Flower and Ornamental Plants (2023), 7(2): 239-260
Research article
DOI: 10.61186/flowerjournal.7.2.239

Qualitative response and vase life of carnation (*Dianthus caryophyllus*) cut flowers treated with salicylic acid and benzyl adenine under cold storage conditions

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Received: 2022/01/21, Revised: 2022/05/08, Accepted: 2022/05/08

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Abstract

Prolonging the vase life and delaying the onset of senescence of cut flowers is one of the most important goals of experts, producers, and activists in ornamental plants. According to the high production of ethylene in carnation petals and its negative effect on the postharvest life of carnation, the present study investigated the effect of preservatives containing salicylic acid (0, 2, and 4 mM) and benzyl adenine (0, 25, and 50 mg L^{-1}) and their interaction on maintaining the quality and vase life of carnation flowers stored at 4 ± 1 °C. The results showed that the benzyl adenine treatment (25 mg L^{-1}) increased the vase life, affected the carnation stem's diameter, and cut flowers significantly. Absorption of solution of salicylic acid 4 mM treated carnation flowers significantly caused an increase in their weight and showed a significant increase compared to control carnation flowers. Salicylic acid treatment decreased the percentage of ion leakage. The interaction of salicylic acid and benzyl adenine treatments also maintained flower quality by increasing the total sugar content, decreasing the pH of the preservative solution, and reducing the bacterial population in the preservative solution. The treatments of the current study increased photosynthetic pigments. The highest chlorophyll a, total chlorophyll, and carotenoids were obtained in benzyl adenine at 25 mg L^{-1} and 50 mg L^{-1}. Salicylic acid 2 mM caused them to increase in T2 compared to T1. The reaction of chlorophyll b was different so that it reached its maximum in salicylic 2 mM and increased it at T2 with the help of benzyl adenine 25 mg L^{-1}.

Keywords: Vase Life, Cut Flowers, Carnation, Salicylic Acid, Benzyl Adenine.

Introduction

Carnation (*Dianthus caryophyllus*) belonging to the *Caryophyllaceae* family is grown as an ornamental garden plant, cut flower, or potted flowering plant (Dole and Wilknis, 2005). The vase life of cut flowers is one of the most important quality factors and the long life of flowers has a great impact on consumer demand and the value of cut flowers. Ethylene, known as a plant hormone effective in the senescence of carnation flowers (Pun *et al*., 1999) causes folding of petal edges and carnation wilting (Fharokhzad *et al*., 2005). Thus, it is important to know the optimal carnation post-harvest care techniques (Witte and van Doorn, 1991).

Salicylic acid is a natural plant hormone and one of the substances used to maintain the post-harvest quality of flowers and to increase the vase life. In addition, it is involved in defense and resistance to disease conditions and plant stresses. External use of salicylic acid can help the plant to maintain

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quality and increase longevity and resistance to stress and disease. However, determine the effective concentration and the best time of application is very important and requires research. Salicylic acid with the chemical name of 2-hydroxybenzoic acid belongs to a group of phenolic compounds in plants and as a hormone-like substance plays an important role in regulating plant growth and development (Khan *et al*., 2003). This compound is also observed in the leaves and reproductive organs of plants. With its highest concentration found in tropical inflorescence (Ruskin, 1992) Evidence suggests that salicylic acid plays an important role in the physiological processes such as plant growth and development, photosynthesis, transpiration, ion uptake, protein synthesis (Amin *et al*., 2008), fruit ripening, and senescence (Zhang *et al*., 2003). Many studies have also shown that this substance is involved in plant resistance to pathogens (Gaur *et al*., 1982) and interferes with ethylene biosynthesis (Zhang *et al*., 2003). In a related study, the use of acetylsalicylic acid was found to improve the vase life of cut gerbera branches (Kazemi *et al*., 2012). Also, the use of salicylic acid in roses increased flowering life and water uptake (Ghadimian and Danayi, 2020) and its application in Narcissus flower increased relative fresh weight (Gan and Ozturk, 2020).

Benzyl adenine, as a synthetic plant growth regulator, belongs to a group of cytokinins that inhibits activity and production of ethylene and prevents premature senescence (Raven *et al*., 1981). Application of cytokinin on isolated carnation petals showed that pretreatment with benzyl adenine and kinetin inhibits the conversion of ACC[1] to ethylene. Benzyl adenine also inhibits endogenous ACC accumulation and ethylene production in petals (Wawrzynczak and Goszczynska, 2003). Foliar application of benzyl adenine to tuberose cut flowers (*Polianthes tuberosa* L.*)* increased the number of days required for flower opening, the number of flowers, stem length, length of inflorescence, fresh and dry weight, the diameter of inflorescence, and also increased essential oil in flowers (Eid *et al*., 2010). Besides, application of this compound in Alstroemeria cut flowers caused a significant increase in leaf nitrogen and chlorophyll content of the flowers and finally delayed flower senescence and prolonged flowering life and maintained flower quality (Mutui *et al*., 2001).

Carnation is currently one of the ten most important cut flowers in the world. The post-harvest life of cut flowers is one of the most important quality factors. Considering the positive effects of salicylic acid and benzyl adenine treatments on growth characteristics, antioxidant system, and vase life of cut flowers, the present study aimed to investigate the effects of salicylic acid and benzyl adenine on some quality characteristics of carnation under cold storage conditions.

Materials and Methods

Plant materials and chemicals

Cut flowers of white carnation standard cultivar at the bud stage were purchased from greenhouses located in Karaj, Alborz province, and transferred to the laboratory at the Faculty of Agriculture and Natural Resources of Hormozgan University. Salicylic acid (138.121 g/mol), benzyl adenine (225.25 g/mol), and sucrose (Merck, Germany) were purchased.

Preparation and treatments

The flower branches were cut to the same height (45 cm) diagonally under the water to prevent air bubbles from forming in the stem vessels. The leaves were removed on the lower 10 cm of the stem for easy placement in the vase and to prevent decay and accumulation of pathogens. Salicylic acid solutions (2 and 4 mM), benzyl adenine solutions (25 and 50 mg L^{-1}), and flower preservative solution containing 4% sucrose were prepared. To apply the treatments, carnation shoots were first divided into three groups of 3 branches and placed in distilled water and 25 and 50 mg L^{-1} benzyl adenine for 48 hours, respectively (short-term pulsing treatment) (Meir *et al*., 1998; Capdeville *et al*., 2003). Then, each group of flowers treated with benzyl adenine was divided into three groups of 3 branches and

1. Amino cyclopropane 1-carboxylic acid

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placed in disinfected (with 0.5% sodium hypochlorite) containers with 250 ml salicylic acid solution (0, 2, and 4 mM) containing 4% sucrose to the end of the experiment (long-term, permanent treatment) (Solgi *et al*., 2009). The flowers were then refrigerated at a temperature of 4±1 °C and a humidity of 70-80% with 12 hr light/12 hr dark conditions with fluorescent lamps and light intensity 12 micromoles per square meter per second. To prevent water evaporation, the mouths of the bottles were covered with cotton and aluminum foil. Evaluation of different flower characteristics was performed for 36 days with an interval of 12 days as follows:

Physical quality

Flower quality was recorded in terms of days until the petals completely lost their turgor pressure and freshness. The physical quality was measured on a scale ranging from 1 to 5 (5 = The stage when the petals do not change color and retain their freshness; 4 = The onset of quality loss in 15% of the petals; 3 = Decreased quality in 15-30% of petals; 2 = Decreased quality in 30-60% of petals; and 1 = Decreased quality in 60-100% of petals). The end of vase life was determined by a 30% reduction in physical quality (Shahabi Rabri, 2020).

The absorption rate of preservative solution and relative fresh weight

The absorption rate of the preservative solution was measured before the treatments and also at specified intervals using Eq. (1). Furthermore, to measure the relative fresh weight (RFW) of the flowers Eq. (2), the cut flowers were weighed before treatment and at specified intervals (Solgi *et al*., 2009):

$$\text{Absorption rate (\%)} = (\text{Secondary weight} - \text{initial weight}) \quad (1)$$

$$\text{RFW} = (\text{initial weight/secondary weight}) \times 100 \quad (2)$$

Flower and stem diameters

A digital caliper (in millimeters) was used to evaluate the effects of different vase solutions on flower and stem diameter (at a distance of 3 cm from the base of carnation inflorescences). Initial evaluation was performed 24 hours after treatment, and then flower and stem diameter sizes were examined and recorded every 12 days.

Solution pH and soluble solids content of petals

The pH of the preservative solution was measured directly at the beginning and end of the experiment by a pH meter (HANA, Romani). To measure the percentage of soluble solids content, one gram of the petal tissue was randomly selected from each experimental unit and was extracted and measured by placing one drop of the extract on the prism plate of digital refractometer (EZDO, Taiwan).

