



Extending the vase life of rose cut flower cv. Bakara using inhibitors of physiological vascular occlusion

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ABSTRACT

Purpose: Rose cut flowers have a short postharvest life, which can be increased using different treatments. Thus, an experiment was designed to determine the effect of hot water (one min) and chemical solutions (pulse treatment for 20 h) on the postharvest quality of cut rose flowers cv. Baraka. **Research Method:** Hot water treatments contain 50, 55, and 60 °C, chemical treatments consist of catechol (5 and 10 mM), sodium azide (0.05 and 0.1 mM) and sodium metabisulfite (5 and 10 mM). Afterward, for the evaluation of associated traits with longevity, the flowers were kept in a vase solution containing sucrose (3%) and hydroxyquinoline (8-HQ at 200 mg. L⁻¹). **Findings:** The results showed that the vase life of cut flowers extended about four days by the application of chemical treatments as compared with control. The maximum vase life (9.9 days) observed in 0.05 mM sodium azide and 10 mM catechol (9.7 days). Also, the vase life of cut flowers increased 3.7 days by hot water treatments (at 50 and 55 °C) in comparison to the control. Moreover, the results revealed that the catechol, sodium azide and sodium metabisulfite treatments delayed flower senescence and maintained leaf chlorophyll and petal anthocyanin content. The lowest content of lignin was obtained in 10 mM catechol, and 0.1 mM sodium azide. **Research limitations:** There was no significant limitation to the report. **Originality/Value:** Overall, the results showed that catechol and sodium azide were the most effective treatments to increase the vase life of rose cut flowers.

INTRODUCTION

In recent decades, many advances have been made in the management and post-harvest processes of horticultural products, especially cut flowers. The necessity of proper and timely marketing, as well as the supply of products in global markets, is influential factors with great contribution to the development of new and appropriate methods of packaging, storage and post-harvest physiology of cut flowers (Reid & Jiang, 2012).

One of the most important goals after harvesting of cut flowers is to provide solutions for increasing the postharvest quality, through which the floriculture industry can deliver more attractive flowers with more longevity to consumers (Scariot et al., 2014). Roses are the most high-demand cut flower in the world (Fanourakis et al., 2013). The vase life rose cut flower is relatively short due to water loss (Ichimura et al., 2006; Hassan et al., 2014). For short vase life of this flower, various causes have been noted, such as stem end occlusion by microbes, physiological occlusion caused by wound and the presence of air in the xylem vessels (Fanourakis et al., 2013). The occlusion of the vessels causes the synthesis of wound healing compounds and insufficient water uptake by the stem which can destroys the vascular tissue (Damunupola et al., 2010). Water stress is the main reason for senescence of rose, which causes early petal wilt, disturbance in bud opening, shoots and leaves wilt, as well as bending of the stem (Ahmad et al., 2011). Bolla et al. (2010) reported that water stress in cut roses reduced the content of leaf chlorophyll. Recently, many efforts have been made to increase the vase life and maintain the quality of cut flowers using various techniques. Today, the application of thermal treatments is used in a wide range of products to enhance postharvest quality. Thermal treatment is carried out in the form of hot steam, hot air and hot water (Lurie & Pedreschi, 2014). Woolf et al. (2012) reported that hot water treatment at 50 °C for 5 min or 52.5 °C for 2.5 min effectively reduced leaf yellowing and increased the vase life of *Lilium* cut flower.

On the other hand, the use of chemicals is one of the strategies developed along with other methods. Wounding in the plant tissue activates the mechanisms for healing wounds. The deposition of substances such as suberin, lignin, and tannin in the xylem is a physiological response to the wound healing (Van Doorn & Vaslier, 2002). In this regard, the peroxidase enzymes by binding hydroxyl groups to phenolic compounds, play a vital role in the biosynthesis of lignin and suberin. The enzymes of the peroxidase group are present in all plant organs and are involved in many growth processes, including cell wall development, lignin synthesis, and auxin catabolism (Vanholme et al., 2010). Chemical compounds, including catechol, sodium metabisulphite, and sodium azide act as inhibitors of peroxidase, phenoloxidase and laccase enzymes, respectively (Celikel et al., 2011; Patel et al., 2014). In recent studies, the use of these inhibitors in the vase solution of various cut flowers such as lisianthus (Sharifzadeh et al., 2014), gerbera (Gerabeygi, 2018; Ghafouriyan et al., 2019) and acacia (Celikel et al., 2011) has had reported promising results to increase the vase life. Application of catechol as a vase solution for lisianthus showed that it significantly improved water uptake and relative fresh weight, also increased the vase life of the cut flower. Among these concentrations, catechol 10mM was more effective than other treatments and increased its vase life by about 5 days compared to control (Sharifzadeh et al., 2014). In evaluating the effect of catechol and 8-HQC on the maintenance of the quality of gerbera flower, it was determined that the application of 1 mM catechol reduces blockage of stems and bacterial growth in the vase solution and increases the vase life (Wang et al., 2014). The treatment of *Acacia holosericea* with peroxidase inhibitors such as catechol, p-phenylenediamine and copper sulfate had a positive effect on solution uptake and fresh weight of cut stem (Celikel et al., 2011). According to what was stated, the present study was conducted to evaluate the role

of anti-wound healing treatments in control of physiological vascular occlusion and postharvest quality of the rose cut flower.

