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# Comparison of Plant Growth Regulators and Exogenous Ethylene Effects on Two Types of Cut Carnation (*Dianthus caryophyllus* L.)

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Abstract

The main objective of the present study was to investigate the effect of plant growth regulators on the prolongation of vase life of two types of cut carnation under normal conditions and ethylene stress. Carnation is one of the most popular flowers with a short life due to its sensitivity to ethylene. The effect of plant growth regulators on several physiological traits under ethylene stress and normal conditions was also studied. The effects of short-term treatment with benzyl adenine (BA), salicylic acid (SA), and methyl jasmonate (MeJA) were investigated on two types of cut carnations (*Dianthus caryophyllus* L.), one-headed standard cut carnation and multi-flower mini cut carnation, under ethylene exposure. The short-term treatment with 100 µM benzyl adenine had the strongest effect on total chlorophyll content. The treatment with 100 µM SA enhanced the soluble carbohydrates and prolonged the cut carnation vase life. The plants treated with 400 µM MeJA showed an elevated level of catalase and peroxidase enzymes activity and an increase in the proline content. All considered traits were reduced by exogenous ethylene. None of the short-term treatments could repel the inappropriate exogenous ethylene effects. One-headed standard carnations were also more ethylene tolerant than multi-flower mini carnations.

**Keywords:** Antioxidant, Longevity, Senescence.

Abbreviations:
Benzyl adenine (BA), Methyl jasmonate (MeJA), Multi-flower mini carnation (Mn carnation), One-headed standard carnation (Os carnation), Plant growth regulator (PGR), Salicylic acid (SA).

## INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) belongs to the family Caryophyllaceae, and more than 300 ornamental species have been identified in the genus *Dianthus* (Jurgens *et al.,* 2003). The cultivars used for producing cut flowers are different in terms of the inflorescence type. In Os cultivars, the cut flowers should have single coarse flower while in miniature cultivars, flowers are in a cluster shape. Carnation is one of the most popular cut flowers produced considerably in the world, but it has climacteric behavior which shortens its vase life. To allow its transportation from producers to customers, the vase life of carnation cut flowers should be prolonged, expectedly to as long as two weeks (Boxriker *et al.,* 2017).

Senescence involves systemic metabolic and physiological changes including the breakdown of cell walls, ion leakage, the degeneration of chlorophyll, the cessation of photosynthesis, and the loss of proteins (Mansouri, 2012). Senescence is mainly controlled by plant growth regulators (PGRs) (Munne-Bosch and Alegre, 2004). Flower senescence is accompanied by the loss of commercial value of cut flowers (Varshney *et al.,* 2013; In *et al.,* 2013). Prolonging cut flower longevity has been a challenge for producers.

Cytokinins and ethylene have antagonistic effects in plants. Cytokinins suppress the senescence symptoms. It has been revealed that benzyl adenine (BA) plays an important role in the maintenance of anthocyanins in the petals of *Eustoma grandiflorum* (Mariachii cv. 'Pink'). Also, BA delays ethylene synthesis and increases vase life (Karimi *et al.,* 2012). The application of exogenous cytokinins prevented chlorophyll degeneration in harvested broccoli (Gapper *et al.,* 2007). The role of salicylic acid (SA) in expressing genes involved in responding to stresses during senescence has been well-documented (Mansouri, 2012). In another research, senescence has delayed in rose and gladiolus when SA has been applied (Ezhilmathi *et al.,* 2007; Alaey *et al.,* 2011). Jasmonates have been introduced as signaling compounds that induce resistance to stresses (Yu *et al.,* 2002). It has been reported that postharvest application of methyl jasmonate (MeJA) to berries increased their antioxidant capacity. Also, MeJA application suppressed postharvest diseases in peaches and grapefruits (Wang *et al.,* 2009). We studied the extent to which these PGRs can suppress the symptoms of senescence in two types of carnation (multi-flower mini carnations and one-headed standard carnations).

## MATERIALS AND METHODS

The study was intended to determine PGRs mechanisms and their effects on cut flower appearance and biochemical traits associated with the prolonged longevity of carnation cut flowers. The experiment was conducted in the crop years 2014- 2015 in an experimental greenhouse in Varamin, Iran (51°64' E, 35°32' N).

