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The Interactive Effect of Sodium Benzoate and Ethanol on the Vase Life of Cut Roses cv. 'Avalanche'

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Abstract

The vase life of cut roses is decreased by ethylene and water stress. Ethanol and sodium benzoate are among the antimicrobial and anti-ethylene compounds that are applied to vase solutions. This research investigated the effect of different rates of ethanol (0, 2, 4, and 6 %) and sodium benzoate (0, 150, 200, and 250 mg l^{-1}) on the vase life of cut roses cv. 'Avalanche' in a factorial experiment based on a randomized complete block design with 16 treatments and 3 replications. The experimental treatments were applied as a 24-hour pulse. Distilled water was used as the control. The results showed the significant interactive effect of "ethanol × sodium benzoate" on improving vase life and other studied traits. The longest vase life (13 days), water uptake (3.54 ml g^{-1} F.W.), dry matter (44.38 %), petal protein (35.08 %), and total chlorophyll (13.09 mg g^{-1} F.W.) were obtained from the application of "ethanol 4% × 150 mg l^{-1} sodium benzoate". This treatment also exhibited the lowest fresh weight loss (1.98%), stem-end bacteria population (8.33 Log_{10} CFU ml), vase solution bacteria population (23.33 Log_{10} CFU ml), and ethylene synthesis (0.4 nl l^{-1} h^{-1} g^{-1} F.W.). According to the results, it is recommended to apply "ethanol 4 % × 150 mg l^{-1} sodium benzoate" to preserve the quality and extend the vase life of cut roses cv. 'Avalanche'.

Keywords: Climacteric flower, Ethylene, Postharvest aging, Vascular blockage, Vase solution.

INTRODUCTION

After cut flowers are excised from their material plants, they are exposed to many physiological and pathological issues and stresses, such as xylem blockage by microorganisms and air, water stress, and ethylene sensitivity, which directly influence their longevity and postharvest quality. Various guidelines and techniques have long been proposed for preserving the longevity and commercial value of cut flowers during the marketing period. One common technique is the use of preservatives or longevity-extending solutions. In addition to supplying the energy requirement of cut flowers for their survival, vase solutions contribute to preserving their freshness and extending their longevity by reducing stem-end microbial load, sustaining water uptake, and suppressing ethylene in climacteric flowers (Gebremedhin, 2020; Nguyen *et al*., 2022).

Ethylene is a volatile chemical compound known as a safe and environmentally-friendly substance and used in different industries. Research has established that ethanol is a proper compound for preserving the postharvest quality of horticultural produce (Lin *et al*., 2020). Ethanol is an anti-ethylene and antimicrobial compound whose application in vase solutions of cut flowers extends their postharvest longevity (Sadeghi Hafshejani and Hashemabadi, 2016; Yaghoubi Kiaseh and Yadegari, 2016). The positive effect of ethanol on maintaining postharvest quality and freshness has been reported for tomatoes, apples, cut petunia (Lin *et al*., 2020), and cut *Alstroemeria* (Sadeghi Hafshejani and Hashemabadi, 2016; Yaghoubi Kiaseh and Yadegari, 2016).

Sodium benzoate is a white, odorless, water-soluble salty solution that is safe for humans. This compound is extensively used in food, pharmaceutical, cosmetic, and health industries and is regarded as the first preservative permitted for food products. Sodium benzoate is an antifungal, anti-yeast, and antibacterial compound that performs much better in acidic pH. In addition to its antimicrobial activity, sodium benzoate is used as a growth regulator in agriculture (Kaur *et al*., 2019; Liang *et al*., 2020). Kella *et al*. (2018) report that as a strong antimicrobial compound, sodium benzoate reduces the activity of microorganisms in the vase solutions of cut flowers. There are reports about the positive effect of sodium benzoate on the postharvest longevity of pears (Kaur *et al*., 2019) and cut *Limonium sinuatum* (Kella *et al*., 2018) and roses (Ketsa and Sribunma., 1985).

The postharvest longevity of cut roses is limited by ethylene and water stress (Ha and In, 2022). Given the proven anti-ethylene and antimicrobial effects of ethanol and sodium benzoate, the present research aims to investigate the interactive effects of these two compounds on the vase life of cut roses cv. 'Avalanche'.

