jalapeño fruit with the maximum weight (46.36 g) recoded at the first harvesting dates and the lowest (33.87 g) with control (untreated plants) at the last harvest cycle. Using data from all harvest cycles, the average fruit weight for Capsicum annum from control plants was 37.03 and 33.87 g, while those from MT-sprayed 50 and 100 µM were 42.97, 38.97 g and 46.36, 39.30 g, respectively. Then, as a result of these treatments, fruit quality improved, because larger pepper fruits are more valued by consumers and command higher prices in markets than smaller fruits (Frank et al., 2001; Valero et al., 2014).

Fruit yield per plant (kg) was significantly higher ($p < 0.05$) in treated plants than the control plants Table 1.with values of 0.71, 0.82, and 0.86 kg per plant for control, and MT 50 and 100 sprayed plants, respectively. Taking into consideration a plant density of 2.5 plant/m^2 and the fruit yield (ton/ha) in control and treated plants, the treatments with MT 50 and 100 would result in an increase in fruit yield of 17.75 and 21.50 ton/ha, respectively, representing a yield enhancement of approximately 17.44 %197.

Table 1: Effect of melatonin on fruit weight (g) in two harvest time, fruit yield/plant (kg), and fruit yield (ton/ha) of Capsicum annum L. at two consecutive years.

Treatments (T)	Control	MT 50	MT 100	F test
Fruit weight (g) 1st harvest	37.03^{c}	42.97^{a}	46.36^{a}	**
Fruit weight (g) last harvest	33.78^{b}	38.94^{a}	39.30^{a}	**
Fruit Yield/plant (Kg)	0.71^{c}	0.82^{b}	0.86^{a}	**
Fruit Yield/ha (ton)	17.75^{c}	20.50^{b}	21.50^{a}	**

Means with the same letters within rows are not significant at $P \leq 0.05$. Each value is the mean of three replicates. MT 50 (melatonin at 50 µM), MT 100 (melatonin at 100 µM), and Control (untreated). *, and ** no Significant, Significant at $P \leq 0.05$

This yield potential could be related to the high photosynthetic efficiency and a delay in leaf senescence in MT-treated plants, result in a higher level of photoactivities fragmentation between plant sinks, as demonstrated in capsicum plants (Okunlola et al.,

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2022; Djanaguiraman et al., 2009) on cotton. In addition, soaking tomato seeds in 0.1 mM MT prior to germination was related with an increase in fruit production by 13%, when compared to untreated seeds. Also, irrigating plants with a MT-supplemented raised yield by 4% (Liu et al., 2016). Therefore, melatonin is thought to have influenced signal transduction as well as regulating plant physiological and biological processes. Melatonin can be thought of as a biological plant growth regulator that improves a plant's production capacity (Sharif et al., 2018). Melatonin application significantly increased pomegranate tree productivity at harvest, with higher yield, number of fruits per tree, and average fruit weight (García-Pastor et al., 2017).

3.2 Hot pepper fruit weight loss%, firmness, thickness, diameter and length response to melatonin application and storage time

1. Weight loss%

After 45 days of storage, the control samples (untreated plants) lost significantly more weight (4.38%) than the MT-treated capsicum annum plants ($P \leq 0.05$). The MT 100 treatment resulted in the smallest weight loss (1.12%), which was ($P \leq 0.05$) lower (1.83%) than the MT 50 treatment. At either storage time, there were no statistically significant differences between the MT 100 treatments Table 2.

Postharvest weight loss in fruits and vegetables is caused by metabolic activity, respiration, and transpiration. Weight loss increased as the storage period progressed, regardless of whether the fruits were treated with melatonin or not (Bal, 2019). These findings suggest that foliar application of melatonin could improve preservation through a process involving inhibition of the respiration rate. Similar results were observed by (Wang et al., 2019) on Sweet cherries (Bal, 2019) on plum, who reported the least amount of weight loss in M1000 and M100 treatments throughout storage M1000 (4.5%) and M100 (4.6%) treatments had the lowest weight loss at the end of storage. A similar finding was obtained by (Liu et al., 2018), the weight loss of both control and melatonin-treated fruit increased during storage.