### Plant materials and treatments

Os and Mn carnations were harvested at the same growth stage and were transferred to a laboratory. The stems were cut under water to 50 cm length. The cut carnation flowers were placed in 250 ml of PGR solutions (50 or 100 µM SA and BA, 200 or 400 µM MeJA, and control) for 24 hours. After the PGR short treatments (day 0), ethanol 2% was used as the preservation solution for all cut flowers (day 1). Two days after the short-term treatment, the carnation cut flowers were exposed to 1000 ppm ethylene for two hours and then were subject to trait measurements.

### Fresh weight changes

Carnation cut flowers were weighed immediately after harvesting to record the primary fresh weight. When wilting symptoms such as the bending of peduncle and the rolling of petal edges appeared, the carnation cut flowers were weighted again. Fresh weight change was determined as the percentage of weight difference.

**Cell membrane stability (CMS)**

Cell membrane stability is a criterion to assess the extent of oxidative damage in membrane phospholipids. CMS was measured by following the protocol of Ben Hamed *et al.* (2007) on the 4th day. Equation: CMS (%) = $[(1-(T_1/T_2))/(1-(C_1/C_2))]$ C=control sample, T= heat-treated sample.

**Chlorophyll content**

According to Arnon (1967)'s description, chlorophyll concentrations were estimated on the 3rd day. Concentrations of pigments (mg/ml) were calculated using the following equation: Chlorophyll a + b = 0.0202 (A645) + 0.00802 (A663).

**Soluble carbohydrates**

The content of soluble carbohydrates of petals was determined according to the method of Schlegel (1986) on the 5th day.

**Antioxidant enzymes activity**

The peroxidase activity (EC 1.11.1.7) was measured based on Dias and Costa (1983)'s described procedure. The catalase activity (EC 1.11.1.6) was measured by the method of Kato and Shimizu (1987) on the 6th day.

**Proline content**

As described by Bates *et al.* (1973), proline (PRO) content was determined on the 6th day.

**Vase life**

Vase life duration was determined from the harvesting of the flowers until the rolling of the petals and the bending of the stalk (Fig. 1).


Fig. 1. Vase life ending in Mn (A) and Os (B) cut carnations.

**Statistical analysis**

The experiment was conducted in a completely randomized design. All values represent the mean of three replicates. The differences between treatments were calculated by multi-analysis of variance which was considered significant when they were equal or more than the least significant difference (Duncan) computed at the 5% level using the SAS version 9.1.3 software package.

## RESULTS AND DISCUSSION

### Fresh weight changes

The effects of PGRs on fresh weight of carnation cut flowers were significant (Table 1). The highest fresh weight was observed in the cut flowers treated with 50 µM BA, while the lowest was related to the control cut flowers (Table 2). Fresh weight in the Os carnations was higher than that in the Mn carnations (Table 2). Additionally, the effect of exogenous ethylene on the carnation cut flower was tested along with the other mentioned factors (PGRs and carnation type). It was revealed that the decrease in fresh weight was significantly less pronounced in the untreated flowers than in the flowers exposed to ethylene (Table 2). On the other hand, fresh weight loss under ethylene exposure in the carnation cut flowers treated with 50 µM BA was less noticeable than the control cut flowers (Table 2). It should also be noted that the Os cut flowers compared with the Mn cut flowers under ethylene exposure showed greater resistance to fresh weight loss (Table 2). In this study, the highest fresh weight was observed in the cut flowers treated with 50 µM BA. As mentioned by Alexopoulos *et al.* (2007), fresh weight of potato sprouts treated with GA+BA was increased versus control. Mansouri (2012) contended that the increase in respiration rate followed by senescence process and the loss of water uptake could lead to fresh weight loss. As it was observed in this study, fresh weight loss in the Mn carnation was more pronounced and was also accompanied by stem bending and leaf drying.