MATERIALS AND METHODS

Plant materials and inhibitor treatments

Rose cut flowers were harvested in the bud stage when the sepals were separated from each other and completely turned back (Yamada et al., 2009). The harvest of cut flowers was carried out in the early morning hours from a commercial greenhouse in Pakdasht, Tehran, Iran. The cut flowers, with suitable packaging, were transported dry to the laboratory of the Horticultural Sciences in the shortest possible time. In the laboratory, the cut flowers were re-cut to 40 cm long under distilled water and then subjected to different treatments. In this experiment, 12 treatments including control, sucrose (3%), 8-Hydroxyquinoline (8-HQ, 200 mg. L⁻¹), catechol (5 and 10 mM), sodium azide (0.05 and 0.1 mM), sodium metabisulfite (5 and 10 mM) and hot water (50, 55, and 60 °C) were used. Pulse treatment with chemicals was applied for 20 h. For hot water treatments, the base of the cut stem was exposed to hot water for one minute. After applying the treatments, the flowers were transferred to flasks contain distilled water, sucrose and 8-HQ and kept at the room with a temperature of 20±2 °C, 60% humidity and 12 hours light period. The vase solution was replaced with a fresh solution every two days.

Vase life and flower diameter

To evaluate the vase life, the placement time of flowers in preservative solutions was considered until the onset of their aging symptoms. Observation of symptoms such as wilting, withering, color change of petals, petal abscission, bent neck and similar symptoms that lead to poor marketability of flowers were used to evaluate the end of the longevity of cut flowers. On this basis, once these symptoms were observed in each flower, that day was considered as the vase life end (Jiang et al., 2015).

Flower diameter was measured daily using a caliper (Guanglu Digital Caliper, 300 mm, resolution 0.01 mm). The change in the flower diameter during vase life was defined as the percentage of a difference to the initial flower diameter (Jowkar et al., 2013).

Relative fresh weight and solution uptake

The relative fresh weight and solution uptake were measured daily and up to the ninth day. Relative fresh weight (RFW) of cut stems was calculated using the formula $RFW (\%) = (W_t/W_0) \times 100$, where, W_t is the weight of cut stem (g) at $t = \text{day } 0, 1, 2, \dots$, and W_0 is the weight of the same cut stem (g) at $t = \text{day } 0$. Solution uptake (ml/g FW) was calculated using the formula $(S_{t-1} - S_t)$, where, S_t is the volume of vase solution (ml) at $t = \text{day } 1, 2, 3, \dots$, and S_{t-1} is the solution volume of the previous day. FW is the fresh weight of cut stem (g) at $t = \text{day } 1, 2, 3, \dots$ (He et al., 2006).

Chlorophyll and anthocyanin content

Pigments measurement was performed every three days for each treatment. To measure the leaf chlorophyll, 5 ml acetone 80% was used as a solvent (Lichtenthaler & Buschmann, 2001). To measure the anthocyanin content in petals by the pH difference method, 3 ml of acidic methanol (1% HCl in methanol) was used as a solvent (Giusti & Wrolstad, 2001).

Lignin content

Measurement of lignin content at the base of cut stems (0.2 g) was performed using [Schenk and Schikora \(2015\)](#) method during three consecutive days. The standard curve of lignin was prepared by dissolving various concentrations of lignin in 0.5 M sodium hydroxide and the soluble lignin content was calculated in mg g⁻¹ FW.

Statistical analysis

The experiment was conducted in a completely randomized design with five replications. Statistical analysis of data was performed using SAS program (version 9.1) and the mean comparison was done using Duncan's multiple range test at a probability level of 0.05.

RESULTS AND DISCUSSION

Vase life

The results showed that all treatments had a significant effect on increasing the vase life of rose cut flowers. The highest vase life was observed in 0.05 mM sodium azide, which increased the vase life by 4.6 days compared to the control and did not have a significant difference with 0.1 mM sodium azide, 10 mM catechol and 5 mM sodium metabisulfite. The use of hot water treatment was effective in increasing the vase life of the cut flowers, and it was able to extend the vase life by 9 days, but the increase in temperature of water to 60 °C was not associated with an increase in the longevity of cut flowers ([Fig. 1](#)).

