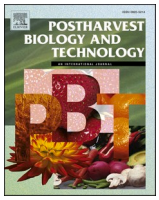
Postharvest Biology and Technology 184 (2022) 111759

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio



Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* L.) vase life

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ARTICLE INFO

Keywords:
Cut carnation
Petal
Antioxidant activity
Chlorophyll
Total phenolic content
Melatonin

ABSTRACT

The marketability of cut flowers is directly affected by their vase life, which determines acceptability for commercial purposes. In carnations and other species of cut flowers, corolla is one of the most affected parts during flower senescence due to the petal withering which is accelerated by metabolic processes occurring after separation from the mother plant. Melatonin (MT) is a compound with antioxidant properties, naturally present in plant tissues that plays important roles in the regulation of different metabolic processes. In this research work the effect of different MT concentrations (0.01, 0.1 and 1 mM) on the vase life of cut carnations flowers cv. Baltico was evaluated. The greatest delay in senescence was observed with 0.1 mM MT concentration, increasing vase life up to 10 days more as compared to control carnations. Although all MT concentrations assayed significantly ($P < 0.05$) maintained initial levels of fresh weight, membrane stability index, bioactive compounds and antioxidant activity for longer time, the lowest concentrations were those that had the most relevant impact on vase life. The highest dose evaluated (1 mM) maintained all the parameters evaluated but showed the wilting symptoms earlier. For this reason, 0.1 MT concentration could be a tool capable of improving carnation vase life for longer time, increasing the commercial potential of this cut flower.

1. Introduction

Carnation (*Dianthus caryophyllus* L.) is a flower with important economic and ornamental values in Mediterranean countries and worldwide (Ranjbar and Ahmadi, 2015). In addition, white carnation is one of the most demanded in comparison with the rest of coloured carnations that can be found for commercial purposes (Ebrahimzadeh et al., 2008). This plant species is very sensitive to exogenous ethylene but the response to ethylene exposure is also cultivar dependent (Serrano et al., 1991, 1999). Flower senescence in most carnation cultivars is characterized by autocatalytic ethylene production and subsequent petal in-rolling (Serrano et al., 1991, 2001; Satoh et al., 2005). However, some carnation cultivars have low or even absence of ethylene production (Wu et al., 1991; Serrano et al., 1991; Ebrahimzadeh et al., 2011), thus influencing their vase life (Nukui et al., 2004). Vase life is the most important quality criteria that influence the consumer demand of cut carnations although appearance, colour and uniformity of the corolla are also remarkable factors (Reid and Jiang, 2012; Scariot et al., 2014).

Cut flowers are metabolically active after harvest, carrying out all their vital processes by using available substrates in their tissues (Yakimova et al., 1997). These energy requirements are partially provided by starch hydrolysis but some techniques as the application of ethylene inhibitors can be a useful tool to delay flower metabolism specially in carnations with a climacteric pattern (Serrano et al., 2001; Ebrahimzadeh et al., 2008). Increasing vase life and delaying flower senescence can be achieved by maintaining the normal rate of water absorption, preventing carbohydrate depletion, and reducing the oxidative stress, which are specially stimulated during postharvest senescence period (Halevy and Mayak, 1981; Ebrahimzadeh et al., 2008). Indeed, the maintenance of a strong antioxidant potential to scavenge reactive oxygen species (ROS) is associated with a longer vase life period in cut flower species (Ezhilmathi et al., 2007; Hassan and Ali, 2014a, 2014b; Aalifar et al., 2020; Rashidiani et al., 2020). In this sense, many advances have been made and the number of studies regarding the

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https://doi.org/10.1016/j.postharvbio.2021.111759
Received 25 June 2021; Received in revised form 13 October 2021; Accepted 15 October 2021
Available online 26 October 2021

effect of new compounds on the antioxidant potential to eliminate ROS has increased in recent years. Special interest has received some natural compounds as alternatives to common chemicals (Solgi, 2018; Akhtar et al., 2021) since they do not compromise human health and have been reported as environmentally friendly. Some of these natural substances such as essential oils and moringa leaf extract, have been described as potential providers of extra energy demanded during development by cut flower tissues increasing carbon sources availability (Salmi et al., 2018; Hassan and Fetouha, 2019) and thus, delaying senescence process. In this context, melatonin (MT), polyamines or γ-aminobutyric acid (GABA), have been proposed as responsible of GABA shunt pathway activation, providing extra ATP and decreasing accumulation of ROS in vegetal tissues (Aghdam and Fard, 2017; Karimi et al., 2017; Mohammadi et al., 2020) specially under metabolic stress conditions (Aghdam et al., 2016a; Arnao and Hernández-Ruiz, 2019).