MATERIALS AND METHODS

To study the interactive effect of ethanol and sodium benzoate on the vase life and some related traits of cut roses cv. 'Avalanche', a factorial experiment was conducted based on a randomized complete block design with three replications. The cut flowers were purchased from a commercial greenhouse in Amol, Iran when their flowers were half-open and were transferred to the study site in commercial packages. In the laboratory, the flowers were cut short to a length of 60 cm and the lower leaves of the stems were removed. Then, 2 cm from the stem end was re-cut under tap water to prevent vascular blockage. In the next step, the flowers were treated with different levels of ethanol (0, 2, 5, and 6%) and sodium benzoate (0, 150, 200, and 250 mg l^{-1}) for 24 hours (pulse treatment). Distilled water was used as the control. After the pulse treatments, the cut flowers were kept in vases containing 500 ml of 8-hydroxy quinoline sulfate and sucrose 3% until the experiment was ended. The experiment was conducted in a controlled room at a temperature of 20 ± 2 °C, relative humidity of 60-70 percent, and a daylight duration of 12 hours with a light intensity of 15 µmol m^{-2} s^{-1}.

Assessment of traits

The vase life was calculated by counting the number of days from the initiation of the treatments until neck bending and the withering of two-thirds of the petals in the rose (Seyf *et al*., 2012; Farazmandi *et al*., 2020). Solution uptake was obtained from the following equation:

$$\text{Solution uptake (ml/g F.W.)} = \frac{V_{t0}-(E_t+V_{t1})}{\text{F.W.}}$$

in which V_{t0} represents the initial solution volume, V_{t1} represents the final-day solution volume, E_t represents the final amount of evaporation from the solution surface, and F.W. represents the cut flower's fresh weight on the first day.

Fresh weight loss was calculated by the following equation:

Fresh weight loss (g) = First-day fresh weight - (Last-day fresh weight + Weight of re-cuts)

Dry matter (%) was calculated at the end of the vase life by the following equation:

$$\text{Dry weight (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Vase solution and stem-end bacteria were estimated by Liu *et al*.'s (2009) procedure for which 24 hours after the application of the pulse treatments. Total protein contents were calculated by the Kjeldahl indirect method and the following equation:

$$\text{Nitrogen(\%)} = 0.56 \times T \times (A-B) \times \frac{V}{W} \times \frac{100}{DM}$$

in which T represents the concentration of the acid used for titration (mol L^{-1}), A represents the quantity of the acid used for the sample (ml), B represents the quantity of the acid used for the control (ml), V represents the volume of the extract derived from digestion (ml), W represents the plant sample weight for digestion (g), and DM represents plant dry matter.

$$\text{Total protein (\%)} = \text{Nitrogen} \times 6.25$$

To measure ethylene production, 24 hours after the application of the pulse treatments, the amount of ethylene synthesized was measured by the gas chromatography method. The ethylene content of the samples was measured with a Shimadzu gas-chromatography device (Japan) and reported in nl l^{-1} h^{-1} g^{-1} F.W.

To determine total chlorophyll, 0.5 g of the leaves were extracted with 80% acetone. Then, the absorbance of the resulting samples was read at 642 and 660 nm with a spectrophotometer (Shimadzu UV-120-02, Japan). Afterward, the following equation was used to calculate chlorophyll content in mg g^{-1} F.W. (Mazumdar and Majumder, 2003):

$$\text{Total chlorophyll} = 7.12\,(A_{660}) + 16.8\,(A_{642})$$

Data analysis

The data were analyzed in the SPSS 19 statistical software package. The means were, also, compared by the LSD test at the $P < 0.05$ level.

RESULTS

Vase life

Vase life is the most important index in assessing the quality and commercial value of cut flowers. As is evident in Fig. 1, the combined application of "ethanol × sodium benzoate"

increased longevity versus the control flowers whose vase life was 9.16 days. The treatments E4 × B150, E6 × B200, and E4 × B200 exhibited the longest postharvest longevities of 13, 12.65, and 12.5 days, respectively, showing an improvement of over 3 days compared to the control (Fig. 1).

