

Effects of Grape Marc on Vase Life of Carnation Flowers

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ABSTRACT

In carnation, vase life is shortened due to ethylene and water stress, resulting in petal curling, browning, and wilting symptoms. Preservative solutions are used to prolong the vase life of cut flowers in the world, and natural substances with antimicrobial properties have been preferred as preservatives in recent years. It is thought that benefiting from the antimicrobial properties of plant wastes will be beneficial for both the cut flower industry and waste management because they are natural. This research was carried out to determine the effect of grape marc extract (GME) on the vase life of cut carnation. *D. caryophyllus* cv. 'Baltico' was used as a plant material. The plants were placed in vases containing two different concentrations of GME (100 μ L L⁻¹, 200 μ L L⁻¹). The vase life, relative fresh weight (RFW), daily solution uptake (DSU), lipid peroxidation, proline content, and antioxidant enzyme activities were measured during and at the end of the experiment. In the study, it was determined that GME was effective on the vase life of cut carnation flowers and GME at 200 μ L L⁻¹ concentration (22.67 days) extended the vase life by 6.50 days and 40.2% compared to the control [(16.17 days), (distilled water)]. At the same time, GME was found to be effective on the post-harvest stress mechanisms of cut carnation flowers. GME improved vase life by increasing both DSU, and antioxidant enzyme activities, and reducing RFW loss. It also reduced the accumulation of MDA and proline.

Üzüm Cibresinin Karanfil Çiçeklerinin Vazo Ömrüne Etkileri

ÖZET

Karanfillerde taç yapraklarda içe kıvrılma, kahverengileşme ve solma semptomları ile sonuçlanan yaşlanma ve su stresi nedeniyle vazo ömrü kısalmaktadır. Dünyada kesme çiçeklerde vazo ömrünü uzatmak amacıyla koruyucu solüsyonlar kullanılmakta ve son yıllarda koruyucu olarak antimikrobiyal özelliklere sahip doğal maddeler tercih edilmektedir. Doğal olmaları nedeniyle bitkisel atıkların antimikrobiyal özelliklerinden faydalanılmasının hem kesme çiçek sektörü hem de atık yönetimi açısından faydalı olacağı düşünülmektedir. Bu araştırma, üzüm cibresi ekstraktının (GME) kesme karanfilin vazo ömrüne etkisini belirlemek amacıyla yürütülmüştür. Bitkisel materyal olarak D. caryophyllus türüne ait 'Baltico' çeşidi kullanılmıştır. Bitkiler, iki farklı konsantrasyonda GME (100 µL L⁻¹, 200 µL L⁻¹) içeren vazolara yerleştirilmiş ve bitkilerde deneme süresince ve deneme sonunda vazo ömrü, oransal taze ağırlık (RFW), günlük solüsyon alımı (DSU), lipid peroksidasyonu, prolin içeriği ve antioksidan enzim aktiviteleri belirlenmiştir. Çalışmada, GME'nin çiçeklerin vazo ömrü üzerine etkili olduğu ve 200 μ L L⁻¹ konsantrasyonundaki GME'nin (22.67 gün) vazo ömrünü, kontrole [(16.17 gün), (saf su)] göre 6.50 gün ve %40.2 oranında uzattığı belirlenmiştir. Aynı zamanda kesme çiçeklerin hasat sonrası stres mekanizmaları üzerinde GME'nin etkili olduğu saptanmıştır. GME, DSU ile antioksidan enzim aktivitelerini artırarak ve RFW kaybını azaltarak vazo ömrünü iyileştirmiş; MDA ve prolin birikimini azaltmıştır.

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INTRODUCTION

Carnation (Dianthus caryophyllus L.) is one of the most popular plants that belongs to the Dianthus genus in the family of Caryophyllaceae (Sevik & Saruhan, 2010). It is native to the Mediterranean region and is produced in many countries as cut flowers, pots, bedding, border, and rock garden plants (Tah & Mamgain, 2013). Carnation, especially as a cut flower has great commercial value because of its excellent keeping quality, a wide array of colors, and forms. According to the data of 2020, 1.4 billion pieces of cut carnation with a value of 217 million € were exported worldwide (AIPH, 2021). However, the vase life is the most critical criterion for determining the commercial value of cut carnation. The vase life varies considerably according to the carnation varieties, and it is stated that the vase life varies between 7-14 days on average (Anonymous, 2022). Depending on the storage and transportation conditions, a 15% reduction in vase life can be observed at carnations (Kazaz, 2015). Improving the cut carnations' vase life is the main issue for increasing their market value in the floral industry (Patel et. al., 2016).