Petal ion leakage

To measure the membrane stability, 0.1 g of petal tissue with 10 ml of distilled water was placed in test tubes. First, the tubes were placed in a hot water bath at 40 °C for 30 minutes and after cooling, the initial electrical conductivity of the samples was measured using an EC meter (Weilheim, Germany). The samples were then placed in a hot water bath at 100 °C for 15 minutes after cooling the solution the secondary electrical conductivity was measured. The membrane stability index was calculated using the following formula:

$$\text{Ion leakage (\%)} = (\text{Secondary electrolytic conductivity/primary electrolytic conductivity}) \times 100 \quad (3)$$

Total phenol

Total phenol content was measured using Singleton, Rossi's (1965) method. To do so, 0.5 g of petal tissue was homogenized with 3 ml of 85% methanol and 300 µl of it with 1500 µl of diluted Folin-Cocaltive reagent (10%). After five minutes, 1200 µl of 7% sodium carbonate was added to the mixture and then placed on a shaker for 90 minutes. Its absorption was measured using a microplate reader (Epoch, USA) at a wavelength of 760 nm and compared with the standard curve of gallic acid in different concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, and 500). Then, the total phenol content was calculated based on mg of gallic acid per 100 g of fresh weight (Singleton and Rossi's, 1965).

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Antioxidant activity

To measure the antioxidant activity, 30 µl of methanolic extract of petal tissue was mixed with 270 µl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM) and stirred by vortexing and placed in a dark place for 30 minutes. The absorbance rate of the samples was read by a microplate reader (Epoch, USA) at 517 nm and calculated using the following formula (Williams *et al*., 1995):

Antioxidant activity (%) = (Control absorbance-sample absorbance)/Control absorbance × 100

Total flavonoids

To determine the total flavonoids, 0.5 g of petal tissue was homogenized and centrifuged with 3 ml of methanol. Then, 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of potassium acetate (one molar), and 2.8 ml of distilled water were combined with each of the plant extracts. The solutions were placed at room temperature for 30 minutes. The absorbance of the samples was measured at 415 nm with a microplate reader (Epoch, USA) and compared with standard quercetin curve in different concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, and 500). Then, the total flavonoid content was measured based on quercetin (mg) per 100 g fresh weight (Chang *et al*., 2002).

Chlorophyll and carotenoids

To measure the content of chlorophyll and carotenoids, 0.5 g of petal tissue was grounded with 5 ml of 80% acetone using a porcelain mortar. After homogenization, it was centrifuged at 4 ° C for 13 minutes at 13,000 rpm (Micro R 220, Germany). The supernatant was separated and its volume was increased to 10 ml with 80% acetone. Then, the absorbance rate of the samples was read using a microplate reader (Epoch, USA) at wavelengths of 645, 663, 510, and 480 nm. Finally, the concentrations of a, b, total chlorophyll, and carotenoids were measured using the following equations (Lichtenthaler, 1987):

Chl a (µg/ml) = [(12.25×A663) - (2.69×A645)] ×V/W

Chl b (µg/ml) = [(20.31× A645) - (4.91×A663)] ×V/W

Chl total (µg/ml) = [(20.20×A645) + (8.02×A663)] ×V/W

Carotenoid (µg/ml) = [(7.6×A480) – (1.49×(A510)] ×V/W

The bacterial population of preservative solutions

To measure the total number of bacteria grown (regardless of their type) in the vase solutions, 1 mL of the vase solution was removed using a pipette and its absorption rate was read with a spectrophotometer (Cecil , Engl) at a wavelength of 600 nm. Finally, the bacterial population in the vase solution was calculated according to the following equation and the amounts of changes were expressed in percentage (Farsani and Zolala, 2008):

Bacterial population = 8×10^8/ absorption number

Data analysis

Data analysis was performed using Duncan's new multiple range test (MRT) to compare the mean of the data at the probability level of $P<0.01$ with SAS software (version 9.4). Besides, the correlations between the traits in questions were evaluated using R software (R version 3.5.0) and the curves were drawn by Excel and R software.

Results and Discussion

Physical quality

The results of analysis of variance (ANOVA) showed that the quality of carnation cut flowers was not affected by the simple effect of salicylic acid, but its interaction with benzyl adenine was significant at the level of 5%. The benzyl adenine treatment ($P<0.05$) and the storage time ($P<0.01$)

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had a significant effect on the physical quality of carnation cut flowers as confirmed by the analysis of variance. Accordingly, 25 mg L^{-1} benzyl adenine increased the quality of the cut flowers (Figure 1-a). Moreover, the interaction of 25 mg L^{-1} benzyl adenine with 2 mM salicylic acid led to the best quality of carnation cut flowers (Figure 1-c).

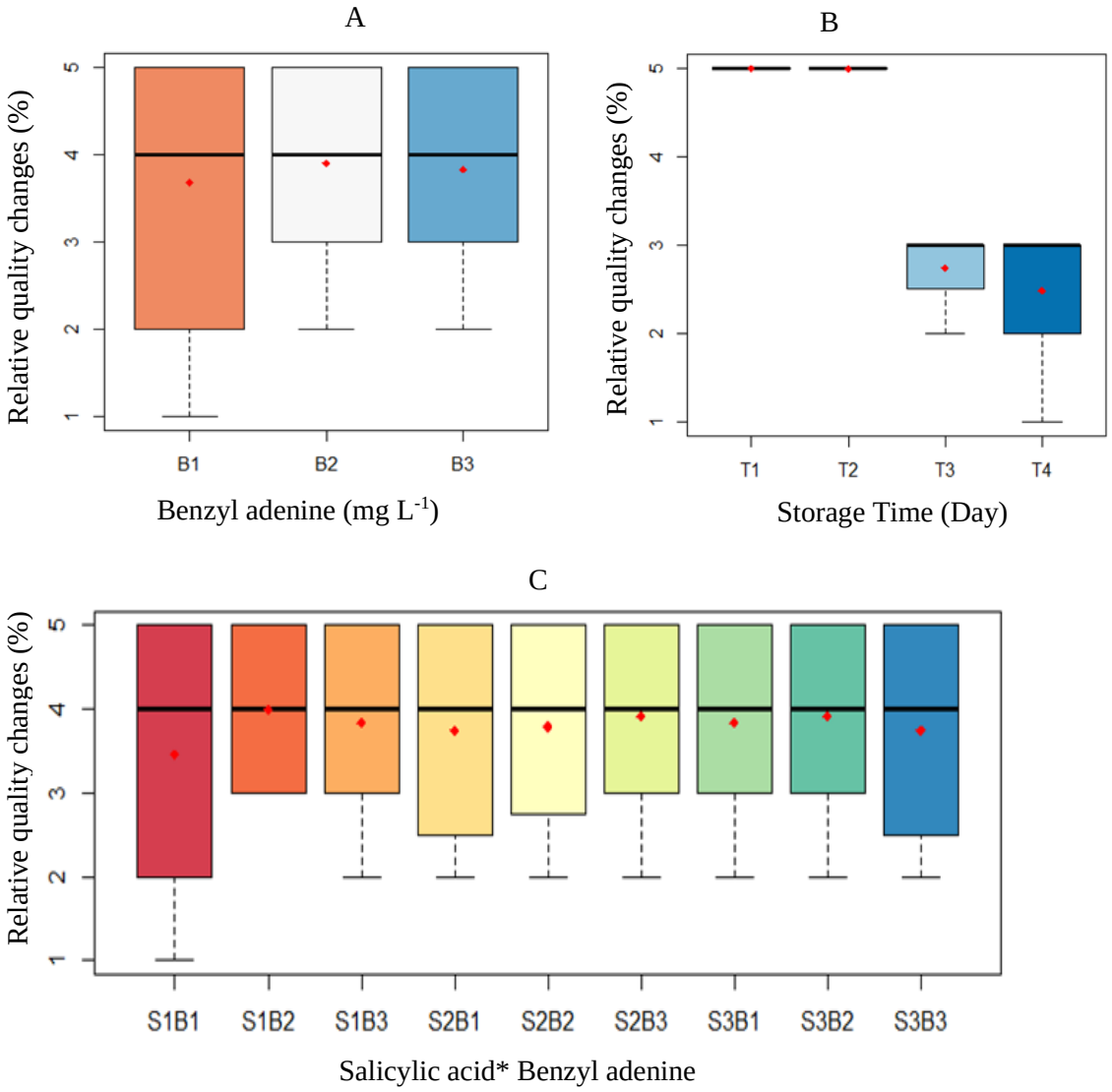

Figure 1. The simple effect of benzyl adenine (a), storage period (b), and the salicylic acid-benzyl adenine interaction (c) on the trend of relative changes in the quality of carnation cut flowers at 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (S1), 2 mM (S2), and 4 mM (S3). Benzyl adenine was applied at three concentrations: 0 (B1), 25 mg L^{-1} (B2), and 50 mg L^{-1} (B3). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

Carnation is in the group of climacteric flowers and if it is not kept at good post-harvest conditions, due to its high sensitivity to ethylene and its production, the senescence and decay process begins quickly. Contact of these flowers with external ethylene causes them to become tubular and ultimately decrease their post-harvest life (Zadehbagheri *et al*., 2010). Accordingly, this study showed that benzyl adenine contributed to improving the quality of carnation. Furthermore, 1.5 mM salicylic acid in the gerbera flower solution improved vase life and the quality of the flowers (Kazemi *et al*., 2011).