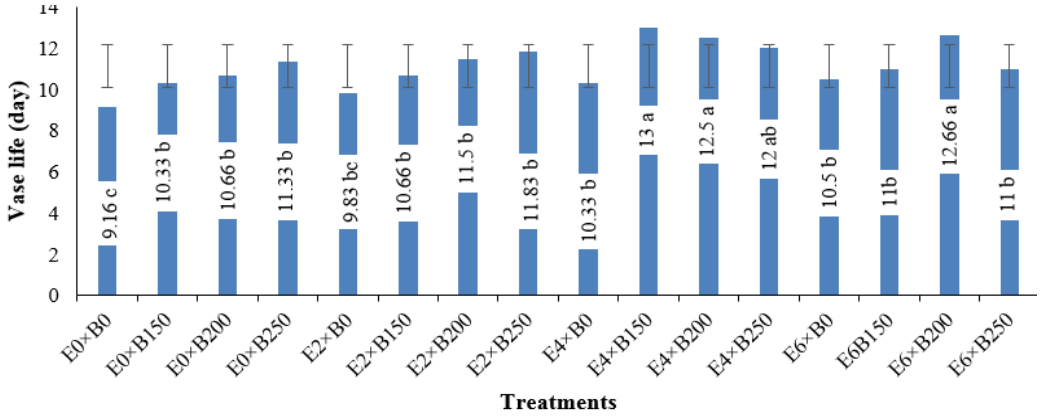

Fig. 1. The interactive effects of "ethanol × sodium benzoate" on the vase life of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

Water uptake

Postharvest longevity had a direct relationship with the water uptake of the cut flowers. The research revealed the positive effect of "ethanol × sodium benzoate" on the water uptake so that all treatments increased this trait versus the control (2 mg g^{-1} F.W.). The highest water uptake was obtained from the application of E4 × B150 (3.54 ml g^{-1} F.W.) and E6 × B200 (3.49 ml g^{-1} F.W.), so they were the best treatments for preserving and increasing the water uptake (Fig. 2).

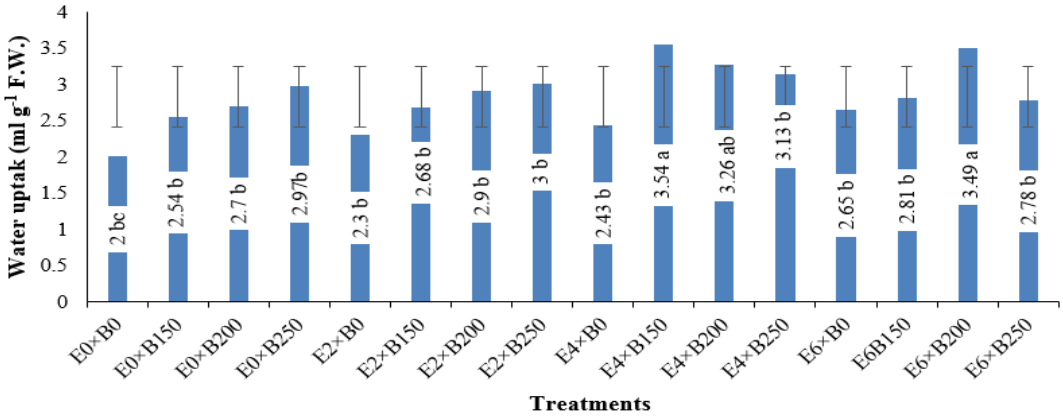

Fig. 2. The interactive effects of "ethanol × sodium benzoate" on the water uptake of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

Fresh weight loss

Fresh weight loss signals senescence and the end of cut flowers' vase lives. Fig. 3 depicts that the combined application of "ethanol × sodium benzoate" was effective in maintaining the fresh weight of the cut flowers. The treatment of E4 × B150 was related to the lowest and the control to the highest fresh weight losses of 1.98 and 9.59 g, respectively (Fig. 3). Since E4 × B150 exhibited the highest water uptake, the preservation of fresh weight and the extension of vase life with this treatment were quite expected, as recorded in the present work.