The cut carnations' vase life is mainly affected by two factors that cause loss of flower quality, such as inrolling and wilting of petals and leaves or even falling (Satoh et. al. 2005). One of the factors is the blockage of the vascular system (Khenizy et. al., 2014). The cut flower stems that are placed in a vase solution develops a negative water balance due to occlusion in the xylem by microorganisms, air emboli, and physiological responses of stems to cut. When water uptake becomes lower than the transpiration rate, it results in water stress (Damunupola et. al., 2010; Elhindi, 2012). The other factor is ethylene. Carnation is a climacteric flower that is highly sensitive to ethylene (Kazemi et. al., 2012). Ethylene is responsible for inducing many of the biochemical processes leading to programmed cell death (Ebrahimzadeh et. al., 2013), such as the acceleration of senescence, increasing respiration rate, and production of reactive oxygen species (ROS) (Saeed et. al., 2014). Ethylene production causes a sharp increase in the production of ROS (Zamani et. al., 2011) and the ROS react with and degrade lipids, proteins, and nucleic acids leading to the ultimate death. The involvement of ROS in the senescence process has gained considerable attention recently (Ezhilmathi et. al., 2007).

It is possible to prolong the vase life by using different solutions, which reduce microbial growth and vascular blockage, increase water uptake of the stem, and decrease the negative effect of ethylene (Shanan, 2017; Ghadimian & Danaei, 2020). Many chemical

such silver thiosulfate. 8compounds as hydroxyquinolines, and aluminum sulfate are used in the vase solution. They are effective on vase life extension (Elhindi, 2012; Amin, 2017). But some of these chemicals have side effects on human health and the environment. Also, some of them have a high cost. Substitution of chemicals with natural compounds is important to maximize the damage control and to minimize their side effects and costs (Ebrahimzadeh et. al., 2019). For these reasons, natural plant extracts or essential oils, which have antifungal, antibacterial, and antioxidative properties have recently been preferred to be used in the vase solution (Rahman et. al., 2012; Adam, 2021).

Grape (Vitis spp.) is one of the most famous species among plants that have antimicrobial and antioxidative activities (Gökçen et. al., 2017). It is a crucial crop for human nutrition in the world and has different usage areas such as fresh fruit, wine, and molasses. The waste material comes out while making wine and molasses. Three tons of grapes for winemaking yield about one ton of waste. This waste material is called grape marc and it can cause environmental pollution due significant to accumulation at production areas (Eleonora et. al., 2014). However, grape marc represents an important source of resveratrol and other bioactive compounds that can be a valuable source of antioxidants and antimicrobials due to their poor extraction during the winemaking process (Luchian et. al., 2019). Moreover, it is cheap and is not harmful to human health. For these reasons, grape marc is thought that using as a vase solution is important in terms of its potential to reduce/inhibit microbial growth and to prevent senescence by decreasing ROS affect. This study has been aimed extending the vase life of the carnation using different concentrations of grape marc extract (GME) in the vase solution and to be the opportunity to use a waste material which is in large quantities as winery waste every year.

MATERIAL and METOD

Plant Material

Flowers of *D. caryophyllus* L. cv. 'Baltico' was used as a plant material. Flowers were obtained from a commercial company and were grown under the greenhouse covered with plastic and located in Antalya (30°93'75.6"N 30°76'23.1"E). The flowers were harvested in the early morning at the paintbrush stage, which is a recommended harvesting period for long-distance transportation (Jawaharlal et. al., 2007).

Vase Life Room Conditions

This study was conducted out at the vase life room in the Department of Horticulture, Faculty of Agriculture, Yozgat Bozok University in Yozgat, Turkey in May. Vase life room conditions were a photoperiod of 12 h light/dark cycles, 1000 lux light intensity, $22 \text{ °C} \pm 2 \text{ °C}$ room temperature and $55 \% \pm 5$ % relative humidity.