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Salicylic acid has also been reported to prevent senescence by reducing ACC oxidase activity and converting ACC to ethylene (Raskin, 1992). Thus, this valuable compound can delay the onset of the senescence process. Cytokinin prolongs the life of cut flowers by delaying fresh weight loss and increasing the water uptake (Chamani *et al*., 2018). However, the best concentration should be selected carefully because the effects of cytokinin depending on its concentration can slow or accelerate the senescence process. At high concentrations of cytokinin, aminocyclopropane (ACC) (an ethylene precursor) accumulates in the petals of cut flowers, resulting in signs of senescence (Skutnik *et al*., 2006). At suitable concentrations, benzyl adenine increased the lifespan of the Pink Castellaro carnation cultivar by increasing its soluble sugar and chlorophyll content (Hamidimoghadam *et al*., 2014). In fact, cytokinins delay the senescence of cut flowers and leaves by playing a role in cell division, preventing chlorophyll degradation, chloroplast development, leaf growth, and opening and closing of stomata. In addition, cytokinins have anti-ethylene properties and increase the vase life of cut flowers by inhibiting ethylene activity (Talla *et al*., 2016). The prolonged vase life of *Cyperus alternifolius* after the use of benzylamine may be due to its ability to maintain the integrity of the tonoplast membrane and prevent leakage of proteases that hydrolyze soluble proteins of the chloroplast membrane and mitochondria. Cytokinins also prevent chlorophyll degradation, enhance the absorption and storage of amino acids in plants, and prevent senescence by stimulating cell division in plants (Soffar and Taha, 2019).

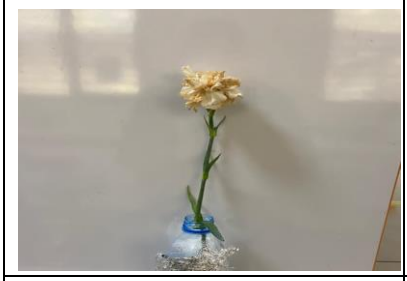
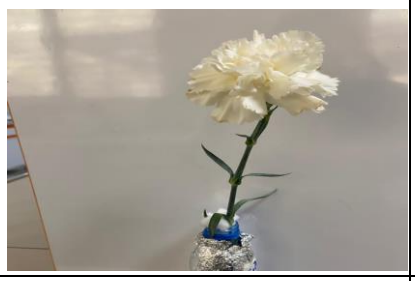
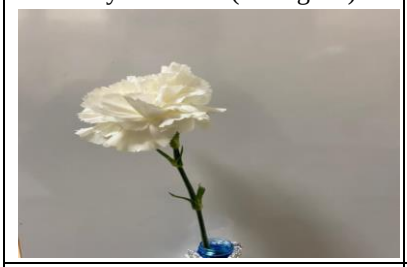
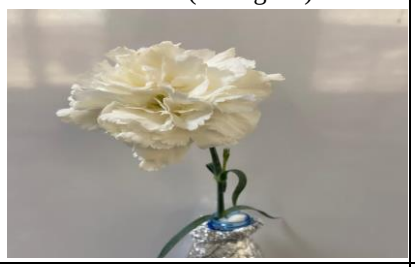
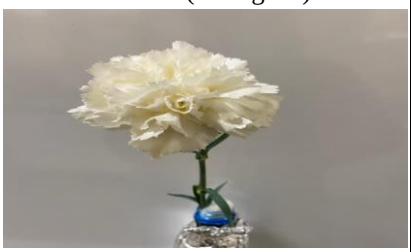
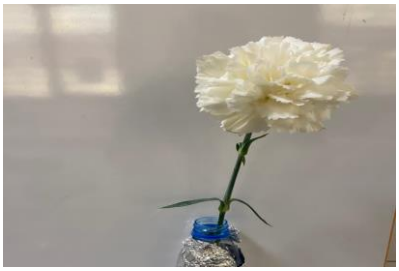
Control	Salicylic Acid (2 Mm) And Benzyl Adenine (0 Mg L^{-1})	Salicylic Acid (4 Mm) And Benzyl Adenine (0 Mg L^{-1})
		
Salicylic Acid (0 Mm) And Benzyl Adenine (25 Mg L^{-1})	Salicylic Acid (2 Mm) And Benzyl Adenine (25 Mg L^{-1})	Salicylic Acid (2 Mm) And Benzyl Adenine (25 Mg L^{-1})
		
Salicylic Acid (0 Mm) And Benzyl Adenine (50 Mg L^{-1})	Salicylic Acid (2 Mm) And Benzyl Adenine (50 Mg L^{-1})	Salicylic Acid (4 Mm) And Benzyl Adenine (50 Mg L^{-1})
		

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Figure 2. Effect of treatments used on physical quality of carnation cut flowers at the end of the storage time.

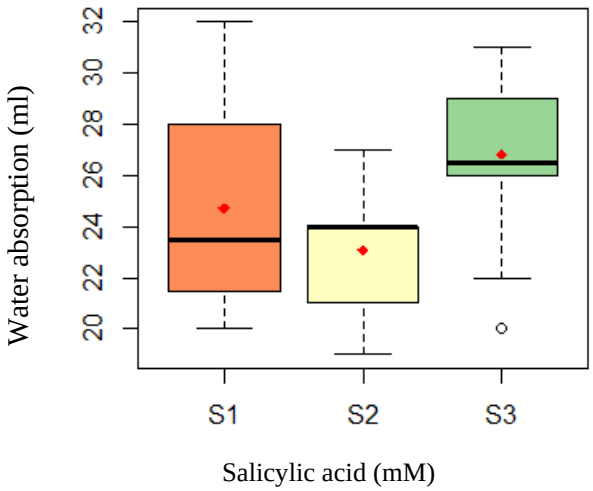

Figure 3. Effect of salicylic acid on the weight of carnation cut flowers stored at 4 ± 1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (S1), 2 mM (S2), and 4 mM (S3). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

It is important to note that the effect of salicylic acid on many physicochemical properties, including flower longevity, is concentration-dependent and the best results are obtained within a small range of concentrations. Moreover, the addition of this valuable compound to the preservative solution affects the activity of catalase and ACC oxidase and plays an effective role in reducing the final ethylene production, and can delay the senescence process (Heydari Alamdarlou *et al*., 2017).

The absorption rate of preservative solution and relative fresh weight

The flower weight was significantly affected by the 2mM salicylic acid and reached its maximum at the concentration of 4 mM (Figure 3). The flower weight data prove that the water absorption of salicylic acid-treated flowers increased their weight and showed a significant increase compared to the control flowers. The sufficient salicylic acid contained in water can significantly extend the vase life of the flowers (Reid and Jiang 2012). A study of salicylic acid and its physiological responses on two gerbera cultivars showed that water uptake was higher compared to control plants (Shabanian *et al*., 2018). It is estimated that salicylic acid has some effect on increasing the number of cells or their weight and also possibly affects the activity of cytokines (Khan *et al*., 2010). Thus, one of the reasons for weight gain in flowers under the influence of salicylic acid seems to be the stimulation of cytokinin synthesis (Hayat *et al*., 2010). Stimulatory effects of salicylic acid on growth can be due to factors such as increased division in meristematic regions and cell growth that promotes growth and another reason is the effect of this hormone on other plant hormones (Shakirova *et al*., 2003).