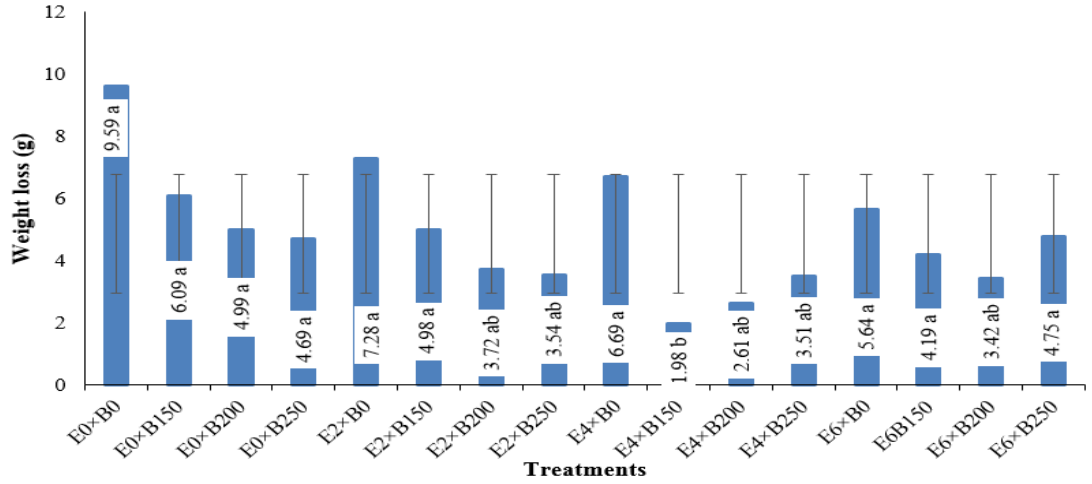

Fig. 3. The interactive effects of "ethanol × sodium benzoate" on the fresh weight loss of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

Dry matter percentage

According to Fig. 4, the treatment of the cut roses with "ethanol × sodium benzoate" influenced dry matter preservation positively. As is observed, all treatments outperformed the control (25.47%) in dry matter. The highest dry matter was observed in the flowers treated with E4 × B150 (44.38%) and those treated with E6 × B200 (40.74%) (Fig. 4).

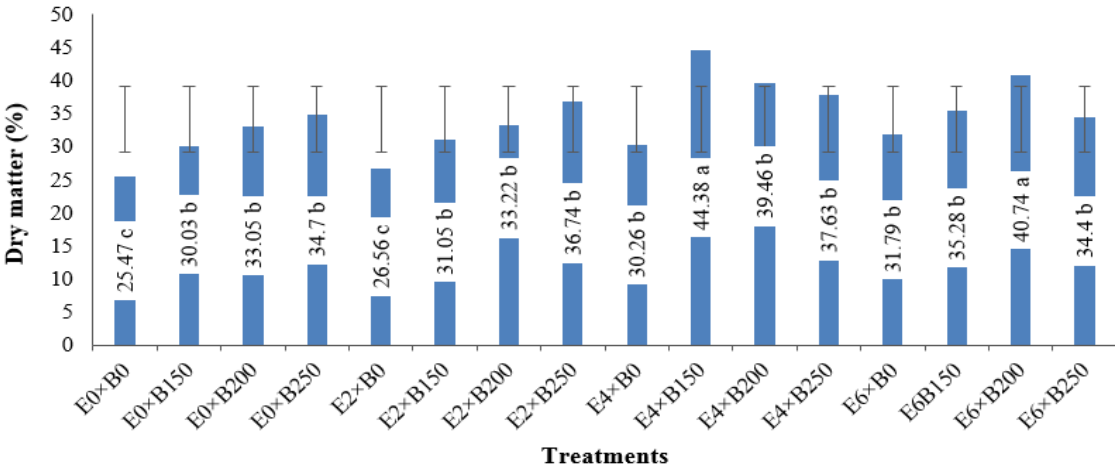

Fig. 4. The interactive effects of "ethanol × sodium benzoate" on the dry matter of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