MT (N-acetyl-5-methoxytryptamine), is an indole derivative involved in a wide range of cellular and physiological actions in plant processes, such as seed germination, root development, flowering, photosynthesis, leaf senescence and protective effect against biotic or abiotic stress (Arnao and Hernández-Ruiz, 2019; Debnath et al., 2019; Sharma and Zheng, 2019). However, little information is available regarding the effect of MT on cut flowers vase life and senescence. In fact, as far as we know, only one report (Aghdam et al., 2019) studied the effect of MT treatment on increasing anthurium cut flowers resistance to chilling injury during cold storage. For this reason, carnation flowers were selected in this report since this specie is considered a model plant for the ornamental industry (Aalifar et al., 2020). Different MT concentrations were applied to cut carnation flowers, to study their effects on different cut flower quality parameters, with particular attention to the effect on delaying petals and sepals senescence.

2. Experimental design

2.1. Plant material and treatments

Cut carnation flowers (*Dianthus caryophyllus* cv. Baltico) were harvested from a commercial orchard in Murcia (Spain) at commercial harvesting stage. Then, flowers were transported to the laboratory with stems immersed in tap water on the harvest day. At the laboratory, 66 flowers were selected (discarding any flower with visual defects) for each treatment (MT at 0.01, 0.1 and 1 mM and distilled water as control) at the development stage 3 (Fig. 1). To select the optimal MT doses to test in the entire flower we carried out a previous study and different MT doses were tested on 'Baltico' carnation petals. 20 petals per MT dose (0.005, 0.01, 0.1 and 1 mM) were individually evaluated at room temperature in comparison with 20 petals in distilled water as control. Petal fresh weight (FW) was reduced, specially, for the 0.1 mM MT dose (Fig. S1). Melatonin (Sigma-Aldrich, USA, > 98 % M5250) was previously dissolved for each concentration with 0.5 mL ethanol. Similar ethanol volume was added to the control solution. Stems were cut to 10 cm and placed individually in falcon tubes with 10 mL of the different treatments. Carnations were kept at room temperature of about 20 °C, relative humidity 65–70 % and a 12 h photoperiod using white fluorescent light with an intensity of 80.5 μmol m^{-2} s^{-1}. These conditions were maintained for four weeks, and distilled water or MT solutions were added to falcon tubes when necessary. Non-destructive measurements as vase life, FW and vase solution uptake (VSU) were evaluated almost daily in one lot of 30 flowers per treatment. For respiration rate and ethylene production another lot of 6 flowers per treatment was used exclusively to evaluate these parameters. The different solutions in each tube were replaced when necessary. Additionally, 30 flowers per treatment were divided in 5 lots of 6 flowers, to evaluate destructive parameters as membrane stability index (MSI), total polyphenol content (TPC) and total antioxidant activity (TAA) in petals or total chlorophyll content (TCC) in sepals after 5, 10, 15, 20, and 25 d of storage at room temperature. Also, the different parameters were evaluated in an extra lot of 10 carnations at the day of harvest. All parameters were evaluated in fresh samples except TAA and TPC content which were measured in petals separated from 6 flowers for each treatment, mixed, powdered in liquid nitrogen and stored at −80 °C for later analysis.

2.2. Vase life

Vase life was evaluated daily in each flower individually. This parameter was determined by the number of days in which flowers maintained their decorative properties, until carnations had no ornamental value due to the petal in-rolling or tip browning appearance (Satoh et al., 2005). This period in our study match with the 7th senescence stage (Fig. 1). Results were expressed as the means ± SE of 30 flowers per treatment.

2.3. Fresh weight and vase solution uptake

FW of each flower was expressed as percentage with respect to its initial FW which was assumed to be 100 %. Results were the mean ± SE of 30 flowers. To evaluate the VSU the falcon tube without the flower was weighted, as well as the weight of the flower and the solution. Results were expressed as the mean ± SE of 30 flowers per treatment and the following formula was used to calculate the VSU (mL d^{-1} g^{-1} FW) = $(W_{(t-1)}-W_{(t)}) / FW_{t=0}$), where W_t = solution weight (g) at t days (3, 4, 5, etc.), W_{t-1} =water weight (g) on the previous day and $FW_{t=0}$ = FW of the flower (g) on day 0.

2.4. Respiration rate and ethylene production

Respiration rate and ethylene production were measured in 6 flowers by placing each flower in 1 L glass jar hermetically sealed with a rubber stopper for 2 h. After that, one mL gas sample was taken from head space and injected into a Shimadzu TM 14A gas chromatograph (Kyoto, Japan) equipped with a thermal conductivity detector under the chromatographic conditions previously described by Medina-Santamarina et al. (2021) to quantify CO_2 concentration. Respiration rate was expressed as nmol of CO_2 released by kg^{-1} s^{-1} and results were the mean ± SE (n = 12).