Transporting Flowers and Placing Them in Vases

Harvested flowers were immediately placed in a bucket containing tap water in cold storage at 2-4 °C overnight. After that, they were transported dry to the vase life room within 12 hours. At the vase life room, the stems were re-cut to a length of 40 cm and only one upper leaf was held on each stem to decrease contamination. They were then put in glass bottles containing 100 ml of vase solutions, which were GME (100 μ L L⁻¹, and 200 μ L L⁻¹) and distilled water as control.

Preparation of Grape Marc Extract (GME)

The extraction of the grape marc was made according to Kiselev et. al. (2007). The marc of the 'Cardinal' grape variety was dried at 65 °C and pulverized. After the oil removal process, 50 g of sample was taken and 100 ml solvent that included acetone: water: acetic acid (90: 9.5: 0.5) mixture was added. After extracting the Soxalette for 8 hours, the liquid part was removed, and evaporation was carried out in the rotary evaporator. The remaining part was dissolved in 10 ml of methanol.

Measurement and Analysis

The measured traits in the experiment were vase life, RFW, DSU, lipid peroxidation, proline content, antioxidant enzyme activities, bacterial activity, and pH value of vase solutions. Vase life was recorded daily and was terminated when flowers wilt and formed necrotic points on petals (Ebrahimzadeh et. al., 2013). RFW and DSU recorded daily measurement of the weights of the vase with and without flowers. The RFW change and DSU were calculated using formula that were given by He et. al. (2006) and RFW was expressed % of initial and DSU was g stem-1 day-1. Lipid peroxidation was determined by the method modified according to Madhava Rao & Sresty (2000). The amount of MDA that is the last product of lipid peroxidation was calculated from the extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as M kg⁻¹ FW. Proline content was estimated by the method modified according to Bates et. al. (1973). The amount of proline was calculated from a previously plotted standard curve and expressed in M kg⁻¹ FW. Catalase (CAT; EC 1.11.1.6) enzyme activity was estimated by the method modified according to Cakmak et. al.

(1993), superoxide dismutase (SOD; EC 1.15.1.1) was by the method Gong et. al. (2005), ascorbate peroxidase (APX; EC 1.11.1.11) was by the method Nakano and Asada (1981). The amount of enzyme that reduces the absorbance by 1 M in 1 min at 25 °C was accepted as 1 enzyme Unit and the results were expressed as enzyme U kg⁻¹ protein. In the measurement of biochemical/physiological analysis except antioxidant enzyme activities; all petal samples from glass bottles were taken from at the end of the vase life. For antioxidant enzyme activities, samples were taken from glass bottles at four different stages: the period when 1/3 of the flowers opened (Stage 1) and the period when the symptoms such as necrotic points and wilting begin to appear (Stage 2). Bacterial activity of vase solutions was determined according to Kazemi and Ameri (2012)'s method has been modified and plant count agar was used as nutrient solution. The samples were taken two different times (1st day of the vase life and end of the vase life) and the results were given average CFU mL⁻¹. The pH value of vase solution was measured beginning the experiment by pH meter (Mettler Toledo pH/Ion meter s220).

Antimicrobial Activity and pH Value of GME

In the study, antimicrobial activity of GME was also determined. The antimicrobial activity of the GME was made as described below: Bacterial strains (Mc Farland OD: 0.5, 6.0x108 bacteria mL⁻¹), used in the study were inoculated into nutrient broth and activated for 24 hours at 37 °C. Yeast strains (1.5x106 yeast mL⁻¹) were inoculated into sabouraud dextrose broth and activated for 24 h at 30 °C (Anonymous, 1999). Mueller hinton agar, which was sterilized in test tubes and cooled down to 45-50 °C, was inoculated with 24 h broth culture of bacterial strains prepared as above, and yeast strains in 24 h broth on sabouraud dextrose agar medium. The homogenized medium mixture was transferred to sterile petri dishes with a diameter of 9.0 cm. To determine the antimicrobial activities of GME, 100 µL of methanol extracts were taken and absorbed into sterile antibiotic disks with a diameter of 10 mm. Disks impregnated with essential oil were placed on solidified agar by pressing lightly (Bağcı & Dığrak, 1997). Petri dishes prepared in this way were incubated at 4 °C for 2 h, then bacteriainoculated petri dishes were incubated at 37 °C for 24 h, and yeast-inoculated petri dishes were incubated at 30 °C for 24-48 h (Bradshaw, 1992; Collins et. al., 1987; Bağcı & Dığrak, 1996). At the end of the period, the inhibition zones formed on the medium were evaluated in mm.