Compared to the control and 0.01 mmol L^1 melatonin treatments, the 0.1 and 1 mmol L^1 melatonin treatments significantly reduced strawberry fruit weight loss from day 9 to day 12. This could be attributed to the strawberry fruit's superior skin strength, which was critical in reducing weight loss. Furthermore, Gao et al. (2016), who demonstrated that treated plants with melatonin significantly reduced weight loss percentage in peach fruit during storage.

2. Fruit firmness (N)

The firmness of Capsicum annum cv. Jalapeño fruit reduced significantly over both storage time (0 and 45 d) Table 2. The firmness values of the Control, M50, and M100 groups were 29.06 N, 38.31 N, and 33.49 N, respectively, at the end of storage (45 d). These results are in line with obtained (Wang et al., 2021). Exogenous melatonin significantly preserved the firmness of pepper fruit ($p \leq 0.05$). All exogenous melatonin preharvest spraying inhibited Jalapeño fruit softening, with no noticeable difference between the MT-treated groups at both storage time for the same treatment. In addition, Wang et al., (2019) reported that melatonin treatment significantly inhibited the decline of cherry firmness. Furthermore, 100 mol L^1 of melatonin suppressed the firmness decline of peach during postharvest storage. The initial

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strawberry fruit firmness was 3.99 N and declined during storage, but the melatonin treatment efficiently delayed this reduction (Liu et al., 2018). According to Gao et al., (2016), melatonin treatment significantly maintained firmness in peach fruit during storage. Sun et al., (2015), on the other hand, revealed that an exogenous melatonin dipping at 50 mol L^1 for 2 h significantly accelerated softening in green mature tomato fruit. The differences in melatonin response between our study and that of Sun et al., (2015) could be attributed to different ripening stages.

3. Fruit Thickness (mm)

The thickness (mm) of the "Jalapeno" pepper gradually improved during storage (0 and 45 days), and melatonin treatment significantly ($p < 0.05$) inhibited the increase Table 2. At the end of storage (45 days), the thickness of treated pepper with control, MT 50, and MT 100 was 23.02, 17.02, and 13.99 % less than that of untreated pepper (0 d). Fruit thickness did not change significantly ($P > 0.05$) at harvest time, but significant differences were observed at the end of storage time (45 d). Fruit thickness decreased due to tissue dehydration, but differences between control and treated peppers persisted at the end of storage. A similar result was obtained by (Valero et al., 2014).

4. Fruit diameter (cm)

Fruit diameter was measured at harvest (0 d) and at the end of storage time (45 d), and the results are shown in Table 2, the fruit diameter affected significantly by MT treatments and storage time. Furthermore, the findings revealed that a MT 100 treatment stimulated fruit growth resulted in significantly larger pepper fruit ($p \leq 0.05$) in MT 100 -treated plants (3.32 cm) outperformed MT 50 or control plants (3.07 and 2.78 cm, respectively), at harvest (0 day) , respectively. While, after 45 d from storage the values were 2.73, 3.05, and 3.19 for control, MT50 and MT 100 respectively. These result was in line with findings by (Medina-Santamarina et al., 2021). This effect was caused by an increase in peel width rather than an increase in aril portion.

Table 2: Effect of melatonin on weight loss (%), Firmness (N), Thickness (mm), fruit diameter (cm), fruit length (cm) Capsicum annum L. at harvest (0-day) and after storage (45-days), at two consecutive years.

Storage Time (ST)	Treatments (T)	weight loss %	Firmness N	Thickness mm	Fruit diameter cm	Fruit length cm
0-Day	Control	-	29.06^{c}	4.17^{a}	3.07^{b}	8.31^{a}
	MT 50	-	29.03^{c}	4.23^{a}	3.19^{ab}	7.91^{a}
	MT 100	-	33.85^{b}	4.29^{a}	3.32^{a}	8.79^{a}
45-Days	Control	4.38^{a}	33.49^{b}	3.21^{c}	2.73^{c}	8.47^{a}
	MT 50	1.83^{b}	38.31^{c}	3.51^{bc}	2.78^{c}	8.98^{a}
	MT 100	1.12^{c}	37.10^{c}	3.69^{b}	3.05^{b}	8.95^{a}
LSD 0.05		**0.047**	**3.320**	**0.401**	**0.242**	**NS**

Means with the same letters within columns are not significant at $P \leq 0.05$. Each value is the mean of three replicates. MT 50 (melatonin at 50 µM), MT 100 (melatonin at 100 µM), and Control (untreated). *, and ** no Significant, Significant at $P \leq 0.05$

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5. Fruit length (cm)

Data as shown in Table2. The fruit length was increased insignificantly for melatonin treatments and storage. Fruit pepper treated with MT 100 recoded the highest value for fruit length (8.98 and 8.95 cm), at harvest (0 day) and after storage (45 d) compared with (8.32 and 7.91cm) for untreated plant in both storage time.