Ethylene production was determined by injecting another mL gas sample taken from the same atmosphere into a Hewlett-Packard TM model 5890A gas chromatograph (Wilmington, DE), equipped with a flame ionization detector and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. Chromatographic conditions were similar to those previously reported (Medina-Santamarina et al., 2021). Ethylene production was expressed

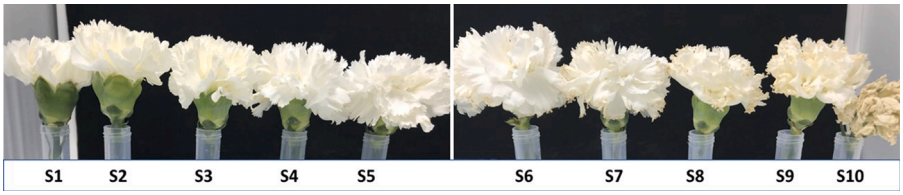

Fig. 1. Ten stages of flower development in carnation cultivar Baltico during vase life (growth: S1-S5, Senescence: S6-S10).

as nmol $kg^{-1}s^{-1}$ and results were the mean ± SE (n = 12).

2.5. Membrane stability index

Four external petals were taken from each one of the 6 flowers sampled from each treatment and sampling date and from each petal one disc was cut with a corkborer (measuring 1 cm in diameter) and placed in testing tubes as indicated by Rashidiani et al. (2020) with small modifications. Thus, 12 testing tubes per treatment and sampling date containing 2 petal discs were used. These tubes were added with 15 mL ultrapure water and 6 tubes were placed in a warm bath (40 °C) for 30 min, cooled down to 25 °C and then the electrical conductivity (EC) was measured (C1). Then, a second series of 6 testing tubes was placed in a warm bath (100 °C) for 20 min and then EC was read after being cooled down rapidly in ice bath until 25 °C (C2). MSI was calculated using the following formula: MSI= [1 - (C1/C2)] 100 where C1 = EC after heat treatment at 40 °C and C2 = EC after exposure to 100 °C. (n = 6)

2.6. Total chlorophyll content

For chlorophyll measurement in sepals, six calix disks, each of 6.25 mm in diameter, were punched from each flower. The disks were placed immediately into 8 mL of 100 % methanol, and pigments were allowed to be extracted in the dark at 30 °C for 24 h. Absorbance of the extract was measured using spectrophotometer (1900 UV/ Vis, Shimadzu, Kyoto, Japan) at 652 and 665 nm (Porra et al., 1989). Results were the mean ± SE of six individual flowers per treatment (n = 12).

2.7. Total polyphenol content

To extract phenolic compounds, 0.5 *g* of petal tissue were homogenized with 20 mL of water: methanol (2:8, v/v) containing 2 mM NaF by using a homogenizer (Ultraturrax, T18 basic, IKA, Berlin, Germany) for 60 s. The extracts were centrifuged at 10,000 *g* for 10 min at 4 °C and the supernatant was used to quantify total phenolics (in duplicate in each extract) by using the Folin-Ciocalteu reagent as previously described for petals and other tissues by Lezoul et al. (2020). The results were expressed as g gallic acid equivalent (GAE) kg^{-1} and are the mean ± SE of measures performed in 6 individual flowers (n = 12).

2.8. Total antioxidant activity

The determination of the antiradical activity by the ABTS test was carried out using the method described by Lezoul et al. (2020). 10 mL of 50 mM phosphate buffer solution and 10 mL ethyl acetate were added to 1 *g* of petal tissue in three replicates and the mixture was homogenized for 1 min and then centrifuged at 10,000 rpm for 20 min at 4 °C. Then, the determination of the TAA was evaluated in the hydrophilic fraction and lipophilic fraction. The results were expressed in g trolox equivalent kg^{-1} FW with reference to the trolox calibration curve (n = 6).

2.9. Statistical analysis

The experiment was conducted in a complete randomized design. The analysis of variance (ANOVA) was performed, and data were analysed using SPSS software package v. 20.0 for windows. Means ± SE values were compared by a Least Significant Difference (LSD) test at $P < 0.05$.

3. Results

3.1. Fresh weight and water uptake

FW increased from day 0 to day 5 (Fig. 2) due to petal opening manifested as an apparent corolla growth as flowers evolved from stage S3 to S5 (Fig. 1). From day 5, a slow decrease of FW occurred in control carnations which was accelerated from day 11. Carnations with stems submerged in the most concentrated MT solutions (0.1 and 1 mM) remained with a constant FW, ≈ 107 % of the initial stage, for longer time than the rest of the treatments (Fig. 2A). Nevertheless, it is worth noting that all melatonin treatments delayed significantly ($P < 0.05$) flower weight losses. Also, carnations in MT 0.1 mM solutions showed the highest FW in comparison with the rest of the treatments and especially significant in comparison with control flowers ($P < 0.05$) during the whole experiment. In fact, the FW percentage observed on d 21 of the vase period for control and 0.1 mM MT solution was 66.05 ± 5.47 and 97.27 ± 0.55 % respectively.