Table 1. ANOVA of data for the measured traits.

| S.o.V | df | MS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | FWC | CMS | TChl | TC | CAT | POD | Pro | Vl |
| PGR | 6 | 619.8$^{**}$ | 482.7$^{**}$ | 1.43$^{**}$ | 232.5$^{**}$ | 0.008$^{**}$ | 0.01$^{**}$ | 19.16$^{**}$ | 18.32$^{**}$ |
| Et | 1 | 241.2$^{**}$ | 0.007$^{ns}$ | 16.23$^{**}$ | 669.9$^{**}$ | 0.15$^{**}$ | 0.41$^{**}$ | 8.6$^{**}$ | 192.0$^{**}$ |
| Type | 1 | 829.7$^{**}$ | 530.5$^{**}$ | 0.29$^{ns}$ | 645$^{**}$ | 0.17$^{**}$ | 0.0003$^{ns}$ | 4.3$^{**}$ | 131.2$^{**}$ |
| PGR × Et | 6 | 38.03$^{**}$ | 47.29$^{ns}$ | 0.11$^{ns}$ | 37.9$^{**}$ | 0.007$^{**}$ | 0.003$^{**}$ | 0.65$^{**}$ | 1.15$^{**}$ |
| PGR × type | 6 | 21.09$^{ns}$ | 18.83$^{ns}$ | 0.01$^{ns}$ | 19.54$^{**}$ | 0.004$^{**}$ | 0.0006$^{**}$ | 0. 052$^{ns}$ | 2.55$^{*}$ |
| Et × type | 1 | 153.4$^{**}$ | 252$^{*}$ | 1.1$^{*}$ | 132.3$^{**}$ | 0.11$^{**}$ | 0.0007$^{*}$ | 0.98$^{**}$ | 7.44$^{**}$ |
| PGR × type × Et | 6 | 20.28$^{ns}$ | 6.36$^{ns}$ | 0.03$^{ns}$ | 18.14$^{**}$ | 0.003$^{**}$ | 0.005$^{**}$ | 0.29$^{*}$ | 2.41$^{*}$ |
| Error | 56 | 13.1 | 61.07 | 0.13 | 1.05 | 0.0002 | 0.0001 | 0.095 | 0.08 |
| CV (%) | | 4.06 | 16.49 | 16.9 | 4.61 | 21.31 | 11.79 | 10.56 | 63.57 |

$^{*}$, $^{**}$ and $^{ns}$: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.
Abbreviations: FWC (fresh weight changes), CMS (cell membrane stability),Tchl (total chlorophyll), TC (total soluble carbohydrates), CAT (catalase), POD (peroxidase), Pro (proline), Vl (vase life), No (normal condition), Et (ethylene stress condition).

### Cell membrane stability

The short-term treatment with 100 µM SA significantly diminished the reduction of cell membrane stability (Table 2). As represented in Table 2, the Os carnation cut flowers showed more cell membrane stability as compared to the Mn carnations. Furthermore, the highest rate of cell membrane stability was related to the treatment of the cut flowers with 100 µM SA (Table 2). Also, cell membrane stability in the Os carnation cut flowers was higher than that in the Mn carnation cut flowers (Table 2). It has been found that SA as an ACC-oxidase activity reduction factor plays a key role in improving CMS in carnation cut flowers (Kazemi *et al.,* 2011). The results obtained by Mei-Hua (2008) which further supported our findings on the effect of SA on CMS have indicated that the application of SA as a preservation solution enhanced membrane integrity in gerbera.

Table 2. Means comparison of the measured traits.