Flower and stem diameter

The simple effects of storage time and benzyl adenine were significant on flower diameter at the probability level of 5% and 1%, respectively. However, the simple effect of salicylic acid and benzyl adenine on stem diameter was significant at the probability level of 5% and 1%, respectively. After 12 days of storage, the diameter of carnation flowers reached a maximum and then decreased to the end of the storage period (Figure 4-a). Moreover, as shown in Figure 4-b, the maximum and minimum flower diameters were obtained in 25 and 0 mg L^{-1} benzyl adenine treatments. The maximum stem diameter was obtained by applying 4 mM salicylic acid (Figure 4-c) and 25 mg L^{-1} benzyl adenine (Figure 4-d). The increase in flower diameter with the use of benzyl adenine is probably due to the fact

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that this substance causes the transfer of materials made from leaves to buds and growing flowers, whereby the increased osmotic pressure in the petals results in more water absorption and this, in turn, causes cell swelling and turgor pressure of the flowers and eventually increases the flower diameter (Baniasadi and Saffari, 2013). Benzyl adenine can also increase flower and stem diameter by improving soluble solids and stem water content (Danayi *et al*., 2011). The findings of this study were in line with those reported by Danaei *et al*. (2011) in gerbera cut flowers. The diameter of the flowers first increased until the third day and then showed a decreasing trend. The application of cytokinin compounds also increased flower diameter in carnation (Wawrzynczak and Goszczynska, 2000) and tulips (Kim and Miller, 2008). Besides, the results of experiments conducted by some researchers indicated that benzyl adenine has the potential to increase flower diameter (Solgi *et al*., 2009). A similar result was observed for the effect of benzyl adenine on increasing stem diameter in alstroemeria (Hatamzadeh *et al*., 2012). Since cell proliferation and division are regulated by salicylic acid, it can be said that this hormone balances the growth and senescence of flowers (Popova *et al*., 2003). It is also possible that salicylic acid regulates elongation and cell division in stems and flowers along with other substances such as auxin (Hashemi *et al*., 2010).

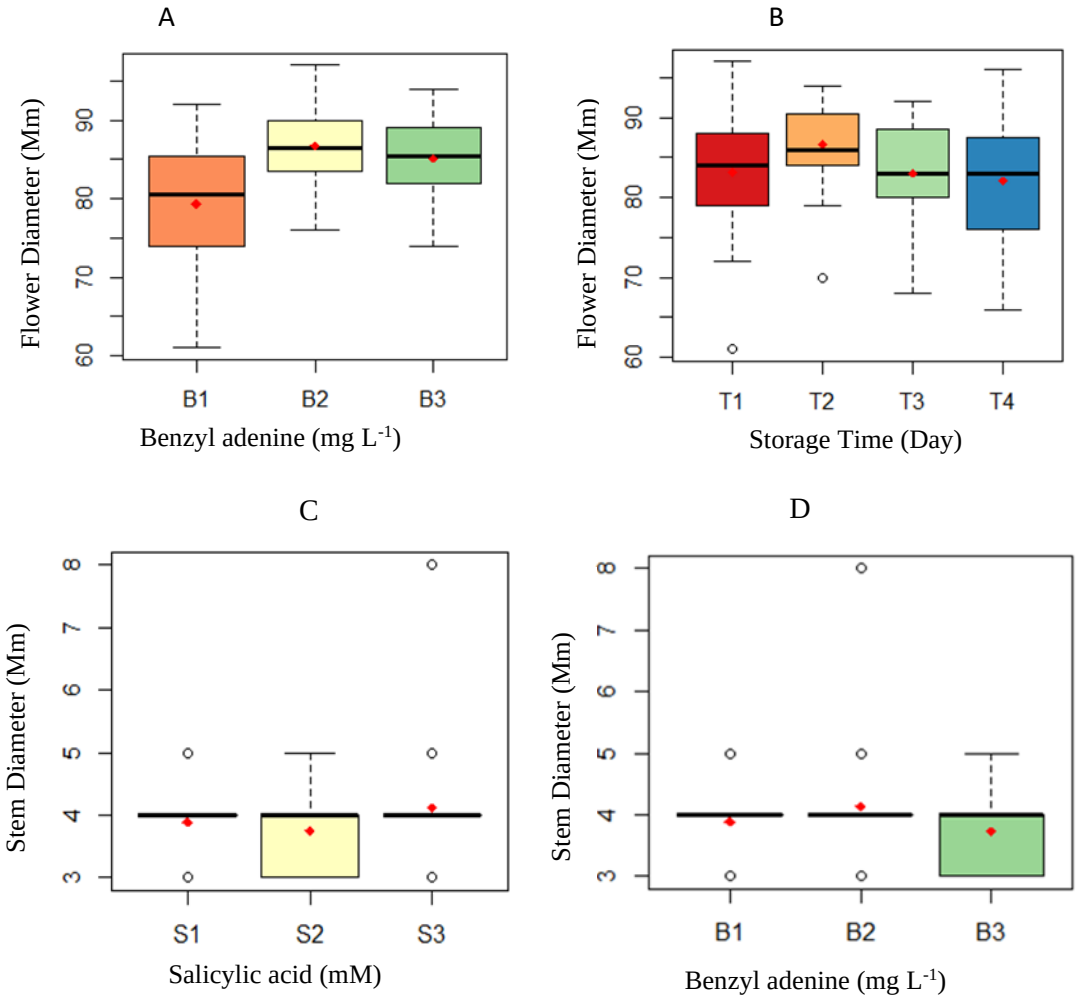

Figure 4. The simple effect of benzyl adenine (a) and storage period (b) on the diameter of carnation cut flowers and simple effect of salicylic acid (c) and benzyl adenine (d) on the diameter of the cut carnation stem stored at 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (S1), 2 mM (S2), and 4 mM (S3). Benzyl adenine was applied at three concentrations: 0 (B1), 25 mg L^{-1} (BA2), and 50 mg L^{-1} (B3). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

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The pH of the preservative solution and soluble solids in petals

The simple effect of salicylic acid, benzyl adenine, and storage time treatments on the pH of the preservative solution in the experiment was significant at the significance level of (P<0.01) (Figure 5-a, b, and c) and their interaction effects were similarly significant (Figure 5-d). Lowering the pH of the preservative solution of cut flowers is considered as an important factor in improving their life. In fact, the lower the pH and the better the quality of the water used, the more obvious its positive effects (Zagory *et al*., 1986). Although the sugar content of the petals was not significantly influenced by the simple effects of salicylic acid and benzyl adenine, the interaction of the two compounds was significant at the significance level of 0.05 (Figure 5-e). Moreover, the salicylic acid-storage time interaction could have a significant effect on the sugar content of carnation cut flowers at the significance level of 0.05 (Figure 5-e). The presence of sugar in cut plants and flowers has important functions such as participation in cell wall formation and respiratory substrate in plant cells (Han *et al*., 2001). In a similar study, the application of benzyl adenine on carnation improved its lifespan by increasing the total sugar content and chlorophyll content (Hamidioghadam *et al*., 2014). According to some studies, cytokines such as benzyl adenine cause adaptation of assimilates from leaves to flower organs, resulting in increased TSS (Total suspended solids) of flower petals (El-Naggar *et al*., 2009). A study found that treatment of cut gladiolus flowers with 200 mg/ml BA increased sugar in petals and leaves up to 10 days after harvest (Hassanpour-Asil and Karimi, 2010). Another study on the effect of growth regulators and foreign ethylene on carnation cultivars showed that 100 µM salicylic acid caused the highest carbohydrate content (Ramtin *et al*., 2018).

Petal ion leakage

According to the analysis of variance, the simple effects of salicylic acid and storage time on the percentage of ion leakage at the significance level of 0.01 were significant (Figure 6-a and b). The salicylic acid-storage time interaction effect on this valuable trait was also significant (P<0.05) (Figure 6-c). The amount of ion leakage indicates the stability of the cell membrane and is, therefore, an important factor in the study of cut flowers. In this study, salicylic acid was able to reduce the percentage of ion leakage by strengthening the cell membrane during storage. Salicylic acid treatment seems to act as a retrofitting process and increases the antioxidant capacity of the cell, protects cell membranes and photosynthetic pigments and ultimately improves growth and quality indices (Momeni *et al*., 2012). Reduction of ion leakage can be associated with the antioxidant potential and capacity of salicylic acid to control free radicals and reduce oxidative degradation of cell membranes, thus controlling ion leakage of cells (Nazarideljoo *et al*., 2013).