Statistical Analysis

The experiment was established in a Completely Randomized Design with three replicates. There were fourteen flowers per replication. Statistical analysis was performed using IBM SPSS Statistics vs. 20.0. Analysis of variance (ANOVA) was applied to the data and Duncan's test was used to determine differences between means (*p<0.05).

RESULTS and DISCUSSION

Results

Effects of GME on the Vase Life, RFW and DSU

Based on the statistical analysis, the GME significantly affected the vase life, RFW, and DSU of the cut carnation 'Baltico'. All concentrations of GME extended the vase life. The highest vase life was found

at 200 μ L L⁻¹ GME. The highest concentration of GME extended the vase life by 6.50 d compared to the control. The control had the lowest vase life (Figure 1). During the vase life period, the RFW of flowers kept in distilled water (control) and 100 μ L L⁻¹ GME increased until day 3, while 200 μ L L⁻¹ GME was until day 4. From d 3 and 4 onwards, RFW gradually decreased with time. 200 μ L L⁻¹ GME was determined more successful than lower concentration and control maintaining RFW. The least RFW loss from day 1 to the end of the vase life was observed at 200 μ L L⁻¹ GME (47.90 %) where the highest RFW loss was in 100 μ L L⁻¹ GME (58.22 %) (Figure 2).



Figure 1. Vase life of cut carnation 'Baltico' according to different concentrations of GME (Error bars represent \pm standard deviation, *P < 0.05)

Şekil 1. Farklı konsantrasyonlarda GME'nin 'Baltico' çeşidinin vazo ömrüne etkileri (Hata çubukları ± standart sapmayı temsil eder, *P<0.05)



Figure 2. RFW of cut carnation 'Baltico' at different GME concentrations (Error bars represent \pm standard deviation, *P < 0.05) Sekil 2. Farklı konsantrasyonlarda GME uygulanan 'Baltico' çeşidine ait OTA değerleri (Hata çubukları \pm standart sapmayı temsil eder, *P < 0.05)

Similar to vase life and RFW results, the 200 μ L L⁻¹ GME showed the best DSU. However, from day 1 onwards, DSU gradually decreased with time. The highest DSU on the1st day belonged to flowers in 100 μ L L⁻¹ GME, where the highest DSU on the last day

was in 200 μ L L⁻¹ GME. From day 6 to the end of the vase life; flowers of control had the lowest DSU. The highest average DSU value (87.30 %) was obtained in 200 μ L L⁻¹ GME with 1.51 ± 1.05 g stem⁻¹ day⁻¹. The lowest average DSU value was 1.25 ± 0.99 g stem⁻¹ day⁻¹ in control (Figure 3).



Figure 3. DSU of cut carnation 'Baltico' at different GME concentrations (Error bars represent \pm standard deviation, *P < 0.05)

Şekil 3. Farklı konsantrasyonlarda GME uygulanan 'Baltico' çeşidine ait GSA değerleri (Hata çubukları ± standart sapmayı temsil eder, *P < 0.05)

Effects of GME on Lipid Peroxidation and Proline Content

The GME treatments on the MDA content of the 'Baltico' variety were found to be statistically significant. The highest MDA content was determined in the control group. The lowest MDA content was recorded in 100 μ L L⁻¹ GME. However, 100 μ L L⁻¹ GME was in the same statistical group as the 200 μ L L⁻¹ GME. The highest GME concentration used in the study reduced MDA content by 34.46 % compared to the control (Figure 4).



Figure 4. MDA of cut carnation 'Baltico' at different GME concentrations (Error bars represent ± standard deviation, *P<0.05)

Şekil 4. Farklı konsantrasyonlarda GME uygulanan 'Baltico' çeşidinin MDA içerikleri (Hata çubukları ± standart sapmayı temsil eder, *P<0.05)

Proline content of the 'Baltico' affected significantly by the vase solutions. All concentrations of GME decreased the proline content. The highest proline content was recorded in the control, whereas the lowest proline content was $200 \ \mu L L^{-1}$ GME. However, 200 μ L L-1 GME and 150 μ L L-1 GME were in the same statistical group. The highest GME concentration decreased the proline content by 41.30 % compared to the control (Figure 5).