3.3 hot pepper fruit proximate composition response to melatonin application at during storage time.

6. Fruit moisture %

The proximate composition results of the pepper Jalapeño are shown in Table (2). Moisture content decreased with 45-day storage compared with (0-day) storage ranged between 83.82 to 91.79% with MT100 under storage 4^0C for 45 days from Jalapeño pepper and Jalapeño pepper control (0-day) storage having the least and highest value respectively. There was a significant difference ($p<0.05$) in moisture content of the pepper Jalapeño except those of control and MT50 in 0-day storage and 45-day. The moisture content of the pepper Jalapeño without treatment fresh or storage were higher than those recorded by Amaechi et al., 2021 they determined moisture content of the pepper foliage and found that ranged between 86 to 88% with foliage from Habanero pepper and Anaheim pepper. The high moisture content of the pepper Jalapeño indicates freshness and perishability due to high water activity.

Table 3: Effect of melatonin on gross chemical composition of Capsicum annum L. at harvest (0-day) and after storage (45-days), at two consecutive years.

Storage Time (ST)	Treatments (T)	Moisture %	Ash %	Fat %	protein %	reducing sugar %	Non-reducing sugar %	Total sugar %
0-Day	Control	91.793 ^{a}	9.94 ^{a}	11.643^{b}	14.96^{a}	18.87^{f}	18.35b	37.22d
	MT 50	91.254 ^{a}	9.98 ^{a}	13.725^{a}	15.223^{a}	22.71^{e}	22.95a	45.66a
	MT 100	89.467^{b}	9.95 ^{a}	8.754^{c}	13.68^{c}	23.37d	19.06c	42.43b
45-Days	Control	90.317^{ab}	9.32^{b}	7.159^{d}	11.99^{e}	25.67c	6.55e	32.22f
	MT 50	90.320^{ab}	9.42^{b}	5.191^{f}	14.186^{b}	30.86a	2.64f	33.51e
	MT 100	83.823^{c}	9.14^{c}	6.478^{e}	13.200^{d}	28.52b	11.82d	40.34c
LSD 0.05		**1.639**	**0.146**	**0.495**	**0.293**	**0.059**	**0.390**	**0.241**

Means with the same letters within columns are not significant at $P \leq 0.05$. Each value is the mean of three replicates. MT 50 (melatonin at 50 µM), MT 100 (melatonin at 100 µM), and Control (untreated). *, and ** no Significant, Significant at $P \leq 0.05$

7. Ash %

Ash is a measure of the mineral content of foods. In this study, ash of peppers increases and decreases depending on the treatment and storage. Crude ash recorded lower values at Jalapeño pepper MT 100 under (45-days) storage 4^0C, 9.14 and higher values Jalapeño pepper with MT50, up to 0.832%. Ash content of the pepper Jalapeño ranged between 9.146 and 9.978%.for MT100 45-day storage and Jalapeño MT50 0- day storage had the highest ash content, there were non-significant effect between treatment in 0-day storage and significant effect between

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0-day storage and 45-day. This result indicates that treated Jalapeño fruit with MT50 it will provide more minerals than the other Jalapeño pepper studied Table 2. The quality of pepper fruits is influenced by many factors: treatments fertilization and storage.

The ash values of the pepper Jalapeño were lower than the ash values of pepper foliage (1.30-3.20 g Fw) (Amaechi, et al., 2021). But lower than ash values of Capsicum frutescens ribe (1.9 %, in Fw) was obtained by Adu, et al., (2021).

8. Fat content %

The Jalapeño fruit fat content is significantly affected by treatment and storage under 4^0C temperature conditions. Statistical analysis demonstrated that the crude fat significant variation among Jalapeño pepper 0-day at harvest and Jalapeño 45-day storage 4^0C .Table 2, shows that Jalapeño MT50 fresh (0day storage) has the highest fat value (13.725%) While the same treatment storage recorded the lowest value (5.191%).The fat content of all the Jalapeño pepper was fairly higher than the fat content of all the vegetables reported by Nadeeshani et al., (2018). The low-fat content of the Jalapeño pepper except treatment1 fresh agrees with the findings of (Adu, et al., 2021) they studied the crude fat content to three pepper varieties ripe and unripe and found that ranged between 1.118- 2.108 % for fresh weight data are shown in Table 2.