VSU was high and without significant changes during the first days of vase life of all carnation flowers and decreased sharply after 10 d in control carnations and after 15, 17 and 18 days in those treated with 0.01, 1 and 0.1 mM MT respectively. Nevertheless, all the concentrations maintained higher VSU during storage (Fig. 2B) in comparison with control carnations though a lower VSU was observed in 0.1 mM MT flowers during first period of storage.

3.2. Vase life

Vase life was affected by the different treatments applied. In fact, vase life was significantly ($P < 0.05$) higher when flowers were stored in falcon tubes containing the different melatonin solutions, compared to controls in distilled water (Fig. 3).

MT 0.1 mM was the best dose for carnations in this study with a vase life of 20 d in which stage 7 of senescence was reached, while the rest of the treatments reached this stage several days earlier (10, 16 and 12 days for control, 0.01 and 1 mM MT solutions respectively). Thus, 0.1 mM MT treatments led to a 2-fold increase in the storage time that flowers maintain an aesthetic value with respect to control ones. The melatonin effect on vase life can be clearly observed in the photographs of the evolution of the senescence process (Fig. 4). However, there was no dose-dependence in the range of melatonin concentration assayed, since 0.1 mM led to higher vase life than 0.01 mM but carnation petals of

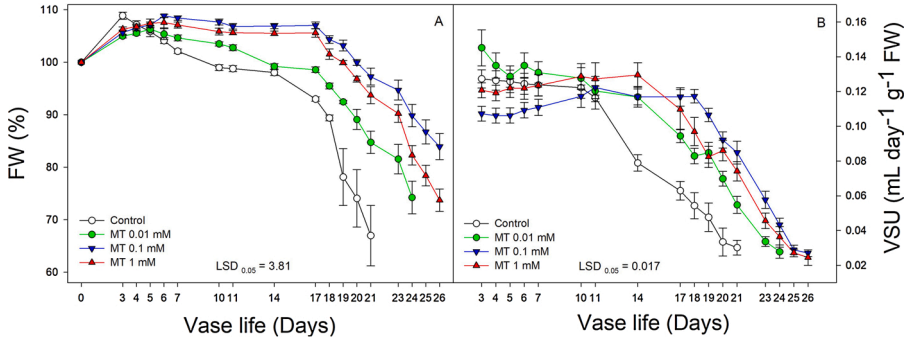

Fig. 2. Carnation fresh weight (FW) evolution (% initial FW) (A) and vase solution uptake (VSU) (B) of control and melatonin (MT, 0.01, 0.1 and 1 mM) in cut carnation flowers cv. Baltico during vase life period. Values are the means ± standard error (SE) (n = 30). LSD at $P < 0.05$ was used for means comparison.

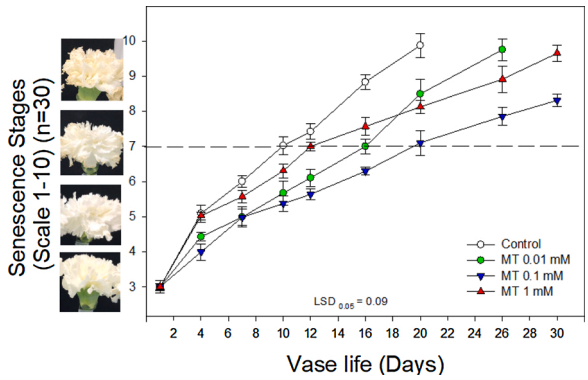

Fig. 3. Effects of melatonin treatments (MT, 0.01, 0.1 and 1 mM) and distilled water (control) on the evolution of the senescence process of carnation flowers during vase life period at room temperature. Values are the means ± standard error (SE) (n = 30). LSD at $P < 0.05$ was used for means comparison.

flowers that received the highest MT dose (1 mM) showed tip burn.

3.3. Ethylene production, respiration rate and membrane stability index

Ethylene production was very low in all carnation flowers during the whole vase life period, with values ranging from 3×10^{-4} to 1×10^{-3} nmol kg^{-1} s^{-1} and no significant differences were observed attributed to melatonin treatments (data not shown). Respiration rate in carnation flowers was similar at the beginning of the storage for all carnation flowers showing an increase until day 4 without significant differences among treatments (Fig. 5A).

However, from day 6 until the end of the experiment, lower respiration values were obtained when MT solutions were applied. The highest respiration rate was measured on control carnations while the lowest values were obtained on d 25 of the vase life period in 0.1 mM MT treatment.

At the beginning of the experiment, the MSI was 90 % and values decreased during vase life period for all evaluated treatments (Fig. 5B). However, compared with control flowers, significantly ($P < 0.05$) higher MSI values were found in the flowers treated with MT from day 5 until last sampling date. For instance, a MSI value of ≈ 53 % was reached on control flowers at day 15, while similar values were reached after 20 d in 0.01 and 1 mM MT treated flowers and after 25 d in those treated with 0.1 mM MT.