Figure 5. Proline content of cut carnation 'Baltico' at different GME concentrations (Error bars represent \pm standard deviation, *P < 0.05)

Şekil 5. Farklı konsantrasyonlarda GME uygulanan 'Baltico' çeşidinin prolin içerikleri (Hata çubukları ± standart sapmayı temsil eder, *P<0.05)

Effects of GME on Antioxidant Enzyme Activity

The antioxidant enzyme activity of the 'Baltico' variety showed a statistically significant difference according to the vase solutions and different stages (Figure 6). In stage 1, the highest CAT enzyme activity was found in 100 μ L L⁻¹ GME, which was the same statistical group as 200 μ L L⁻¹ GME. The highest CAT enzyme activity was recorded in 200 μ L L⁻¹ GME both in stage 1 and stage 2. The greatest increase in CAT enzyme activity from stage 1 to stage 2 was at the highest GME concentration. It increased the CAT enzyme activity 1.3 times compared to the control.

Similar to CAT enzyme activity, SOD enzyme activity also showed an increase from stage 1 to stage 2 at all GME concentrations. However, the SOD enzyme activity of control showed a decrease from stage 1 to stage 2 in contrast to CAT enzyme activity. The highest SOD enzyme activity in stage 1 was found in the control that was the same statistical group with 100 μ L L⁻¹ and 200 μ L L⁻¹ GME whereas the highest SOD enzyme activity was recorded in 200 μ L L⁻¹ GME in stage 2. The lowest SOD enzyme activity in stage 2 was determined in the control. The highest increase in SOD enzyme activity was found at the highest GME concentration.

In terms of APX enzyme activity, it was determined that there was an increase in all treatments from stage 1 to stage 2. Although there was no statistical difference between the treatments within stage 2, the highest APX enzyme activity in stage 1 was found control and 200 μ L L⁻¹ GME. They were in the same statistical group. However, the least increase rate was

found in the control. The highest increase rate was recorded 100 $\mu L \ L^{-1} \ GME$ that was the same statistical group as the 200 $\mu L \ L^{-1} \ GME$.

The Antimicrobial Activity of GME, Bacterial Activity and pH in Vsse Solutions

The data on the antimicrobial activity of GME is given in Table 1. According to Table 1, GME was tested on 10 strains of microorganisms, 6 bacteria, and 4 yeast. Gentamicin, amoxicillin (antibiotic), and nystatin (antifungal) active ingredient was also applied to understand the antimicrobial activity of GME. The results showed that GME was more effective on 5 bacteria (*Bacillus subtilis, Staphylococcus aureus, Bacillus megatherium, Pseudomonas aeroginosa, Enterobacter aerogenes*) than the Gentamicin active ingredient and a bacteria *Staphylococcus aureus* than the amoxicillin active ingredient. Moreover, the GME was at least as effective as nystatin on *Candida albicans.*

The data on bacterial activity of vase solutions are given in Table 2. According to Table 2, the highest bacterial activity was determined at control and 100 μ L L⁻¹ GME respectively, whereas the lowest bacterial activity was found at 200 μ L L⁻¹ GME. The data on the pH value of GME is also given in Table 2. According to Table 2, all concentrations of GME had a lower pH than the control and 200 μ L L⁻¹ GME was the lowest.

DISCUSSIONS

In this study, which was conducted to improve postharvest quality in the cut carnation 'Baltico', it maintaining RFW. Moreover, GME caused lower MDA and proline accumulation.



Figure 6. Antioxidant enzyme activities of cut carnation 'Baltico' at different GME concentrations (Error bars represent \pm standard deviation, *P < 0.05)

Şekil 6. Farklı konsantrasyonlarda GME uygulanan 'Baltico' çeşidinin antioksidan enzim aktiviteleri (Hata çubukları \pm standart sapmayı temsil eder, *P < 0.05)