9. Protein content %

Crude protein in our present study is significantly affected by treatment and storage 45 day under 4^0C temperature conditions. Statistical analysis evaluated that the crude protein significant variation among Jalapeño pepper fresh and Jalapeño storage under 4^0C, except in between MT 50 and control at 0-day storage. The crude protein of treatment Jalapeño fresh and 45-day storage are presented in Table 2. The crude protein content of Jalapeño pepper showed a range of 15.22 to 11.99%. Jalapeño MT50 and Jalapeño control 45-day storage had the maximum and minimum crude protein content respectively. 15 chili peppers crude protein content were ranged between 12.05 – 15.22 g/100 g Ancho and Chipotle Meco, respectively. (Orellana-Escobedo et al., 2013). But higher than the crude protein content of 35 pepper (Capsicum annuum) genotypes of three types group (1) 7.13 group (2) 6.07 and group 3 8.93% (Khan et al., 2019).

10. Reducing and Non-reducing sugar %

The sugars in Jalapeño peppers contribute to their sweet taste Reducing, Non- reducing and Total Sugars of Jalapeño treatments and 45-day storage significantly affected sugar content of Jalapeño pepper fruit. Increase in reducing sugars was observed with the treatment of 45-day storage compared with the same treatment 0 -day storage. However, control storage recorded lower increase compared with other treatment it was 25.67%. These results in the same trend with the rustles found by Abad Ullah et al., 2017 .On the other hand, non-reducing sugars increased for treatment 0 day storage compared with the same treatment 45 day storage, control 0 day (fresh) recorded high value in non-reducing sugar it was 18.35%. Moreover, total sugars showed increasing with the MT 50 and MT100 compared with control 45.66,42 .43 and 37.22 for fresh, respectively (Table 2).

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Table 4: Effect of melatonin on bioactive component of Capsicum annum L. at harvest (0-day) and after storage (45-days), at two consecutive years

Storage Time (ST)	Treatments (T)	Vitamin C mg/100g FW	Total phenol mg/100g galic	Antioxidant activity IC50 g/g
0 Day	Control	216.66^{c}	199.775^{b}	248.64^{b}
	MT 50	306.66^{b}	109.919^{e}	284.64^{c}
	MT 100	346.67^{a}	201.16^{a}	194.19^{a}
45 Days	Control	61.66^{f}	169.717^{d}	557.53^{f}
	MT 50	80.57^{e}	87.459^{f}	508.91^{e}
	MT 100	113.33^{d}	182.571^{c}	451.69^{d}
LSD 0.05		**10.024**	**1.230**	**4.436**

Means with the same letters within columns are not significant at $P \leq 0.05$. Each value is the mean of three replicates. MT 50 (melatonin at 50 µM), MT 100 (melatonin at 100 µM), and Control (untreated). *, and ** no Significant, Significant at $P \leq 0.05$

3.4 Hot pepper fruit bioactive component response to melatonin application at during storage time.

1. Ascorbic acid and total phenolic content

Data presented in Table 3 shows the ascorbic acid as well as the total phenolic content (TPC) and antioxidant activity for the treatment 0-day at harvest of Jalapeno and 45-day after storage. The initial content of ascorbic acid in treatment fresh Jalapeno and control was ranged between 216.66 -346.57 mg /100 g Fw. While, in Jalapeno 45-day storage ranged 61.66-113.33 mg /100 g Fw .It can be seen that a decrease in the storage cold temperature treatment has an important effect on ascorbic acid ($p < 0.05$), thus, a maximum loss of 73.72% ascorbic acid in MT 100 45 day storage is observed. All treatment Jalapeno storage samples contained less ascorbic acid than the fresh Jalapeno pepper due to a combination of oxidation and storage destruction of ascorbic acid during storage period. These results were higher than those reported by (Bhandari et al., 2016; Bicikliski et al., 2018).