3.4. Bioactive compounds and antioxidant activity

TCC values in carnation sepals showed that the effect of different MT concentrations was significant ($P < 0.05$) throughout vase life of carnations (Fig. 6A).

TCC decreased in control carnations from day 0 to the end of vase life, while this decrease started at day 5 in 0.01 mM MT treated flowers and after 10 and 20 d in 1 and 0.1 mM MT treated flowers respectively. Thus, although all MT treatments delayed chlorophyll degradation in the carnation sepals as compared with controls, the highest effect was found for 0.1 mM MT concentration. The highest chlorophyll content was observed when 0.1 mM MT dose was applied after 15 d of vase life while control carnation sepals reached the lowest TCC as compared with MT doses at the same sampling date (Fig. 6 A).

Results showed a decreasing pattern for TPC along the experiment in all petal samples evaluated. However, this decrease evolved sharply in control flowers after 10 d postharvest storage and was significantly ($P < 0.05$) delayed when MT treatments were employed. The highest delay was observed for 0.1 mM MT treatments (Fig. 6 B).

TAA was evaluated in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions of petal extracts and, in general, both showed a decreasing pattern during vase life (Fig. 6 C and D). However, H-TAA and L-TAA values were significantly higher ($P < 0.05$) when different MT solutions were employed in comparison with control samples. There was an increase in H-TAA until the 10 d for control and 0.01 mM MT treatment which was delayed when higher MT doses were applied (Fig. 6C). After 10 days H-TAA decreased for control and 0.01 mM MT treatment while the higher doses employed maintained a higher H-TAA level for a longer period. In this sense, when 0.1 mM MT was used, carnation petals showed the highest H-TAA level during the whole vase life period. A similar pattern was observed when L-TAA was evaluated (Fig. 6D). L-TAA values were higher in carnation petals when different MT solutions were applied in comparison with control flowers, though 0.1 mM MT dose just maintained the highest L-TAA values at the end of the vase life period evaluated in comparison with other MT concentrations.

3.5. Discussion

Several studies reflected that flower senescence could be affected by the endogenous MT content. Despite variations between flower species, it seems that MT levels decreased along the development, specially at latter senescence stages (Murch et al., 2009; Zhao et al., 2017). In the present study we demonstrated that exogenous MT treatments exerted an anti-senescent effect in carnations increasing vase life in MT treated cut flowers and the highest effect was found for 0.1 mM dose. This finding was related with the FW evolution since MT treatments, maintained water relation during postharvest vase life for a longer period. An adequate balance between organs dehydration and the water uptake index is critical to maintain cell turgor in cut flowers (Rot and Friedman, 2010; Reid and Jiang, 2012). 'Baltico' carnations treated with 0.1 mM MT showed a higher FW during vase life period but a delayed VSU as compared with control flowers and other MT doses employed. Senescence evolution in cut flowers is affected through the balance transpiration/water uptake. For this reason, FW decreases when transpiration exceeds the VSU (Rot and Friedman, 2010; Ebrahimzadeh et al., 2011). However, regarding water balance in cut carnations, transpiration rate could be more critical for FW maintenance than VSU during vase life (Solgi, 2018; Hassan and Ali, 2014a). According to this, a lower flower transpiration in petals and other tissues could be affecting the VSU probably through a maintenance of the stomatal properties as has been recently reported (Mohamed et al., 2020).

Carnations are characterized by autocatalytic ethylene production which accelerates flower senescence phenomena also affecting other plant organs (Serrano et al., 1999; Ebrahimzadeh et al., 2008). However, 'Baltico' carnation flowers exhibited longer vase life with a low ethylene production pattern at basal level without a pronounced peak of ethylene synthesis and no associated increases of respiration rate as reported by 'Sandra' 'Killer' and 'Pilar' cultivars (Wu et al., 1991; Serrano et al., 1991; Ebrahimzadeh et al., 2011). However, respiration rate was higher in control than in MT treated flowers, also coincident with advanced senescent stages earlier reached in control carnations. The beginning of the senescence process requires the contribution of metabolic energy (Ebrahimzadeh et al., 2008; Reid and Jiang, 2012). MT postharvest treatments enhanced GABA shunt activity increasing ATP supply in strawberry (Aghdam and Fard, 2017) and electron transport in mitochondria increasing the energy status (Aghdam et al., 2016a, b) in cut anthurium flowers. These associated biochemical processes could explain the lower respiration rate found in MT treated carnation flowers in comparison with control ones, specially, when using 0.1 mM MT dose. According to this, we hypothesize that the additional energy provided by the exogenous MT treatment could be maintaining the energy status in 'Baltico' carnation cell tissues decreasing energetic requirements and respiration increasing vase life. In fact, GABA shunt pathway is involved in maintaining general quality and vase life of cut flowers and several studies have demonstrated that exogenous treatments with solutions capable to stimulate GABA shunt activity increase gerbera vase life (Mohammadi et al., 2020) and confers resistance against abiotic and