| Treatment (Simple effect) | FWC (%) | CMS (%) | TChl (mg/g) | TC (mg/g) | Cat (U/mg pro) | POD (U/mg pro) | Pro (mg/L) | Vl (day) |
|---|---|---|---|---|---|---|---|---|
| SA 50 | 88.82 c | 53.93 a | 2.22 bcd | 24.55 b | 0.065 c | 0.104 b | 2.95 d | 6.91 ab |
| SA 100 | 93.01 b | 54.29 a | 2.51 ab | 30.62 a | 0.09 b | 0.125 a | 4.02 b | 7.5 a |
| BA 50 | 97.25 a | 47.19 bc | 2.30 bc | 20.14 e | 0.06 c | 0.087 c | 2.14 e | 5.5 d |
| BA 100 | 95.89 ab | 43.51 c | 2.62 a | 19.17 f | 0.06 c | 0.077 d | 1.75 f | 5.08 d |
| MJ 200 | 88.16 c | 50.73 ab | 1.95 d | 22.59 c | 0.08 b | 0.103 b | 3.67 c | 6.58 bc |
| MJ 400 | 84.52 d | 45.62 bc | 2.05 cd | 21.54 d | 0.11 a | 0.135 a | 4.67 a | 5.83 cd |
| Ctrl | 76.45 e | 36.36 d | 1.61 e | 17.11 g | 0.04 d | 0.065 e | 1.26 g | 3.83 e |
| No | 90.85 a | 47.38 a | 2.62 a | 25.06 a | 0.115 a | 0.17 a | 3.25 a | 7.4 a |
| Et | 87.46 b | 47.06 a | 1.74 b | 19.4 b | 0.030 b | 0.03 b | 2.61 b | 4.4 b |
| Mn | 86.02 b | 44.86 b | 2.24 a | 19.5 b | 0.027 b | 0.097 a | 2.70 b | 4.64 b |
| Os | 92.30 a | 49.89 a | 2.13 a | 25.1 a | 0.118 a | 0.101 a | 3.15 a | 7.14 a |
| SA50*No | 90.75 def | 57.29 a | 2.84 ab | 27.99 b | 0.10129 c | 0.1775 b | 3.1486 d | 8.83 ab |
| SA50* Et | 86.89 fgh | 50.6 abc | 1.62 gh | 21.10 ef | 0.030259 e | 0.0306 g | 2.7601 ef | 5.0 ef |
| SA100* No | 95.96 abc | 54.83 ab | 2.93 ab | 36.96 a | 0.147268 b | 0.2157 a | 4.0608 bc | 9.0 a |
| SA100* Et | 90.06 efg | 53.75 ab | 2.10 ef | 24.27 d | 0.0326 de | 0.0360 f | 3.9957 c | 6.0 de |
| BA50* No | 101.15 a | 47.4 bcd | 2.72 abc | 22.21 e | 0.090195 c | 0.1471 c | 2.4467 fg | 7.17 c |
| BA50* Et | 93.3 bcde | 47bcd | 1.88 fg | 18.07 ij | 0.029253 e | 0.0277 gh | 1.8433 h | 3.8 ghi |
| BA100* No | 97.52 ab | 44.1 cde | 3.01 a | 20.62 fg | 0.084451 c | 0.1302 d | 2.1634 gh | 6.8cd |
| BA100* Et | 94.26 bcd | 42.9 cde | 2.23 def | 17.72 jk | 0.028520 e | 0.0250 gh | 1.3474 i | 3.33 hi |
| MJ200* No | 90.1 defg | 50.5 abc | 2.39 cde | 25.57 c | 0.134614 b | 0.1760 b | 4.4054 b | 7.8bc |