Vase solution and stem-end bacterial population

Microorganisms are among the main causes of vascular blockage and the induction of early wilting in cut flowers. The application of disinfectants in the present research reduced vase solution and stem-end bacterial population significantly. The best treatment for reducing the vase solution bacterial population was E4 × B150 (23.33 Log_{10} CFU ml^{-1}). It reduced the vase solution bacterial population significantly versus the control (71.66 Log_{10} CFU ml^{-1}) (Fig. 5). The lowest and highest stem-end bacterial populations were related to E4 × B150 (8.33 Log_{10} CFU ml^{-1}) and control (45 Log_{10} CFU ml^{-1}), respectively. This shows the positive effect of ethanol and sodium benzoate in reducing vase solution and stem-end microbial load (Fig. 6).

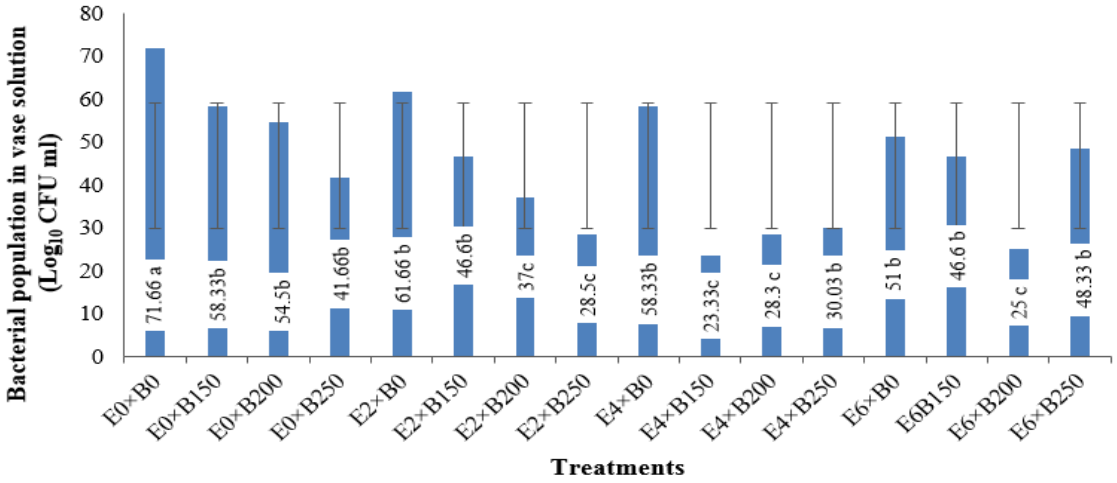

Fig. 5. The interactive effects of "ethanol × sodium benzoate" on the bacterial population in the vase solution of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

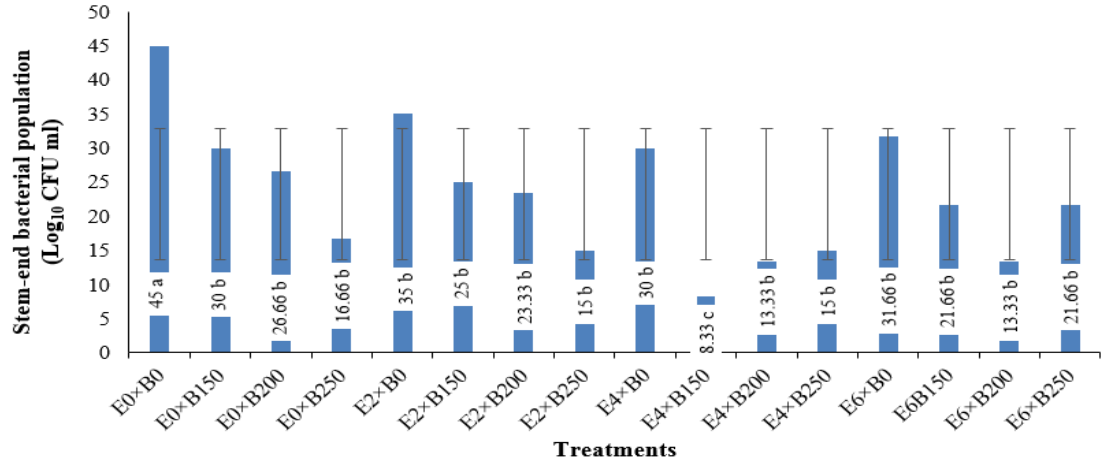

Fig. 6. The interactive effects of "ethanol × sodium benzoate" on the stem-end bacterial population of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