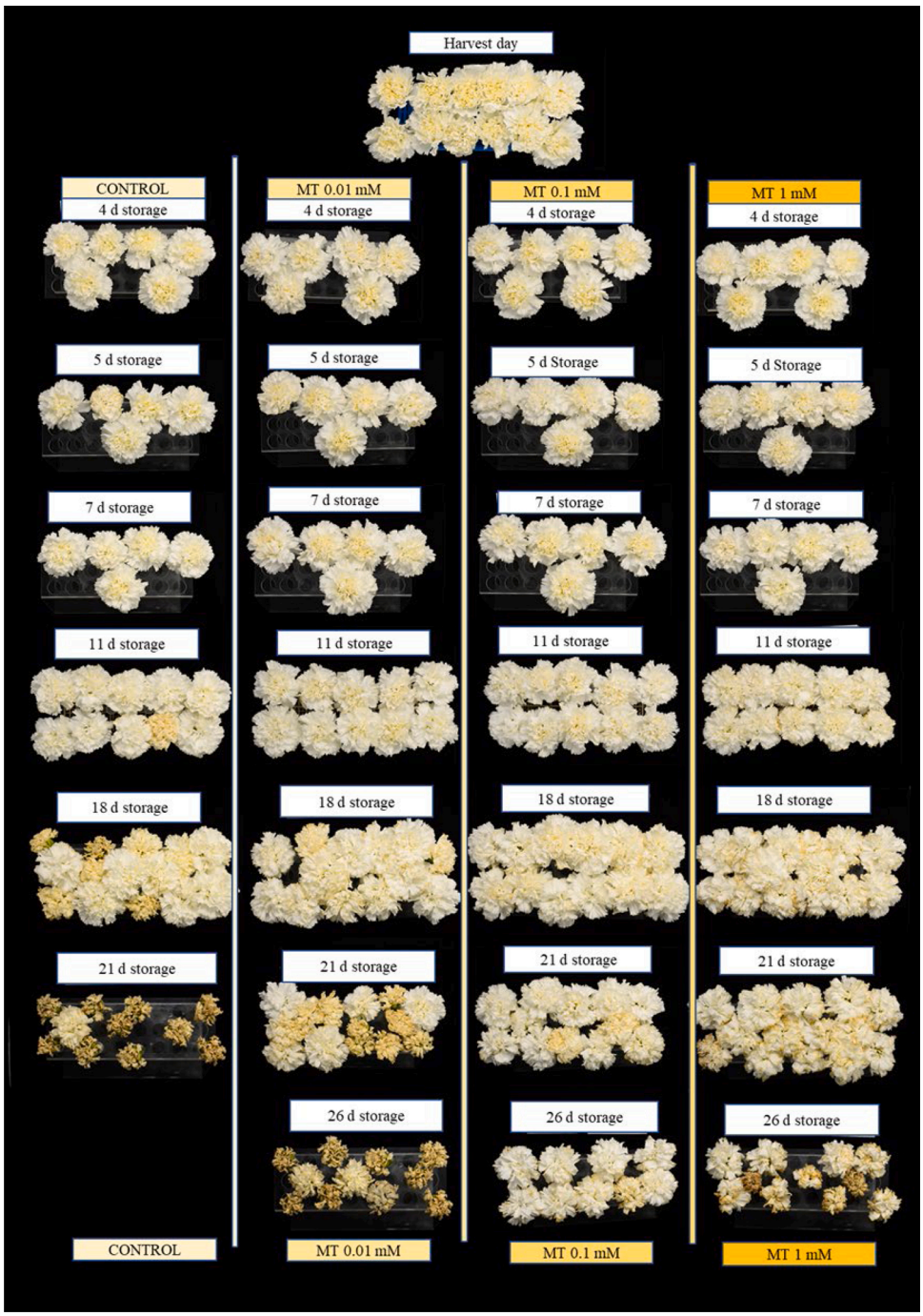

Fig. 4. Carnation cut flowers cv. Baltico appearance during vase life period as affected by different melatonin solutions (MT, 0.01, 0.1 and 1 mM) with respect to control flowers in distilled water.