Table	1. Antir	nicrobial	activity	of	GME	

Microorganisms	GME	Control (Methanol)	Gentamicin 10mcg	Amoxicillin/ clavulanic acid (30mcg)	Nystatin*
Gram Positive Bacteria			0	· · · · · · · · · · · · · · · · · · ·	
Bacillus subtilis	18^{1}	-2	23	-	NT
Staphylococccus aureus	20	-	21	30	NT
Bacillus megaterium	14	-	16	-	NT
Gram negative bacteria					
Klebsiella pneumoniae	19	-	16	-	NT
Pseudomonas aeroginosa	14	-	17	15	NT
Enterobacter aerogenes	15	-	16	-	NT
Yeast					
Candida albicans	36	-	NT	NT	36
Candida utilis	-	-	NT	NT	35
Saccharomyces cereviciae	-	-	NT	NT	36
Yarrowia lipolytica	-	-	NT	NT	37

(*): Each disc contains 10 microliters of 100,000 UNIT nystatin per ml.

(1): inhibition zone, mm; (2): Inhibition zone could not be determined; NT: could not be tested

Table 2. Bacterial activity and pH value of vase solutions

Çizelge 2. Vazo solüsyonlarının bakteriyel aktivitesi ve pH değerleri

Vase Solution	CFU mL ⁻¹	pH value	
Control (distilled water)	$516 a \pm 15.23$	$5.72 a \pm 0.48$	
$100 \ \mu L \ L^{-1} \ GME$	$510 a \pm 14.39$	$5.39 \text{ b} \pm 0.33$	
200 µL L ⁻¹ GME	$492 \text{ b} \pm 10.19$	$5.20 \text{ c} \pm 0.36$	

Solution uptake of cut flowers is one of the most important factors affecting their vase life (Frew et. al., 2018). When solution uptake decreases, the balance of solution uptake and transpiration is disturbed. Because of water stress, early wilting and premature senescence occurred and the longevity of the cut flowers' vase life is shortened (Lou et. al., 2021). In this study, the rate of decrease in solution uptake was higher in the control compared to the highest GME concentration (Figure 3) and it is found that GME decreased microbial activity in vase solutions (Table 2). The enhancement of solution uptake might be related to that GME improved the solution uptake by decreasing or preventing microbial proliferation. Ha et. al. (2019), Kılıç et. al. (2020), and Nguyen and Lim (2021) determined similar results. They reported that some compounds used as vase solutions reduced microbial activity and increased solution uptake because of their antimicrobial properties. In this study, GME was found effective on Bacillus subtilis, Bacillus megatherium, Staphylococcus aureus, Pseudomonas aeroginosa, Enterobacter aerogenes (Table 1). The biodiversity of microorganisms inhabiting vase solutions can change due to the microflora on stems of cut flowers. However, it was determined that microorganisms such as *Bacillus* spp., Pseudomonas spp., Staphylococcus equorum, and Enterobacter agglomerans were found in the samples taken from the vase solutions where different researchers kept cut carnations (Shanan et.al., 2010; Pang et.al., 2021).

RFW plays a critical role in the quality and vase life of cut flowers (Shokalu et. al., 2021) and to improve the vase life, maintaining the RFW is important. RFW of cut flowers changes during the vase life due to respiration and transpiration rate, senescence, water, and nutrient content in cut flowers (Paul et. al., 2021; Song et. al., 2021). In this study, the rate of decreasing RFW of cut carnation flowers was lower at the highest concentration of GME compared to the control. Elansary (2020), Lou et. al. (2021), and Song et. al. (2021) obtained similar results about that some compounds added in vase solutions maintain RFW and reduce RFW loss of cut flowers. The solution uptake helps maintain RFW (Ha et. al. 2019) and there is a positive correlation between solution uptake and RFW (Alkac et. al., 2020). The lower rate of decrease in solution uptake of GME might have resulted in a better RFW in GME. Moreover, the GME might have helped to reduce the transpiration losses by stomatal regulation because of its antioxidant capacity. GME has been determined to increase CAT, SOD, and APX antioxidant enzyme activities and reduce MDA content which is known as a marker of the antioxidant activity (Figure 6, 8, 9, 10). The report of Zhang et. al. (2010) indicates that the antioxidant system might have influenced the stomatal regulation. They found that the channels by which hydrogen peroxide (H_2O_2) mediates the induction of stomatal closure are by increasing the activities of antioxidative enzymes such as SOD, CAT, GPX, APX, and GR in plant tissues. Besides solution uptake and transpiration rate,