Ethylene

The ethylene sensitivity of cut flowers plays a key role in their early withering. The combined application of ethanol and sodium benzoate reduced ethylene production in the cut flowers of roses cv. 'Avalanche' versus the control (0.83 nl l^{-1} h^{-1} g^{-1} F.W.). The lowest ethylene level was related to the flowers treated with E4 × B150 (0.40 nl l^{-1} h^{-1} g^{-1} F.W.) and E4 × B200 (0.50 nl l^{-1} h^{-1} g^{-1} F.W.), which are the most successful treatments in suppressing ethylene synthesis (Fig. 7).

Petal protein content

Fig. 8 displays the positive effect of "ethanol × sodium benzoate" on preserving and increasing petal protein. It is observed that the control had the lowest petal protein content (9.84%) whereas the highest was obtained from the treatments of E4 × B150, E4 × B200, and E6 × B200 whose petal protein contents were 35.08%, 30.86%, and 30.44%, respectively (Fig. 8).

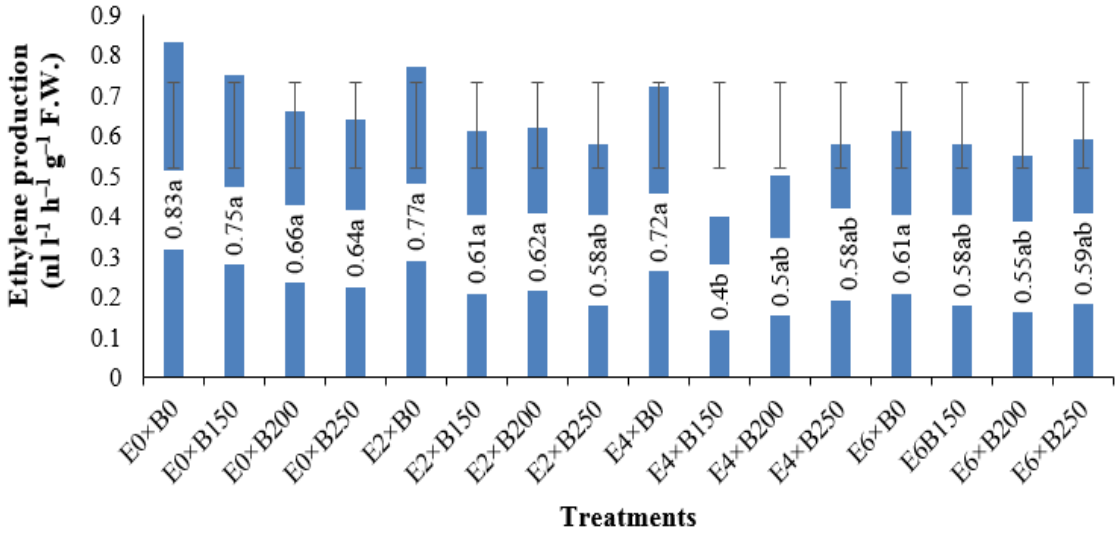

Fig. 7. The interactive effects of "ethanol × sodium benzoate" on ethylene synthesis by cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