biotic stress to anthurium and tuberose cut flowers (Aghdam et al., 2015, 2016a, b; Babarabie et al., 2019) during storage. There are no previous studies regarding vase life in melatonin treated cut flowers though the MT effect on chilling injury in cut anthurium flowers have been demonstrated by Aghdam et al. (2019) confirming our results despite of being a very different ornamental plant species. This positive effect on delaying chilling injury was mainly related to MSI. The present results show that MSI exhibited a positive relationship with flower vase life and FW maintenance, as has been described in previous studies in carnation (Hassan et al., 2020; Rashidiani et al., 2020; Ranjbar and Ahmadi, 2015) and other cut flower species (Hemati et al., 2019; Kazemzadeh-Beneh et al., 2018; Perinban et al., 2015). Based in our results, MT treatments resulted in higher MSI than control flowers during the whole vase life period. These observations could be associated with the maintenance of the membrane unsaturated/saturated fatty acids ratio which is affected by ROS in petals (Wang et al., 2020) and other plant tissues (Aghdam et al., 2016a, b). In this sense, Aghdam et al. (2019) in cut anthurium flowers described the role of MT maintaining redox homeostasis, increasing the antioxidant capacity and thus, modulating reparation of oxidatively damaged proteins. This reported effect would be responsible for maintaining higher MSI in MT treated flowers in comparison with control carnations. Also, for leaves (Ali et al., 2020; Mohamed et al., 2020) and fruit tissues in higher plants, antioxidant system activity was stimulated after preharvest

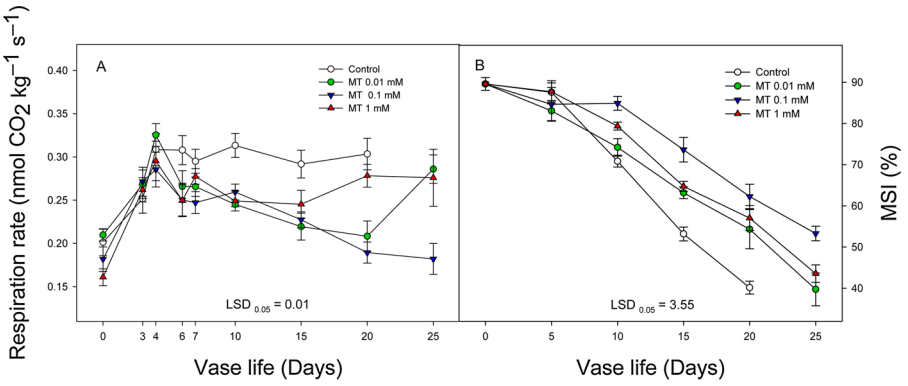

Fig. 5. Respiration rate (n = 12) (A) and membrane stability index (MSI) (n = 6) (B) of control and melatonin (MT, 0.01, 0.1 and 1 mM) in cut carnation flowers cv. Baltico during vase life period. Values are the means ± standard error (SE) (n = 12). LSD at $P < 0.05$ was used for means comparison.

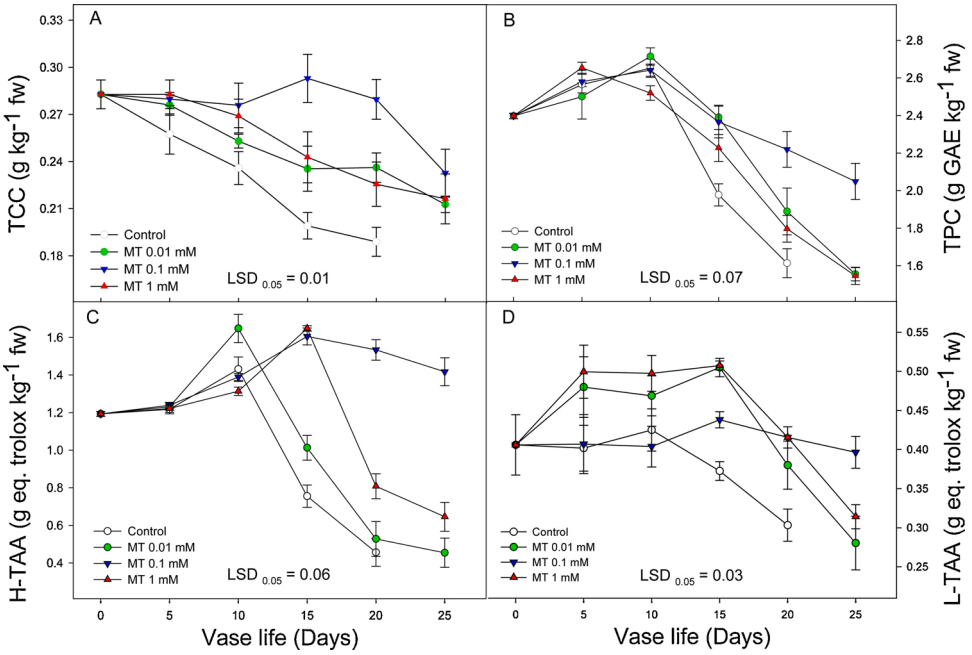

Fig. 6. Total chlorophyll content (TCC) in sepals (n = 12) (A), total polyphenol content (TPC) (n = 12) (B) and total antioxidant activity in the hydrophilic (H-TAA) (n = 6) (C) and lipophilic (L-TAA) (n = 6) (D) soluble phase in petals of carnation cut flowers cv. Baltico treated with melatonin solutions (0.01, 0.1 and 1 mM) and distilled water (control) during vase life period. Values are means ± standard error (SE) and LSD at $P < 0.05$ was used for means comparison.