| MJ200* Et | 86.21 gh | 50.9 abc | 1.51 h | 19.61 gh | 0.031383 e | 0.0306 g | 2.9514 de | 5.33 ef |
| MJ400* No | 85.858 h | 44.1 cde | 2.55 bcd | 24.09 d | 0.200467 a | 0.2259 a | 5.1264 a | 7.33 c |
| MJ400* Et | 83.187 h | 47.1 bcd | 1.55 gh | 18.99 hi | 0.03269 de | 0.0443 f | 4.2233 bc | 4.3gh |
| Ctrl* No | 74.63 i | 33.29 e | 1.91 fg | 18.04 ij | 0.049696 d | 0.1156 e | 1.3894 i | 4.83 g |
| Ctrl* Et | 78.27 i | 39.4 de | 1.31 h | 16.18 k | 0.027643 e | 0.0149 h | 1.1404 i | 2.83 i |
| SA50*Mn | 86.38 c | 57.72 f | 2.16 e | 21.2 e | 0.146 b | 0.103 d | 2.79 ef | 5.83 d |
| SA50* Os | 91.25 de | 50.15 c-f | 2.29 cd | 27.8 b | 0.195 a | 0.105 d | 3.12 de | 8.00 ab |
| SA100* Mn | 90.97 de | 57.39 f | 2.47 bc | 35.9 a | 0.130 b | 0.119 bc | 3.76 bcd | 6.0 d |
| SA100* Os | 95.05 ef | 51.19 def | 2.56 ab | 25.2 c | 0.107 c | 0.132 b | 4.29 b | 9.0 a |
| BA50* Mn | 94.61 ef | 48.93 c-f | 2.29 de | 22.3 e | 0.096 c | 0.089 ef | 1.89 gh | 4.17 ef |
| BA50* Os | 99.90 g | 45.44 b-e | 2.31 bcd | 17.9 i | 0.092 c | 0.086 efg | 2.39 fg | 6.83 cd |
| BA100*Mn | 94.47 ef | 44.30 bcd | 2.57 ab | 21.1 f | 0.063 d | 0.083 fgh | 1.64 ghi | 4.17 ef |
| BA100* Os | 97.31 fg | 42.71 a-d | 2.67 a | 17.9 i | 0.038 e | 0.072 h | 1.87 gh | 6.00 d |
| MJ200*Mn | 83.99 bc | 53.78 ef | 1.84 g | 25.3 c | 0.036 e | 0.096 de | 3.47 cd | 4.67 e |
| MJ200* Os | 92.32 de | 47.69 cde | 2.06 ef | 19.8 g | 0.034 e | 0.11 cd | 3.89 bc | 8.50 a |
| MJ400*Mn | 79.97 b | 49.53 c-f | 1.97 efg | 23.8 d | 0.025 ef | 0.123 bc | 4.37 b | 4.33 ef |
| MJ400* Os | 89.06 cd | 41.71 abc | 2.12 ef | 19.2 gh | 0.024 ef | 0.148 a | 4.97 a | 7.33 bc |
| Ctrl* Mn | 71.69 a | 37.58 ab | 1.55 hi | 18.6 h | 0.021 ef | 0.073 gh | 0.99 ij | 3.33 f |
| Ctrl* Os | 81.20 b | 35.15 a | 1.67 gh | 15.6 j | 0.014 f | 0.058 i | 1.54 ghi | 4.33 ef |
| Mn*No | 86.36 c | 48.16 ab | 2.56 a | 25.7 a | 0.0327b | 0.031c | 2.726b | 5.33b |
| Mn*Et | 85.67 c | 43.12 c | 1.57 c | 13.1 c | 0.0216c | 0.029c | 2.491c | 3.42c |
| Os*No | 95.35 a | 51.61 a | 2.67 a | 25.8 a | 0.1981a | 0.174a | 3.582a | 8.95a |