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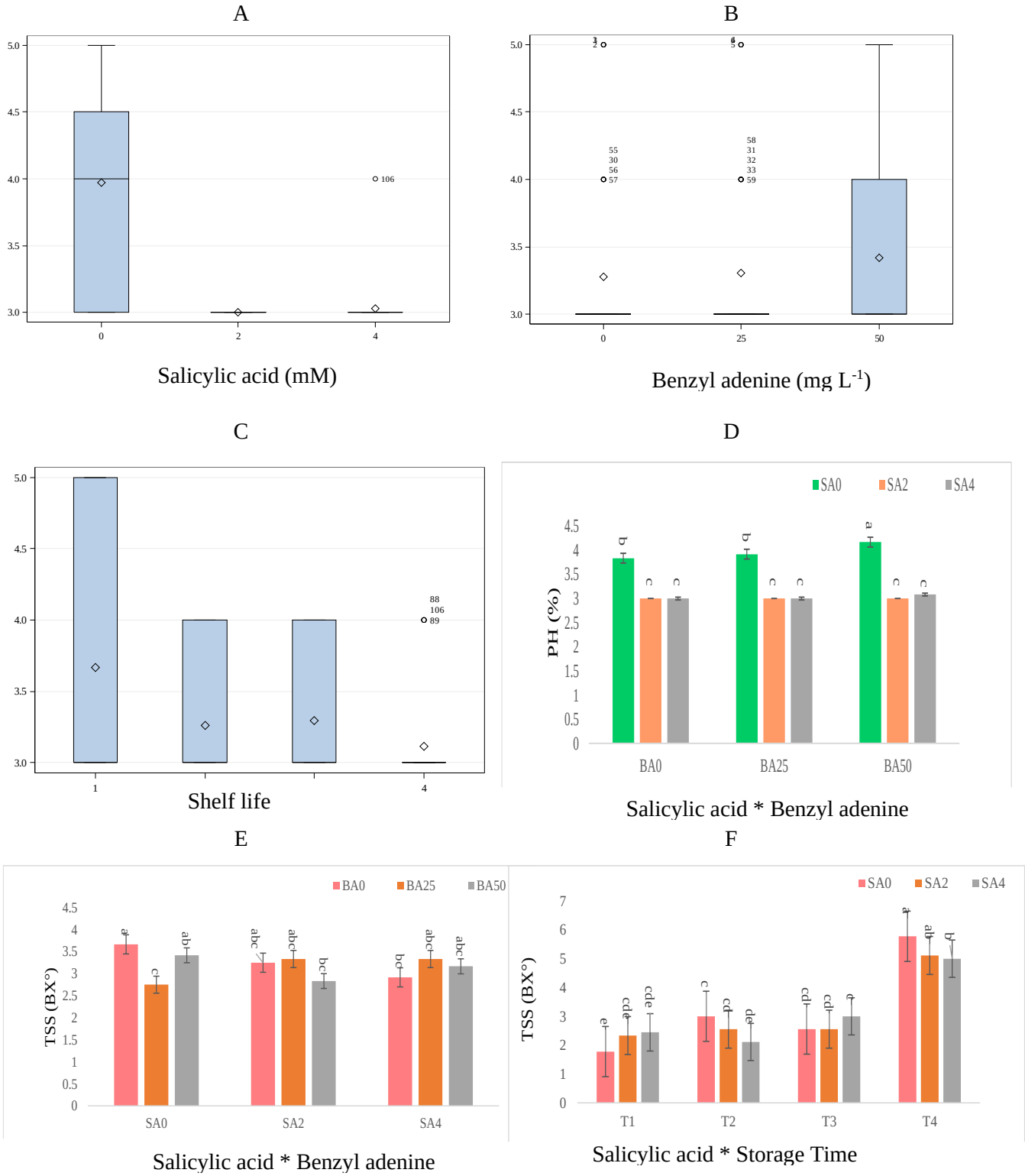

Figure 5. The simple effect of salicylic acid (a), benzyl adenine (b), shelf life (c), and salicylic acid-benzyl adenine interaction (d) on the pH of the preservative solution carnation cut flowers and salicylic acid-benzyl adenine interaction (F) and the salicylic acid-storage time interaction (e) on the number of solids in the petals of carnation cut flowers at a temperature of 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

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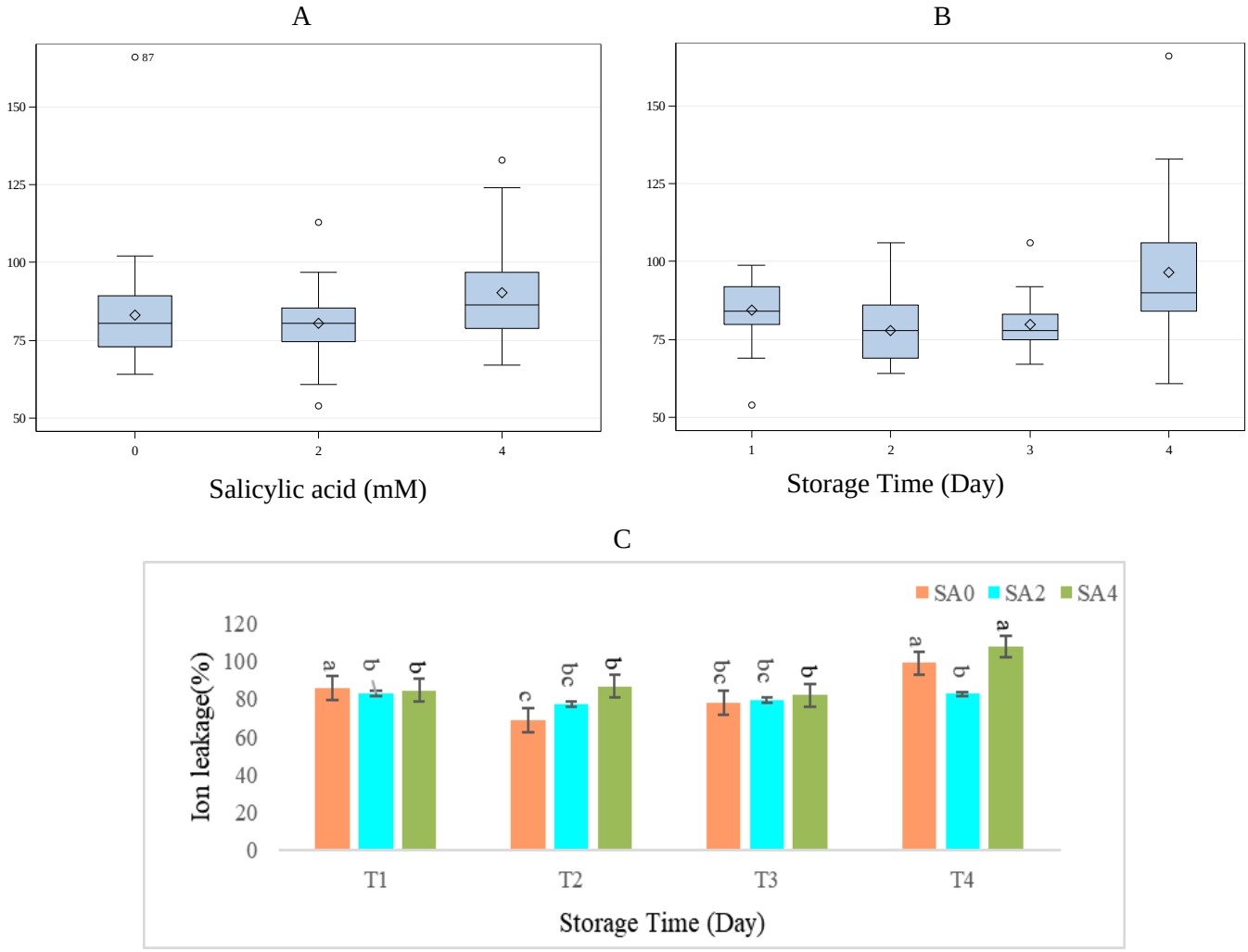

Figure 6. The simple effect of salicylic acid (a), storage time (b), and the salicylic acid-storage time interaction effect (c) on the percentage of electrolyte leakage in carnation cut flowers at 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

Total phenol

The storage time had a significant effect on petal phenol content (P<0.01). After 12 days of storage, phenol content decreased slightly but showed almost no noticeable change until the end of the experiment. The present experimental treatments cannot be considered ineffective in not reducing the phenol content until the end of the experiment. Salicylic acid is known as a key messenger component in activating plant-specific defense responses. Plant defense responses lead to biosynthesis and accumulation of various secondary and medicinal plant compounds. Thus, the induction of salicylic acid is known as an effective way to increase the production of secondary metabolites such as alkaloids, terpenoids, flavonoids, phenolic compounds, and phenocytrins (Mueller *et al*., 1993).