(Medina-Santamarina et al., 2021) or postharvest melatonin applications (Zhang et al., 2018; Liu et al., 2018; Sharif et al., 2018) leading to maintenance of MSI for longer period.

In the current study, the TCC in sepals was maintained during storage in all MT treated flowers but specially, when 0.1 mM MT solution was employed. Chloroplasts are easy targets of ROS-linked damage during various stresses and natural senescence since ROS detoxification systems decrease during postharvest in leaves and other green parts of the plant (Khanna-Chopra, 2012). In this sense, and based on the results, the MT effect on protecting chlorophyll content could be related to an improved water relation through a preserved membrane stability, supported by a higher antioxidant activity system. This is consistent with the finding reported in gardenia MT treated leaves by Zhao et al. (2017) who observed a higher chlorophyll content in comparison with control, with an increased ROS scavenging capacity and membrane integrity. On the other hand, in cucumber seedling similar results were found by Zhao et al. (2016) who also observed an adjusted photosynthetic electron flux capable to suppress the production of ROS in MT treated leaves.

Phenolic compounds protect lipid membrane oxidation avoiding initiation or propagation of oxidizing chain reactions preventing the damage of ROS (Mohammadi et al., 2020). Different flavonoids as kaempferol or isovitexin have been reported in different white carnation cultivars (Iwashina et al., 2010). In this sense, flavonoids and other phenolic compounds can be found in white rose petals including gallic acid, (+)-catechinic acid, caffeic acid, hesperidin and cinnamic acids between others (Yon et al., 2018; Yeon and Kim, 2020). MT treatments delayed carnation polyphenol degradation and 0.1 mM MT treatment maintained TPC content for a longer period. Similarly, higher phenolic concentrations were observed in different cut flower species, such as anthurium, gladiolus and gerbera, after GABA, polyamines or GA3 treatments as well as an extension in their vase life (Aghdam et al., 2015; Sajjad et al., 2015; do Nascimento Simões et al., 2018; Mohammadi et al., 2020). The higher TPC in MT treated flowers could be attributed to a reduction in the PPO activity and to an increase in antioxidant activity as has been described in cut anthurium flowers by Aghdam et al. (2019). Regarding this, the present study showed a higher TAA in both hydrophilic (H) and lipophilic (L) fractions in petals of MT-treated carnations as compared to control ones. Higher antioxidant capacity was observed and for a longer period when 0.1 mM MT was used. Citric acid, vitamins C, B1 and phenolic compounds are major contributors to H-TAA and the

most abundant in plants while carotenoid, vitamin E and terpene compounds are major lipophilic radical-scavenging antioxidants (Niki, 2014; Lezoul et al., 2020). The critical role played by oxidative stress in cut flower vase life, and the stimulation of the antioxidant system activity using different anti-senescent compounds has been studied by several authors (Ezhilmathi et al., 2007; Rashidiani et al., 2020; Mohammadi et al., 2020; Ranjbar and Ahmadi, 2015; Hassan and Ali, 2014a, 2014b). Melatonin has radical scavenge activity by itself and stimulates the antioxidant enzyme system in cut flowers and other tissues also reducing oxidative enzymes activity (Arnao and Hernández--Ruiz, 2019; Aghdam et al., 2019). As far as we know this is the first report in which MT impact on cut flower vase life is described. The longer vase life obtained when MT solutions were applied could be attributed to a lower flower metabolism, improved water relation in flower tissues and maintenance of membrane stability. In addition, the higher antioxidant activity and phenolic content stimulated by MT treatments could modulate oxidative damage in carnation tissues increasing the vase life period. Considering the cost of MT as well as the daily VSU, this postharvest treatment could be cost effective increasing vase life up to 10 days more when using the intermediate concentration (0.1 mM MT) providing benefits for cut-flowers retailers. For this reason, MT as a postharvest treatment could be a useful tool to delay carnation senescence.

Author statement

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Funding sources

This research has been funded by the Spanish Ministry of Science, Innovation, and Universities through Project RTI2018-099664-B-I00 and the European Commission with FEDER funds.

Declaration of Competing Interest

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

Acknowledgements

We would like to thank the Algerian Ministry of Higher Education and Scientific Research for supporting Nour El-Houda Lezoul's visit to the Miguel Hernandez University, with a scholarship from Program National Exceptional (PNE).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2021.111759.

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