* In each column, means with the similar letter(s) are not significantly different (P <0.05) using the LSD test. Abbreviations: FWC (fresh weight changes), CMS (cell membrane stability),Tchl (total chlorophyll), TC (total soluble carbohydrates), CAT (catalase), POD (peroxidase), Pro (proline), Vl (vase life), No (normal condition), Et (ethylene stress condition), Mn (multi-flower mini carnations) and Os (one-headed standard carnations).

Table 2. Continued.

| Treatment (Simple effect) | FWC (%) | CMS (%) | TChl (mg/g) | TC (mg/g) | Cat (U/mg pro) | POD (U/mg pro) | Pro (mg/L) | Vl (day) |
|---|---|---|---|---|---|---|---|---|
| Os*Et | 89.26 b | 46.60 bc | 1.91 b | 24.3 b | 0.0391b | 0.165b | 2.914b | 5.86b |
| SA50*No*Mn | 86.45 e-h | 63.72 g | 2.94 ab | 26.8 d | 0.028 hij | 0.027 klm | 2.828 f | 7.67 c |
| SA50*No*Os | 95.05 k-n | 50.85 c-f | 2.72 bc | 29.1 c | 0.174 d | 0.184 cd | 3.469 de | 10.00 ab |
| SA50*Et*Mn | 86.32 e-h | 51.71 d-g | 1.38 ij | 28.8 c | 0.021 ij | 0.035 jkl | 2.749 f | 4.00 fg |
| SA50*Et*Os | 87.46 f-i | 49.44 b-f | 1.85 gh | 13.3 l | 0.039 hi | 0.171 de | 2.771 f | 6.00 de |
| SA100*No*Mn | 93.1 i-m | 60.34 fg | 3.042 a | 36.7 ab | 0.045 hi | 0.171 de | 3.915 cd | 7.67 c |
| SA100*No*Os | 98.8 mno | 49.32 b-f | 2.81 abc | 37.1 a | 0.250 b | 0.229 b | 4.207 c | 10.33 a |
| SA100*Et*Mn | 88.84 g-j | 54.44 efg | 1.90 gh | 35.1 b | 0.023 ij | 0.036 jk | 3.608 d | 4.33 fg |
| SA100*Et*Os | 91.29 h-l | 53.05 d-g | 2.3 de | 13.3 l | 0.043 hi | 0.202 c | 4.383 b | 7.67 c |
| BA50*No*Mn | 99.14 no | 50.63 c-f | 2.87 abc | 21.6 hi | 0.026 hij | 0.147 fg | 2.157 gh | 5.00 ef |
| BA50*No*Os | 103.16 o | 44.13 b-e | 2.57 cd | 22.8 h | 0.154 d | 0.147 fg | 2.736 f | 9.33 ab |
| BA50*Et*Mn | 90.07 g-k | 47.24 b-e | 1.70 hi | 23.04 fg | 0.021 ij | 0.026 klm | 1.633 ijk | 3.33 gh |
| BA50*Et*Os | 96.64 lmn | 46.74 b-e | 2.06 ef | 13.1 l | 0.037 hi | 0.030 jklm | 2.054 ghi | 4.33 fg |
| BA100*No*Mn | 95.78 k-n | 46.25 b-e | 3.079 a | 19.9 ij | 0.020 ij | 0.121 hi | 1.458 jk | 3.33 gh |
| BA100*No*Os | 99.26 no | 42.01 a-e | 2.95 ab | 21.3 hi | 0.037 hi | 0.140 gh | 1.237 kl | 3.33 gh |
| BA100*Et*Mn | 93.16 i-m | 42.35 a-e | 2.06 ef | 22.4 h | 0.049 h | 0.029 jklm | 3.946 cd | 6.00 de |
| BA100*Et*Os | 95.35 k-n | 43.42 b-e | 2.39 cde | 13.0 l | 0.220 c | 0.032 jklm | 4.865 b | 9.67 ab |
| MJ200*No*Mn | 84.18 d-g | 54.12 efg | 2.37 cde | 24.6 ef | 0.022 ij | 0.161 ef | 2.993 ef | 3.33 gh |
| MJ200*No*Os | 96.02 lmn | 46.87 b-e | 2.41 cde | 26.5 d | 0.040 hi | 0.191 c | 2.910 f | 7.33 cd |
| MJ200*Et*Mn | 83.8 def | 53.44 efg | 1.31 ijk | 26.1 de | 0.052 g | 0.041 jk | 4.563 b | 5.33 ef |
| MJ200*Et*Os | 88.62 g-j | 48.50b-f | 1.71 hi | 13.1 l | 0.349 a | 0.047 j | 5.690 a | 9.33 ab |
| MJ400*No*Mn | 77.86 bc | 49.50 b-f | 2.55 cd | 22.9 gh | 0.024 ij | 0.204 b | 4.191 c | 3.33 gh |
| MJ400*No*Os | 93.85 j-n | 38.81 abc | 2.54 cd | 25.2 de | 0.042 hi | 0.248 a | 4.256 c | 5.33 ef |
| MJ400*Et*Mn | 82.08 c-f | 49.55 c-f | 1.39 ij | 24.7 e | 0.008 j | 0.016 lm | 1.173 kl | 4.33 fg |
| MJ400*Et*Os | 84.28 d-g | 44.61 b-e | 1.7 hi | 13.2 l | 0.091 f | 0.014 m | 1.606 ijk | 5.33 ef |
| Ctrl*No*Mn | 67.98 a | 36.72 ab | 1.86 gh | 17.7 k | 0.020 ij | 0.129 gh | 0.808 l | 2.33 h |
| Ctrl*No*Os | 81.28 b-e | 29.85 a | 1.95 fg | 18.4 jk | 0.036 hi | 0.140 gh | 1.473 jk | 3.33 gh |
| Ctrl*Et*Mn | 75.41 b | 38.43 abc | 1.24 ijk | 19.5 j | 0.020 ij | 0.102 i | 1.458 jk | 3.33 gh |
| Ctrl*Et*Os | 81.12 bcd | 40.45 a-d | 1.38 ij | 12.8 m | 0.037 hi | 0.121 hi | 1.237 kl | 3.33 gh |

*In each column, means with the similar letter(s) are not significantly different (P<0.05) using the LSD test. Abbreviations: FWC (fresh weight changes), CMS (cell membrane stability),Tchl (total chlorophyll), TC (total soluble carbohydrates), CAT (catalase), POD (peroxidase), Pro (proline), Vl (vase life), No (normal condition), Et (ethylene stress condition), Mn (multi-flower mini carnations) and Os (one-headed standard carnations).