another reason why RFW is found to be higher in GME treatment in this study may be delaying senescence. When RFW begins to decline, it marks the beginning of the senescence period (Gomes et al., 2010). So, the fact that GME delayed RFW loss by one day suggests that it has the ability to delay senescence. Carnation is an ethylene-sensitive species however, senescence in cut flowers is not only dependent on ethylene but also oxidative stress and/or water stress and they accelerate the senescence processes (Skutnik et. al., 2020). GME might have delayed senescence by reducing water stress and oxidative stress due to its antioxidant activity. Similar results about some compounds that have antioxidant activity, delay senescence in cut flowers were reported (Lama et. al., 2015; Maity et. al., 2019). In this study, it was also found that the RFW increased up to the first 4 d in 200 μ L L⁻¹ GME and up to the first 3 d in the 100 μ L L⁻¹ and control. The decrease in RFW of the highest GME concentration started one day later than control. Although the reduction of solution uptake, the reason why the RFW increased up at first 4 d in the highest concentration may be that the transpiration rate was lower at the end of the vase life. The lower transpiration rate may be related to the flowers have not reached the full opening. Flowers, which harvested paintbrush stage, were used in the experiment. Azad et. al. (2004) indicated that when petals open, the transpiration rate gradually increased. It is thought that the highest GME concentration might have delayed flowering by one day. There is another research showing that the RFW increases up to a certain time depending on the solution applied, the conditions to keep of flowers, and the cut flower species (Horibe & Makita, 2019; Kılıç et. al., 2020; Yang et. al., 2021).

Lipid peroxidation acts a critical role in the balance of oxidative stress (Bat et. al., 2020) that regulate in the vase life of cut flowers between prooxidants such ROS and antioxidant activities (Aalifar et. al., 2020). Because of lipid peroxidation caused by senescence and/or oxidative stress, cell membranes lose their stability, and MDA that is a toxic aldehyde that is an indicator for determining lipid peroxidation occurs. The damage of membrane lipids and the end-products of its reactions are dangerous for cell viability, even tissues (Mylonas & Kouretas, 1999). In this study, MDA content reached the highest value in the control, while it was lowest at the highest GME concentration. Similar results were obtained by Dar and Tahır (2018), Langroudi et. al. (2019), and Teerarak et. al. (2019). They reported that some compounds that have antioxidant and anti-ethylene properties reduced MDA content. Reducing MDA accumulation by decreasing lipid peroxidation of GME might have been related to its antioxidant activity. SOD, CAT, and APX antioxidant enzyme activity that protects the cell membrane of GME were found higher compared to the control (Figure 8, 9, and 10). Therefore, it reduced lipid peroxidation and stabilized the cell membrane of flowers. Alternatively, the higher electrolytic leakage from petal tissue might have occurred in control due to a result of higher lipid peroxidation. It is indicated that the cell membranes lose their stability; they produce different ROS which results in enhancing the electrolyte leakage (Iqbal et. al., 2017).

Proline is one of the most important soluble osmosisregulator and proteinogenic amino acids (Alvarez et. al., 2021; Yang et. al., 2021). Increment of proline in flowers is a general adaptive response to cellular dehydration caused by water stress. In this study, GME had lower proline content than control flowers. Similar results about some compounds reducing proline accumulation were obtained by Kazemi et. al. (2011) and Skutnik et. al. (2021). The lower proline content might have been related to that GME act as the antioxidant defense factor and protects flowers against cellular dehydration or GME might have increased proline dehydrogenase (PRODH) activity in the petals. Alternatively, the reason for the higher proline content in the control flowers may be the higher protein degradation because of the water and/or oxidative stress. Proline content can increase due to the breakdown of proteins and increase in free amino acids in petal cells with senescence or uplifted stress over time (Mohammadi et. al., 2020; Koentjoro et. al., 2021).