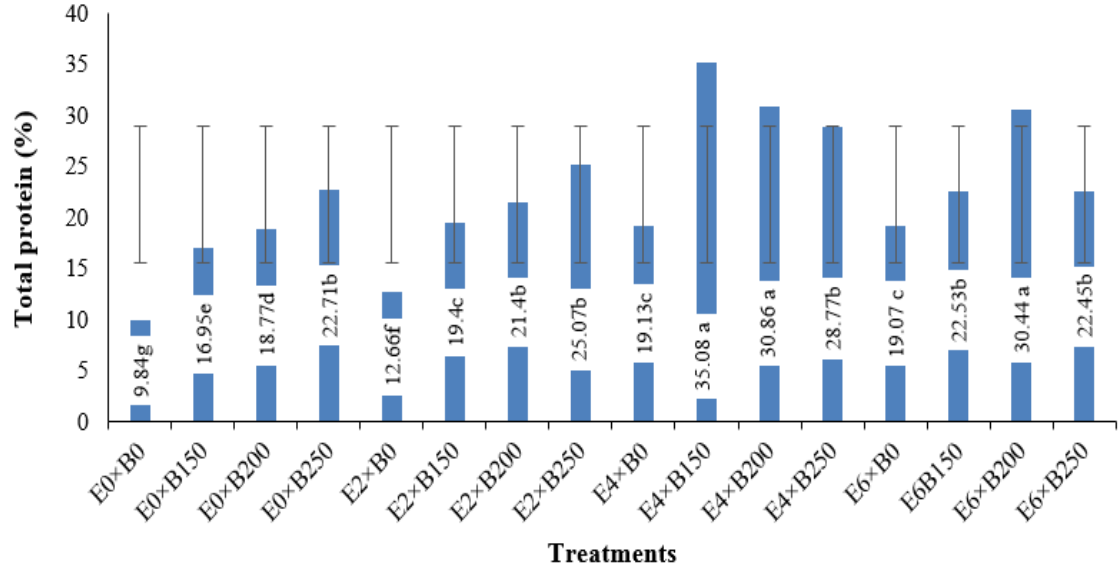

Fig. 8. The interactive effects of "ethanol × sodium benzoate" on total protein content of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

Total chlorophyll content

The interaction of "ethanol × sodium benzoate" inhibited chlorophyll degradation in the cut roses. The control had the lowest chlorophyll content (3.44 mg g^{-1} F.W.) among all studied treatments. The highest total chlorophyll content was obtained from the plants treated with E4 × B150 (13.09 mg g^{-1} F.W.), E4 × B200 (10.00 mg g^{-1} F.W.), and E6 × B150 (9.58 mg g^{-1} F.W.) (Fig. 9).

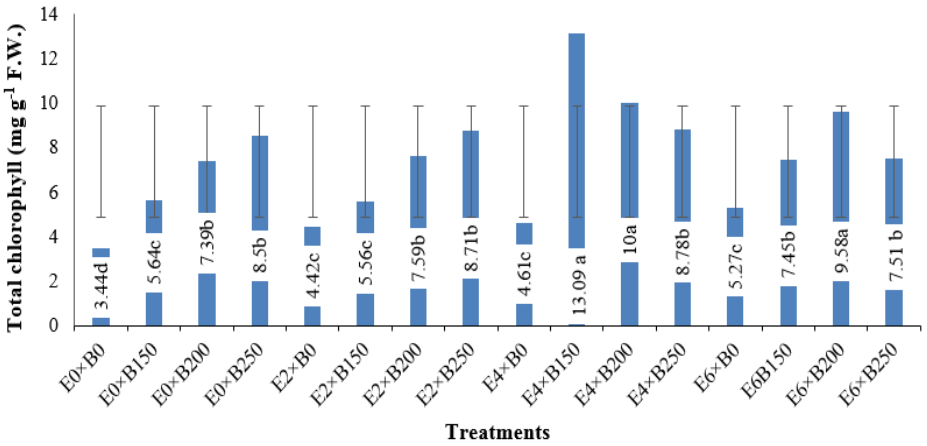

Fig. 9. The interactive effects of "ethanol × sodium benzoate" on total chlorophyll content of cut roses cv. "Avalanche". E0, E2, E4 and E6 represent 0, 2, 4, and 6 percent ethanol, and B0, B150, B200, and B250 represent 0, 150, 200, and 250 mg l^{-1} sodium benzoate, respectively.

DISCUSSION

The rose is one of the most important cut flowers in the world which has short postharvest longevity like most other cut flowers. Water stress and ethylene sensitivity are two factors limiting the postharvest longevity of cut roses. Various methods have been proposed to alleviate the adverse impacts of ethylene and water stress on the postharvest quality of cut flowers. One of the most common methods is the application of vase solutions containing anti-ethylene and antimicrobial compounds. These solutions extend vase life by preserving water uptake and reducing ethylene synthesis (Ha and In, 2022). In the present work, the combined application of ethanol and sodium benzoate as antimicrobial and anti-ethylene compounds contributed to preserving water uptake and fresh weight and finally extending the postharvest longevity of the cut roses cv. 'Avalanche' by reducing ethylene synthesis and vase solution and stem-end microbial population.