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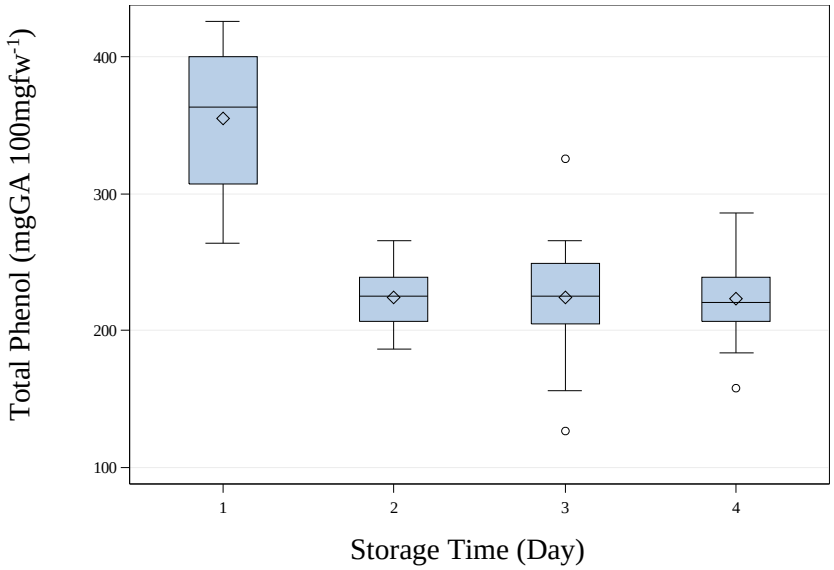

Figure 7. Effect of storage time on phenol content of carnation cut flowers at 4±1 °C. The storage period includes four times: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test (P≤ 0.05) Bars represent SE.

Antioxidant activity

The simple effects of salicylic acid and storage time on antioxidant capacity were significant at the significance level of 0.05 and 0.01, respectively. Moreover, the salicylic acid-storage time interaction, as well as benzyl adenine-storage time interaction, were significant at a 5% level. Benzyl adenine stimulates the biosynthesis of secondary metabolites and leads to increased antioxidant activity (Kaijv *et al*., 2006). Application of this growth regulator in pot marigold increased the antioxidant activity in the petals of this plant (Soltanmoradi and Sedaghatpoor, 2018). Salicylates typically delay senescence in flowers by increasing the activity of antioxidant enzymes and strengthening the cellular antioxidant system (Sood *et al*., 2006). Hatamzadeh *et al*. reported that salicylic acid maintained the quality of cut glycol flowers by increasing the antioxidant capacity of cells and reducing lipid peroxidation. Previous studies have shown that cytokines have the greatest effect in preventing the biological degradation of antioxidant compounds such as anthocyanins during the post-harvest period (Hatamzadeh *et al*., 2012).

Total flavonoids

The salicylic acid-benzyl adenine interaction effect on petal flavonoid content was significant at a 5% level. PAL, as the first enzyme in the phenylpropanoid pathway, converts phenylalanine to 4-coumaroyl coenzyme A, which is an active precursor in the production of flavonoid compounds (Clive *et al*., 1998). Ali et al. (2007) showed that 0.2 mM salicylic acid treatment in Panax (ginseng) doubled the activity of the PAL enzyme. In the foliar spray of salicylic acid at a concentration of 1 mmol on rapeseed, the flavonoid content increased (Mazaheri Tirani, 2007). Benzyl adenine also increases the content of polyphenols including flavonoids as effective antioxidants (Kajiv *et al*., 2006). A study examined the effect of benzyl adenine and salicylic acid on the vase life of Lilium and found benzyl adenine improved the flavonoid content during the storage period of cut Lilium flowers (Abasi *et al*., 2019). Salicylic acid also induces the expression of genes involved in the biosynthesis of some secondary metabolites (Zhao, *et al*. 2005). This compound has been shown to increase the production of secondary metabolites and phenolic compounds, flavonoids, and antioxidants as well as polysaccharides (Wen *et al*., 2019).

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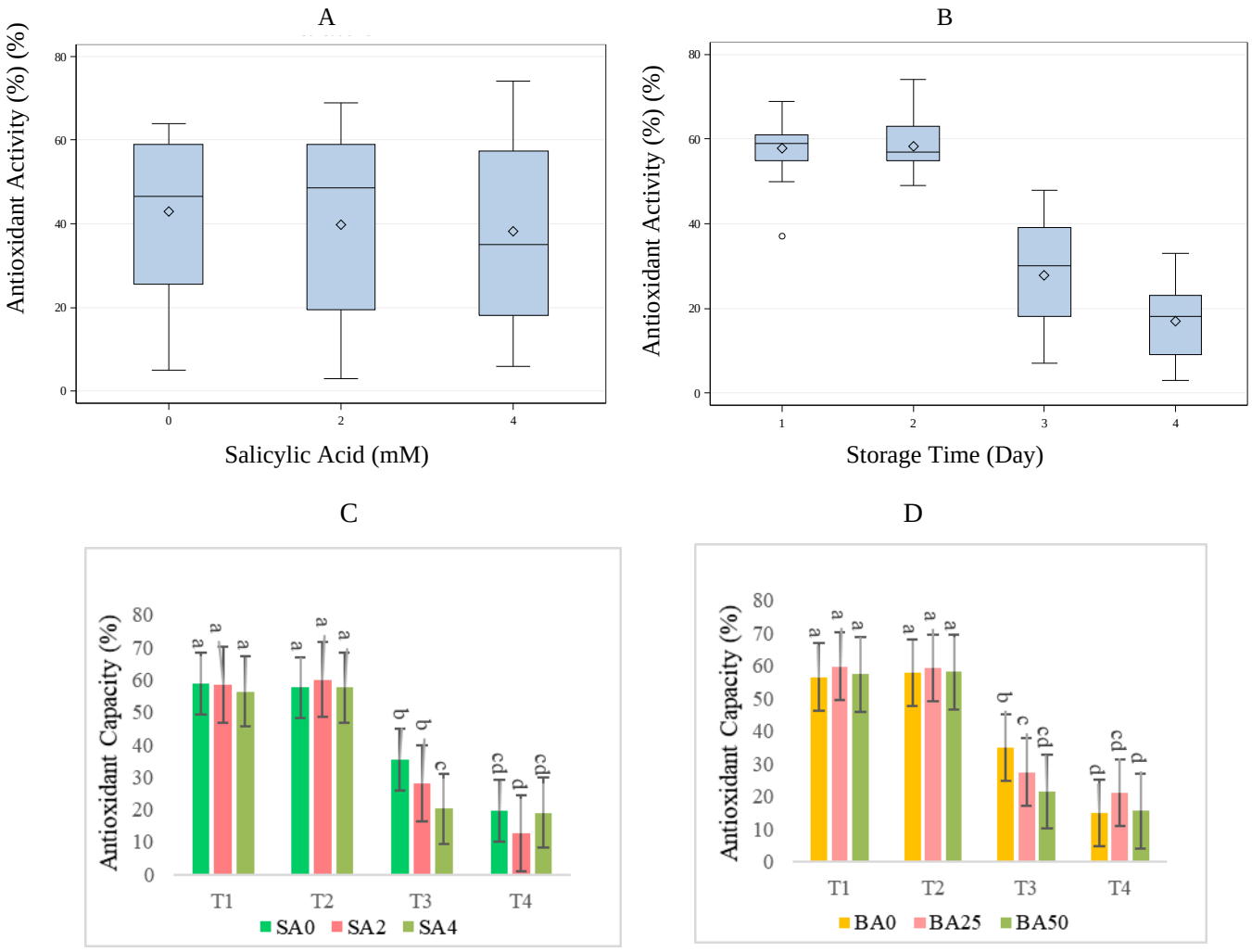

Figure 8. The simple effect of salicylic acid (LF) and storage time (B) on antioxidant capacity, salicylic acid-storage time interaction (C), and benzyl adenine-storage interaction (D) on the antioxidant capacity of carnation cut flowers at 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