## Chlorophyll content

Along with the onset of senescence, chlorophyll content gradually degenerated. However, the highest content of chlorophyll was observed after 3 days in the carnation cut flowers treated with 100 µM BA (Table 2). As far as chlorophyll content was assayed, there was no significant difference in total chlorophyll content between two types of the carnation cut flowers. Further analysis revealed that exogenous ethylene exposure resulted in chlorophyll content reduction (Table 2). It has been found that chlorophyll deterioration under ethylene exposure in Mn carnation cut flowers is more pronounced than in Os carnation cut flowers (Table 2). As reported by Talla *et al.* (2016), cytokinin increased chlorophyll content by up-regulating genes involved in chlorophyll cycle, and thus leaf senescence was delayed. Another study demonstrated a positive correlation between ethylene production and leaf senescence and a negative correlation between cytokinin

levels and leaf senescence (Xu and Haung, 2007). These results corroborate our findings on the role of cytokinin in chlorophyll preservation.

**Soluble carbohydrates**

The results indicated that following the 100 µM SA treatment, an increase was recorded in soluble carbohydrates (Table 2). Soluble carbohydrates were more pronounced in the Os carnation cut flowers than in the Mn carnation cut flowers (Table 2). Also, the results showed that soluble carbohydrates in the carnations exposed to ethylene tended to decrease (Table 2). Further results revealed that the Mn carnations treated with 100 µM SA had the highest soluble carbohydrate content (Table 2). More analysis was done to evaluate the effects of PGR short-term treatments. Soluble carbohydrate was reduced in all the flowers exposed to ethylene (Table 2). Soluble carbohydrate rate was considerably decreased by ethylene exposure in both cut carnation types (Table 2). Further analysis of the interaction of the three factors (carnation type, PGRs, and exogenous ethylene) revealed that the highest soluble carbohydrate content was related to the Os cut carnations treated with 100 µM SA but not exposed to ethylene stress (Table 2). The treatment with 0.5 SA resulted in the highest total soluble solids in kiwifruit (Bal and Celik, 2010). Further studies conducted by Khan *et al.* (2010) demonstrated that the SA treatment increased soluble sugars in hexaploidated wheat leaves in drought stress conditions.

**Antioxidant activity**

Catalase enzyme activity of flowers treated with 400 µM MeJA was increased markedly (Table 2). As observed in the present study, catalase enzyme activity was more pronounced in the Os cut flowers than in the Mn cut flowers (Table 2). According to Table 2, a reduction was observed in catalase activity in all ethylene-treated cut flowers. The comparison of catalase activity in the Os and Mn cut carnations under ethylene exposure showed that ethylene reduced catalase activity in both flower types, which was more pronounced in the Mn cut carnations (Table 2). The results of the interaction between PGR treatments and ethylene exposure represented that ethylene application suppressed the positive PGR effects on catalase activity in cut flowers (Table 2). Also, further analysis on the interaction of PGR treatments and types of carnation cut flowers revealed that catalase activity under the influence of PGR treatments had a similar trend in the Os and Mn cut flowers although the catalase activity was significantly more pronounced in the Mn cut flowers treated with 50 µM SA. The results of three-way interaction (carnation type, PGRs, and exogenous ethylene) represented that the highest catalase activity was obtained from the ethylene stress-exposed Os cut carnations treated with 200 µM MeJA (Table 2).