The propagation of bacteria and fungal agents at the stem end of cut flowers blocks their xylems and disrupts water uptake. It has been reported that many bacterial species stimulate endogenous ethylene, thereby accelerating the aging process in cut flowers (Manzoor *et al.*, 2022; Nguyen *et al.*, 2022). Ethylene has an antimicrobial property. In addition, it disrupts the activity of the enzyme ACC-synthase, thereby reducing the detrimental effect of ethylene, so it acts as a senescence-inhibiting factor in cut flowers (Hossain *et al.*, 2007). Sodium benzoate has antimicrobial activity against a wide range of microorganisms and has also anti-ethylene activity (Mayak *et al.*, 1977). Reportedly, sodium benzoate interferes with the activity of microorganisms by penetrating through their cell membranes. After entering the cells of bacteria or other microorganisms, the molecules of sodium benzoate reduce their pH, restrain the activity of respiratory enzymes and the reaction of the density of acetyl coenzyme A, and disrupt the performance of the bacteria or fungi (Liang *et al.*, 2020). It can, therefore, be said that ethanol and sodium benzoate extend postharvest longevity of cut flowers by a two-fold effect, i.e., reducing microbial population and reducing ethylene sensitivity.

Oraee *et al.* (2011) reported that the treatment of cut gerberas with 250 mg l^{-1} sodium benzoate increased vase life, water uptake, and fresh weight significantly versus the control. There are also reports as to the positive effect of sodium benzoate on postharvest longevity and quality of cut roses cv. 'Cardinal' and 'Whisky Mac' (Younis *et al.*, 2006) and lilies (Yuhua *et al.*, 2008), reflecting the strong anti-microbial and anti-ethylene activity of sodium benzoate in improving postharvest longevity.

We observed that ethanol and sodium benzoate were significantly effective in improving

dry matter, protein, and total chlorophyll positively. The inhibition of chlorophyllase activity contributes to preserving chlorophyll in plant tissues (Ferrant *et al*., 2002). It has been reported that ethanol disrupts chlorophyllase activity by suppressing ethylene synthesis, thereby preserving the structure of chloroplast and chlorophyll (Yaghoubi Kiaseh and Yadegari, 2016). The positive effect of ethanol has been reported on preserving chlorophyll in the leaves of *Alstroemeria* (Mousavi Bazaz and Tehranifar, 2011) and Bougainvillea (Hossain *et al*., 2007).

Yaghoubi Kiaseh and Yadegri (2016) argue that the positive effect of ethanol on preserving dry matter and carbohydrates in cut *Alstroemeria* is associated with its effect on reducing respiration, ethylene synthesis, and microbial population and preserving water uptake. The reports as to the positive effect of ethanol on preserving water uptake, fresh weight, dry matter, reducing ethylene synthesis, and extending vase life of *Alstroemeria* (Sadeghi Hafshejani and Hashemabadi, 2006), carnation (Karimian Fariman and Tehranifar, 2011), and chrysanthemums (Petridou *et al*., 2001) and the positive effect of sodium benzoate in extending vase life and preserving fresh weight of cut roses, gerberas, and carnation (Yuhua *et al*., 2009; Bia *et al*., 2007) support our findings.

CONCLUSIONS

According to the results, the combined application of "ethanol × sodium benzoate" influenced all traits positively. Among different levels of ethanol and sodium benzoate, "ethanol 4% × 150 mg l^{-1} sodium benzoate" was the strongest treatment in extending vase life. In fact, this treatment increased water uptake, preserved fresh weight, and decreased ethylene synthesis by reducing vase solution and stem-end microbial population, thereby prolonging vase life, the most important studied trait, versus the control by over 3 days. Therefore, this treatment is recommended for improving postharvest longevity of cut roses cv. 'Avalanche'.

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