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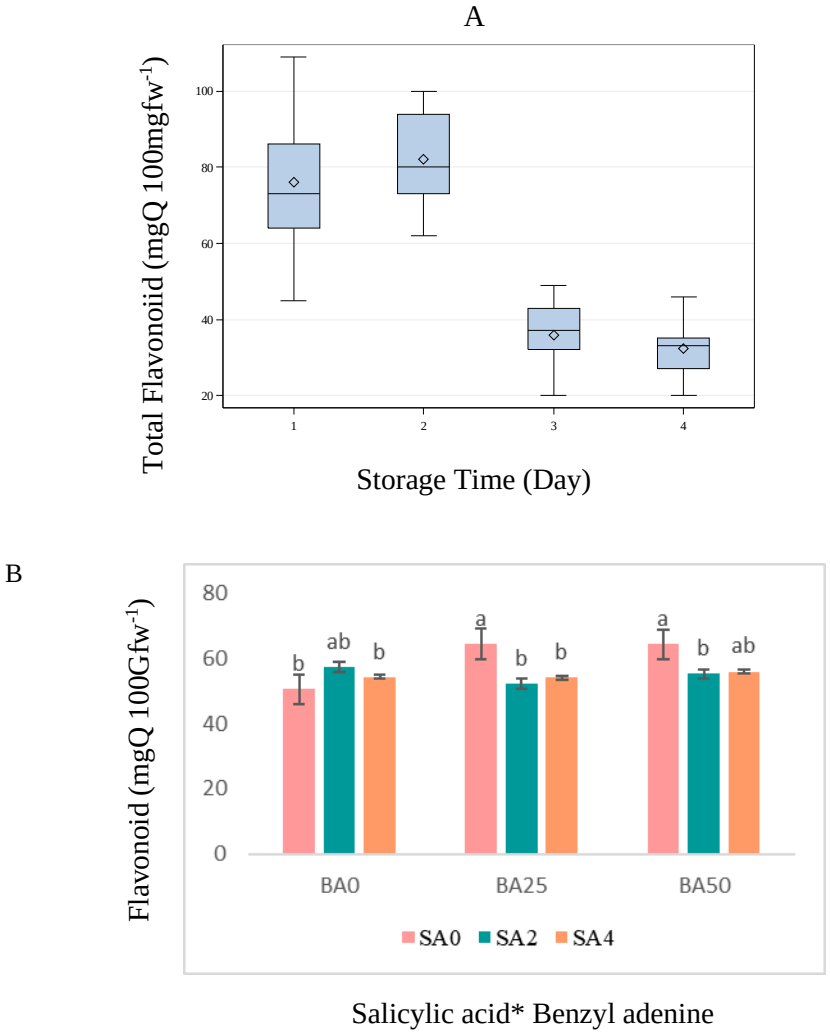

Figure 9. The simple effect of storage time (a) and salicylic acid-benzyl adenine interaction (b) on the flavonoid content of carnation cut flowers at 4±1 °C. The storage period includes four times: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

Photosynthetic pigments

The results of analysis of variance of a, b, and total chlorophyll and carotenoids of the leaves as well as carotenoids of the flowers were significantly affected by the treatments. Thus, chlorophyll a and total chlorophyll were significantly influenced by the simple effects of salicylic acid and storage time at the significance level of 0.01, as well as in the salicylic acid-benzyl adenine, salicylic acid-storage time, as well as benzyl adenine-storage time interaction effects. The triple effects of all three factors were significant at the level of 0.05 (Figure 10-a and c). Almost similar results were found for chlorophyll b, with the difference that the triple effects of salicylic acid, benzyl adenine, and storage time were significant at the level of 0.01 (Figure 10-b). Leaf carotenoids were significantly influenced by the simple effects of salicylic acid and storage time, the salicylic acid-benzyl adenine interaction, as well as benzyl adenine and storage time at the significance level of 0.01. The salicylic acid-storage time interaction and the triple effect of treatments had a significant impact on the carotenoid content of

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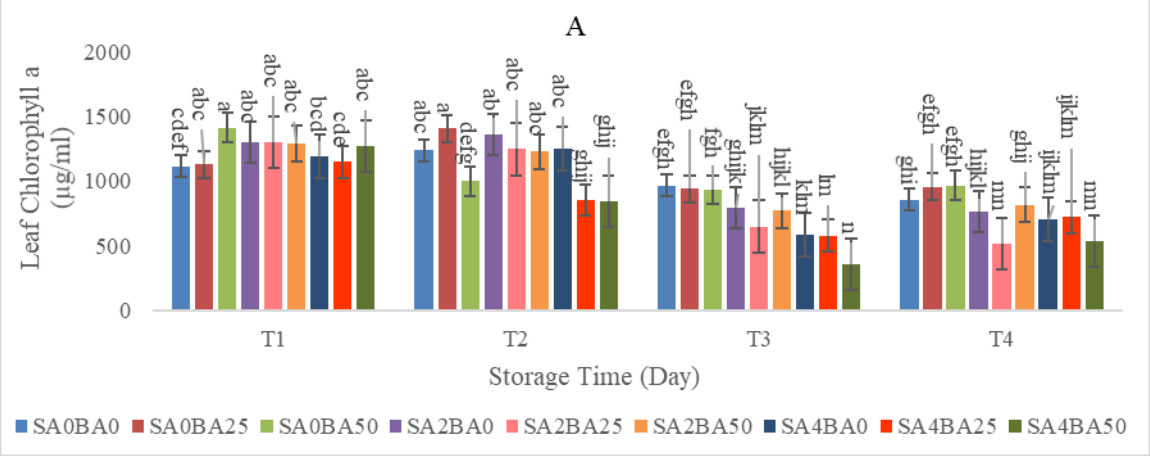
leaves ($p<0.05$). Given that flower color is one of the valuable traits of ornamental plants, carotenoids of carnation flowers were also measured in this study. This trait was significant due to the simple effect of salicylic acid and storage time and their interaction at the significance level of 0.05. External application of the cytokinin combination of benzyl adenine was effective in delaying the senescence of various cells by preventing the degradation of proteins and chlorophyll (Mutui *et al*., 2001). Cytokines can induce a delay in the age-dependent reduction of enzymes involved in photosynthesis and thus increase photosynthesis. These compounds enhance carbohydrate metabolism and cell growth by increasing photosynthesis (Wingler *et al*., 1998). The significant difference between the results and the application of benzyl adenine is attributed to their cytokinin-like activity and stability in plant tissues. Application of cytokinins, especially benzyl adenine, can delay chlorophyll degradation (Zavaleta-Mancera, 2007). Benzyl adenine can increase chloroplast development and chlorophyll synthesis after harvest and during the post-harvest ripening process (Emongor and Tshwenyane, 2004). It should be noted that salicylic acid in suitable concentrations inhibits the activity of chlorophyll oxidase by inhibiting the activity of chlorophyll oxidase enzymes and increasing photosynthesis. Increasing the number of chlorophylls improves photosynthesis and consequently carbohydrate storage (Balkhdi *et al*. 2010). Based on the above findings, it can be concluded that salicylic acid may be indirectly involved in the synthesis and increase of chlorophyll by increasing the activity of nitrate reductase and increasing the absorption of nutrients. The effect that salicylic acid has on chlorophyll is probably due to its effect on ethylene. As mentioned, this hormone increases ethylene synthesis in high concentrations. It also inhibits the synthesis of ethylene at appropriate concentrations and thus can affect chlorophyll content (Mashayekhi, 2007). Salicylic acid is an effective substance to maintain and regulate the function of other hormones such as auxin and cytokinin (Soffar and Taha, 2019). Carotenoids can increase the antioxidant capacity of plants and also support photosynthetic apparatus by increasing the antioxidant capacity of cells and the production of new proteins (Popova *et al*., 2003). This hormone usually regulates many physiological processes and plant growth by acting on the hormones abscisic acid (Senaratna *et al*., 2000) and ethylene (Zhang *et al*., 2002). For example, by acting on abscisic acid and the accumulation of this hormone in the plant, this substance causes plants to become accustomed to environmental stresses (Shakirova *et al*., 2003). Cytokinin increases chlorophyll content by regulating genes involved in the chlorophyll cycle and thus delays leaf senescence. Another study showed a positive correlation between ethylene production and leaf senescence and a negative correlation between cytokinin levels and leaf senescence (Talla *et al*., 2016; Xu and Haung, 2007), confirming the role of benzyl adenine as a cytokinin in chlorophyll retention.