As follows from Table 2, the treatment with 400 µM MeJA caused an increase in peroxidase activity in the carnation cut flowers. The analysis did not identify any significant differences in the peroxidase activities of the two types of carnation cut flowers (Table 1). Ethylene exposure significantly decreased the peroxidase activity of the carnation cut flowers (Table 2). Further analysis for evaluating the interaction of exogenous ethylene and PGR treatments revealed that regardless of PGR-treatment's type, exogenous ethylene stress dramatically reduced the peroxidase activity in all of the cut carnations (Table 2). Table 2 represented that 400 µM MeJA was related to the highest peroxidase activity in the Os and Mn carnation cut flowers, respectively. The study of peroxidase activity in the Os and Mn cut carnations under ethylene exposure demonstrated that ethylene reduced peroxidase activity in the Os cut carnation, while there was no significant effect of ethylene exposure on peroxidase activity of the Mn cut carnations (Table 2). The results of the three-way interaction (carnation type, PGRs, and exogenous ethylene) showed that the highest peroxidase activity was related to the Os cut carnations treated by 400 µM MeJA but not exposed to ethylene stress (Table 2).

In general, 400 µM MeJA induced an increase in proline content (Table 2). Similar to other traits, proline content was higher in the Os carnation cut flowers than in the Mn carnations (Table 2). Proline content was found to be lower in the carnation cut flowers exposed to ethylene (Table 2). Investigating the effects of ethylene exposure on the PGR-treated carnation cut flowers revealed that proline content of the PGR-treated carnation cut flowers decreased in response to exogenous ethylene. The short-term treatment with 100 µM SA and 400 µM MeJA preserved proline content in ethylene exposed carnation cut flowers (Table 2). Also, further results showed that proline content reduction caused by exogenous ethylene was more pronounced in the Mn carnation cut flowers than in the Os carnations (Table 2). Further analysis of the interaction of three factors (carnation type, PGRs, and exogenous ethylene) revealed that the highest proline content was related to the ethylene stress-exposed Os cut carnations treated with 200 µM MeJA (Table 2). It has been documented that 100 and 250 µM MeJA increased protein content and the activity of superoxide dismutase, catalase and peroxidase enzymes in peanut seedlings (Kumari *et al.,* 2006). Moreover, there is some research that shows MeJA treatment elevated the activity of superoxide dismutase, ascorbate peroxidase, catalase, and peroxidase in *Arabidopsis* (Jung, 2004) and canola (Comparot *et al.,* 2002). More evidence about the role of MeJA in increasing proline content under stress conditions has demonstrated that MeJA increases proline content by up-regulating the responsible genes (Wasternack and Kombrink, 2010).

**Vase life**

The vase life of cut flowers was prolonged after PGR treatments. The treatment with 100 µM SA had the strongest effect on the longevity of carnation cut flowers (Table 2). Comparing vase life of two types of carnation cut flowers revealed that the Os carnation cut flowers had more longevity than the Mn carnation cut flowers (Table 2). To understand the role of exogenous ethylene on vase life, the carnation cut flowers were exposed to ethylene as follows in Table 2, and exogenous ethylene reduced the vase life of cut flowers (Table 2). Further assessment about the interaction of ethylene and the type of cut flowers revealed that petal rolling in the Mn carnation cut flowers subjected to ethylene appeared in 3 days after harvest, while petal rolling and wilting symptoms in the Os carnation cut flowers under the effect of ethylene was observed in 6 days after harvest and also, the longevity of the Os carnation cut flowers was 9 days in normal conditions (Table 2). The results of the three-way interaction (carnation type, PGRs, and exogenous ethylene) represented that the longest vase life was obtained from the ethylene stress-exposed Os cut carnations treated with 100 µM SA (Table 2). In the present study, it was found that the treatment with 100 µM SA markedly prolonged the vase life of carnation cut flowers, confirming previous findings. As reported by Zamani *et al.* (2011), evidence points to the enhancement of cut rose vase life by using the age-relating factors in cut roses. Zamani *et al.* (2011) also mentioned that preventing chlorophyll degradation by SA might retard senescence.

## CONCLUSION

It can be concluded from the results of the present study that the short-term PGR treatment enhanced the considered traits in two types of carnations. It has been demonstrated that the Os cut carnations scored better results than the Mn cut carnations in all studied traits under the short-term treatment. Summing up the results shown in all figures, it can be concluded that exogenous ethylene suppressed the proper effects of PGRs on cut carnations. The results imply that the Os cut carnation were also more tolerant of ethylene than the Mn cut carnations.

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