In the present work, phenolic compounds from Jalapeno treatment fresh and 45 day storage varied between 109.92 – 201.16mg GAE/100gFw and 87.45 – 182.57mg GAE/100g Fw, as respectively (Table 3).As for the phenolic content, there were significant differences ($p<0.05$) between the treatment fresh and storage of Jalapeno peppers studied, the variability of these compounds in the peppers evaluated can be explained by treatment factors and storage under cold. The levels found in our study come to an agreement with those reported by Soare et al.**,** (2017), who, evaluating phenolic compounds in six C. annuum peppers, found a range of 128–202.6 mg GAE/100 g Fw. It can be observed from (Table 3) that treatment and storage under cold had an important effect on the total phenolic content ($p < 0.05$). The highest and lowest TPC contents were detected in treatment 2and treatment 1 fresh Jalapeno, respectively.

2. Antioxidant activity

The results expressed as IC50 indicate the concentration of extract able to inhibition 50 % of the DPPH. Therefore, the lower the IC50 value, the higher the antioxidant activity of the

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extract. Thus, it is observed that the MT100 at harvest (0-day) and 45-day after storage showed the highest antioxidant activity, 194.18 and 451 .69 g/g DPPH, while the control storage had the lowest antioxidant activity, 557.53g/g DPPH Table 3. Comparing the antioxidant activity results, it was found that (Carvalho et al., 2015), found values ranging from 1745.18 to 4905.06 g/g in dray weight the genotypes, IAN 186311and IAN 186313, respectively. These values are close to those found for Balady green pepper and yellow bell pepper. Investigating bioactive compounds and the antioxidant activity of 4 traditional tropical fruits from Egypt, do Mennat Ullah E. Abdalla (2019) showed the highest antioxidant activity values, 1114? 17 and 3267.68 µg/ml, respectively.

3. Chlorophyll a, and b content

Chlorophyll a and b were affected by treatment and 45-day storage, interaction between Treatment and 45-day under cold storage. The results on the effect of treatment and storage under cold storage on the Chlorophyll a and b of Jalapeno pepper are as follows: the chlorophyll a content was found to be significantly higher in MT 50 Jalapeno fresh and 45-day storage (Table 4). The values were 0.691 and 0.273. Minimum values were observed in control on fresh and MT 100 storage it was 0.672 and 0.244 mg/g FW. The chlorophyll b was more in control (Table 4) on fresh (0.187). On storage for 45 day, the chlorophyll b content was found to be higher in control (Table 4). The chlorophyll a and b content of Jalapeno pepper under studied for all treatments decreased with storage under cold. The chlorophyll a content due to treatment with and cold storage. These results agree with finding by Żurawik et al., (2021).

Table 5: Effect of melatonin and on Chlorophyll a, and b of Capsicum annum L. at harvest (0-day) and after storage (45-days), at two consecutive years

Storage Time (ST)	Treatments (T)	Chlorophyll a mg/ g Fw	Chlorophyll b mg/ g Fw
0 Day	Control	0.672^{b}	0.187^{a}
	MT 50	0.692^{a}	0.175^{b}
	MT 100	0.674^{b}	0.181^{ab}
45 Days	Control	0.247^{d}	0.093^{c}
	MT 50	0.273^{c}	0.083^{d}
	MT 100	0.245^{d}	0.079^{d}
LSD 0.05		**0.070**	**0.063**

Means with the same letters within columns are not significant at $P \leq 0.05$. Each value is the mean of three replicates. MT 50 (melatonin at 50 µM), MT 100 (melatonin at 100 µM), and Control (untreated). *, and ** no Significant, Significant at $P \leq 0.05$

4. CONCLUSION

This study shows that preharvest melatonin (at 50 or 100 µM) had a significant effect on crop yield and fruit quality of Jalapeno at harvest and during storage (45-days). The 100 µM dose produced the best results for crop yield. Furthermore, after melatonin treatment, the concentrations of sugars and ascorbic acid were higher. Melatonin-treated fruit had lower

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softening, and weight losses during postharvest storage. Overall, melatonin has the potential to be a dependable, feasible, and cost-effective tool for use as a plant bio-stimulant in order to increase pepper crop yield and fruit quality parameters at harvest while also maintaining crop quality.

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Contributions

AAHE, MAE and NAA performed the experiment. AAHE, and MAE analyzed the data. MAE, and AAH conceived the original research plans. AAHE, NAA, and MAEdesigned the experiments and wrote the whole article. All authors read and approved the final manuscript.