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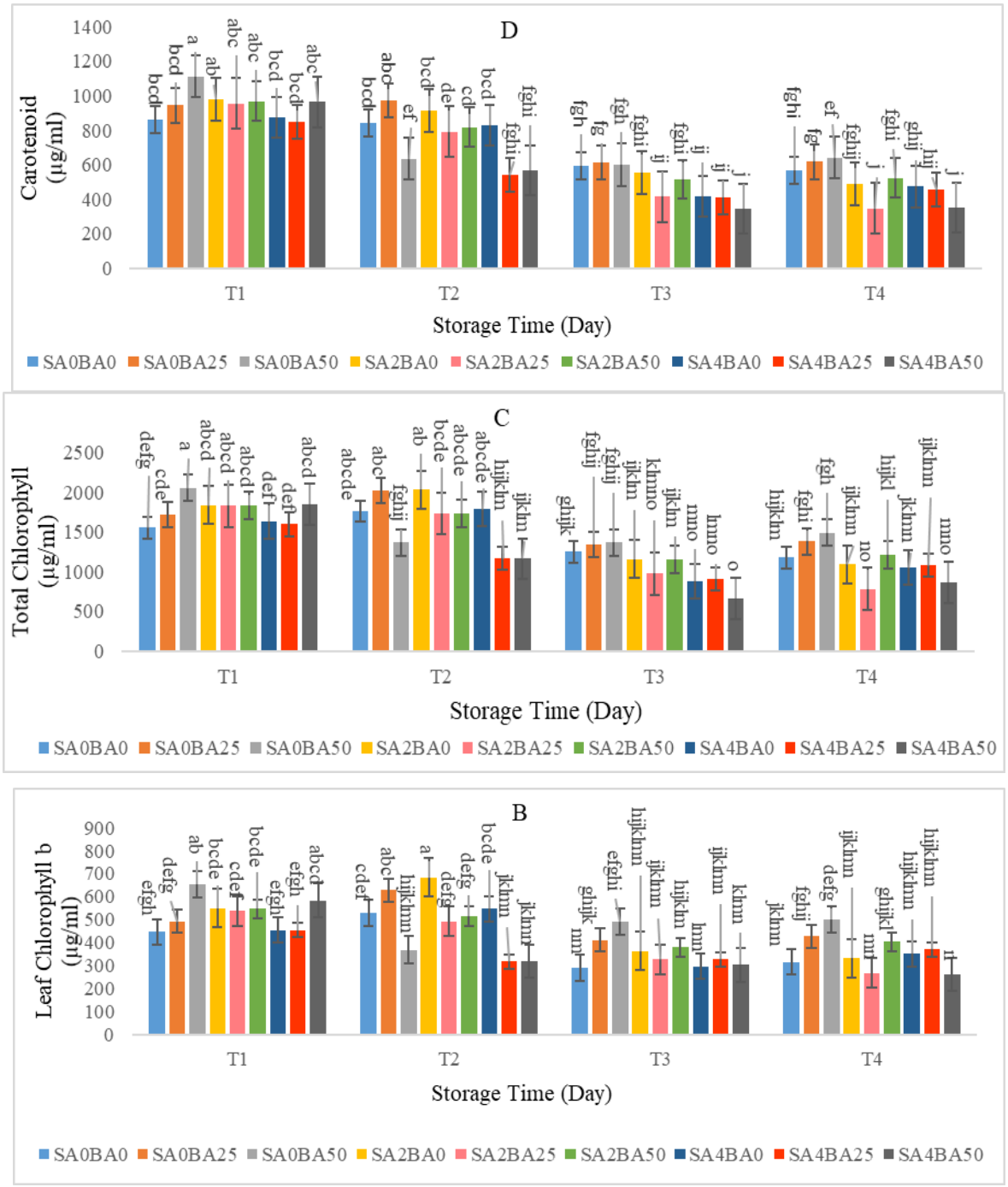

Figure 10. Triple interaction effects of salicylic acid, benzyl adenine, and storage time on leaf chlorophyll a (a) and triple interaction effects of salicylic acid, benzyl adenine, and storage time on leaf chlorophyll b (b), triple interaction effects of salicylic acid, benzyl adenine, and storage time on total leaf chlorophyll content (c), and the triple interaction effects of salicylic acid, benzyl adenine, and storage time on leaf carotenoid content (d) of carnation cut flowers at 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

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The bacterial population of preservative solution

The population of soluble bacteria in the simple effects of all three factors of salicylic acid, benzyl adenine, and storage time as well as their dual and triple interactions were significant at the significance level of 0.01 (Figure 11). The results of the experiments showed that the lowest amount of bacteria was observed in the SA2BA0T3 treatment, and thus caused more sterile conditions than other treatments. Following a review of the literature, the presence of microorganisms in the preservative solution of cut flowers can cause physical blockage of their vessels (Farokhzad *et al*., 2005; Heins *et al*., 1980). The lower rate of water uptake and transport in the stems of cut flowers is related to the obstruction of the woody vessel due to the accumulation of microbes at the base of the stem (Van Doorn, 2008). Obstruction can occur at the end of the stem and woody vessels by microbes, physiological blockage, and the presence of air in wooden vessels that cause water absorption or secretion of cellular enzymes and ultimately damage the vascular tubes (Damunupola *et al*., 2010). On the other hand, the presence of microorganisms in the preservative solution causes the production of endogenous ethylene and accelerates their senescence (Van Doorn, 2008). Therefore, treatments that reduce the population of microorganisms have a special value. Salicylic acid has antimicrobial effects and thus reduces microorganisms and increases solution absorption (Kazemi *et al*., 2011). Thus, increasing the water uptake of cut flowers treated with salicylic acid may be associated with the antimicrobial properties of this compound (Da Rocha Neto *et al*. 2015).

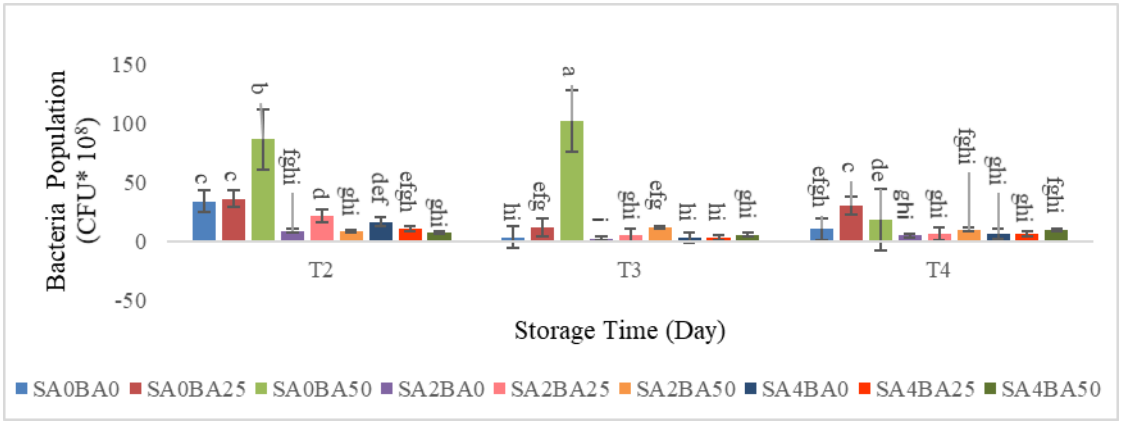

Figure 11. Dual and triple interaction effects of salicylic acid, benzyl adenine, and storage time on the bacterial population in carnation cut flowers at 4±1 °C. The storage time: day 1 (T1), day 12 (T2), day 24 (T3), and day 36 (T4). Salicylic acid was applied at three concentrations: 0 (Sa0), 2 mM (Sa2), and 4 mM (Sa4). Benzyl adenine was applied at three concentrations: 0 (BA0), 25 mg L^{-1} (BA25), and 50 mg L^{-1} (BA50). Distinct letters represent statistically significant differences by the Duncan test ($P \leq 0.05$) Bars represent SE.

Conclusion

The data in this study confirmed that benzyl adenine and salicylic acid can improve the post-harvest vase life and the related parameters of carnation cut flowers. Important parameters such as photosynthetic pigments, the change in which affects the metabolism, and different responses of the plant to the conditions, were improved under the studied treatments. The studied morphological, physiological, and biochemical traits were improved by salicylic acid and benzyl adenine treatments as effective compounds at the cellular level and beyond and played an important role in the resistance of carnation cut flowers over time and under cold storage. The results indicate that the treatment of benzyl adenine at a concentration of 25 mg L^{-1} increased the flower life by increasing the sugar content and minimizing the reduction of flower and stem diameter. Benzyl adenine at a concentration of 25 and 50 mg L^{-1} also increased photosynthetic pigments. Regarding the parameter of solution uptake and

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relative fresh weight of flowers, 4 mM salicylic acid treatment caused the highest uptake and highest weight. The interaction of salicylic acid and benzyl adenine treatments effectively reduced the vase solution's pH and bacterial population. According to the current study results, it seems that the best treatments in our study are 25 mg L^{-1} benzyl adenine to increase morphological and physiological parameters, 2 mM salicylic acid in antioxidant properties and 4 mM salicylic acid to reduce the vase bacterial population. However, it should be noted that the results of each experiment were dependent on specific conditions and cultivars and may not be generally recommended for other flowers. The existence of very complex mechanisms associated with hormones and plant growth regulators has caused many obstacles to applying scientific findings to the ornamental plant industry. The effect induced by salicylic acid depends on the concentration of the substance used. According to most studies, the beneficial effects of plant regulators depend on various parameters such as cultivar, harvest season, type of regulator, and applied concentration. Thus, for any ambiguous issue, an accurate experiment need must be designed and implemented.

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