Antioxidant enzymes, such as APX, SOD, and CAT play a key role in improving the vase life of cut flowers by maintaining the ROS balance (Wang et. al., 2021). When ROS producing rate exceeds that of the cell's defense ability, oxidative stress occurs. ROS such as H_2O_2 is generated, damaging cells, and hastening their death. In this study, all antioxidant enzymes increased at GME treatments from Stage 1 to Stage 2. There are many researchers who have determined that antioxidant enzyme activities increase with substances added to the vase solutions (Maity et. al., 2019; Aziz et. al., 2020; Hassanzadeh-Naemi et. al., 2021). Hassanzadeh-Naemi et. al. (2021) found that CAT and SOD activity decreased during the postharvest life and that this activity was improved with treated plant growth regulators. Similarly, CAT and APX activities increased from Stage 1 to Stage 2, while SOD enzyme activity decreased in the control. It is thought that cut carnation 'Baltico' flowers may have defended themselves against oxidative or water stress by increasing the proline content instead of SOD enzyme activity in the control. As a matter of fact, the proline content in the study was found to be higher in the control. The formation of enzymatic and nonenzymatic antioxidants responsible for ROS detoxification under stress conditions are important defense systems against stress (Hassanuzzaman et. al., 2020). GME might have supported the enzymatic defense system in cut flowers. Although CAT and APX increased in all treatments, the results differed between the treatments and GME is better vase life compared to the control. This might be related to the SOD activity being more distinct and important in the scope of protecting membrane damage by the scavenging of ROS on flowers that exposure to water stress. Du et. al. (2014) reported that the correlation between SOD activity and MDA content was significant compared to CAT and APX enzyme activity, and SOD acted more effectively than ascorbic acid done in the superoxide quenching.

Although the SOD decreased in the control, the increase in the CAT enzyme showed that in the control flowers have occurred H_2O_2 . SOD is a metalloenzyme that catalyzes the dismutation of superoxide to H_2O_2 . H₂O₂ formed directly or by the dismutation of superoxide radicals is detoxified by converting it into the water by GPx and CAT enzymes. The CAT enzyme has significant efficacy in cases where H₂O₂ formation is increased (Koç & Üstün, 2008). CAT increased despite the reduction in SOD at control; indicating that H_2O_2 formation is increased non-enzymatic pathways. Moreover, more oxygen radicals that can cause damage to the cell membrane might have accumulated in control flowers due to the decreasing of SOD activity. This coincides with the increase in MDA content at control. Similar to in this results, Panda and Khan (2004) reported that oxidative damage occurs when the production rate of superoxide radicals exceeds the SOD activity. In this study, SOD enzyme activity may have decreased due to the reduction of synthesis of SOD enzyme or enhancing degradation of this enzyme at control group. Decreasing the SOD activity in the control may have accelerated senescence and caused short vase life. Wang et. al. (2016) reported that the increasing of SOD activity and efficient ROSscavenging capacity contributed largely to the delay of senescence.

There are many substances used to prolong the vase life of carnations, and the commercially preferred STS is a proven substance. In research with STS, it has been determined that the vase life can be extended from 1 to about 14 days, depending on the variety, concentration, and trial conditions (Hassan, 2004). However, STS is harmful to both human health, and the environment. As an alternative to this substance, cheap materials that are friendly to human health and the environment should be determined. The use of plant wastes to increase post-harvest life is important in terms of both overcoming post-harvest problems of cut flowers and ensuring waste management. It is thought that grape marc is important in terms of postharvest studies, especially because it is available throughout the year, can be easily supplied, has no storage problems, is inexpensive, does not have any harm in terms of human and environmental health, and provides an economic return by evaluating a waste material (Sarıçiçek & Kılıç, 2002). Studies are needed to standardize grape marc which affects the stress mechanism in cut flowers after harvest by determining the appropriate doses and to use it as a commercial preparation if proven.

CONCLUSION

In conclusion, GME has the potential to improve the vase life of the cut flowers. The GME treatment prolonged the vase life of cut carnation, and the 200 µL L^{\cdot_1} GME was the best treatment in terms of investigated parameters. GME stimulated the defense ability of cut carnation flowers. It is thought that this is related to the antioxidant activities of GME. Moreover, it decreased the bacterial activity in vase solution due to its antibacterial properties. It is recommended that the effects of higher concentrations of GME should be researched the vase life of cut flowers. Investigation of antioxidant activities of GME is also important to contribute to future research. Different GME obtained from other grape varieties can be researched which has better antioxidant and antibacterial activity. If the effectiveness of GME on vase life is proven, it is possible that it can be standardized and converted into a commercial preparation form. This research also is showing that waste materials can be added to a vase solution.

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Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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