The longevity of cut rose increases with the use of treatments that preserve the water balance. The primary stress of water is one of the main reasons for decreasing the vase life of rose ([Jiang et al., 2015](#); [Fanourakis et al., 2013](#); [Fanourakis et al., 2016](#)). Furthermore, one of the reasons for the decrease of the vase life is vascular occlusion due to the biosynthesis of suberin, lignin and other wound healing compounds ([Ahmad et al., 2011](#)). According to the results, catechol, sodium azide and sodium metabisulfite, had a positive effect on vase life. It seems that the use of these compounds prevents vascular occlusion by reducing lignin formation so that the cut cross-section of the stem is not occluded and the stems can still absorb water solution, which has a significant effect on the vase life of cut flowers. On the other hand, there are several reasons for the positive impact of hot water treatment on the vase life of cut flowers. Among these reasons are the maintenance of chlorophyll by reducing the activity of chlorophyll degrading enzymes ([Woolf et al., 2012](#)), reducing the physical occlusion of the stem ([Ahmad et al., 2011](#)), reducing the activity of the ACC oxidase enzyme, and thus reducing ethylene production ([Fallik, 2004](#)). Our results are consistent with previous studies on the effect of sucrose, catechol and metabisulfite on the vase life of cut flowers ([Celikel et al., 2011](#); [Sharifzadeh et al., 2014](#)).

Lignin of the cut stem

The lignin content of the cut stems was significantly affected by different treatments. All chemical treatments prevented the increase of lignin content of stem end as compared with control. Regarding the amount of lignin formation, 0.1 mM sodium azide and 10 mM catechol treatments had the lowest content at the end of the stem, followed by 5 mM catechol and 0.05 mM sodium azide ([Fig. 2](#)). The results also showed that the use of the chemical compounds of metabisulfite and sucrose had less effect on the control of lignin. In the study of the effects of temperature treatments on the lignin content in roses, it was found that hot water at 50 °C and 55 °C reduced the lignin content of the stem end and prevented its increase compared to the control treatment. By increasing the water temperature to 60 °C, the ability to control the synthesis of lignin was lost and did not differ with the control treatment ([Fig. 2](#)). However,

the effect of temperature treatments on this trait was less than that of chemical treatments. The enzymes involved in the lignification process include peroxidase, polyphenol oxidase, catechol oxidase, and phenylalanine ammonia-lyase (Celikel et al., 2011). Peroxidase inhibitors delay the wilting of chrysanthemum and increase solution uptake, which may be due to the reduction of vascular occlusion (Van Doorn & Vaslier, 2002). Other studies also reported a positive role for peroxidase inhibitors in reducing stem occlusion (Van Doorn & Vaslier, 2002; Celikel et al., 2011).

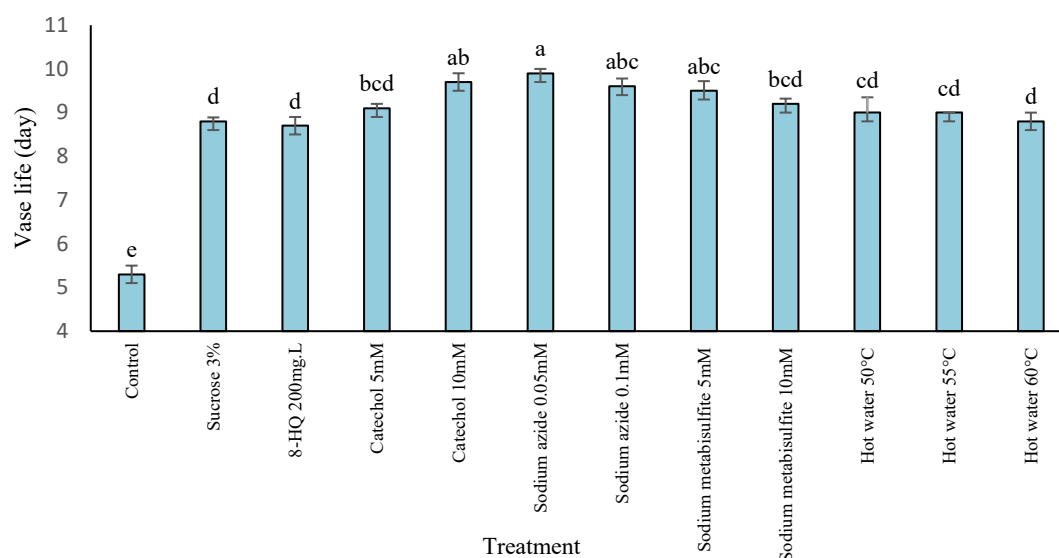


Fig. 1. Effect of different treatments on the vase life of rose cut flowers cv. Bakara. The same letters indicate no significant difference with Duncan's multiple range test at $P \leq 0.05$.

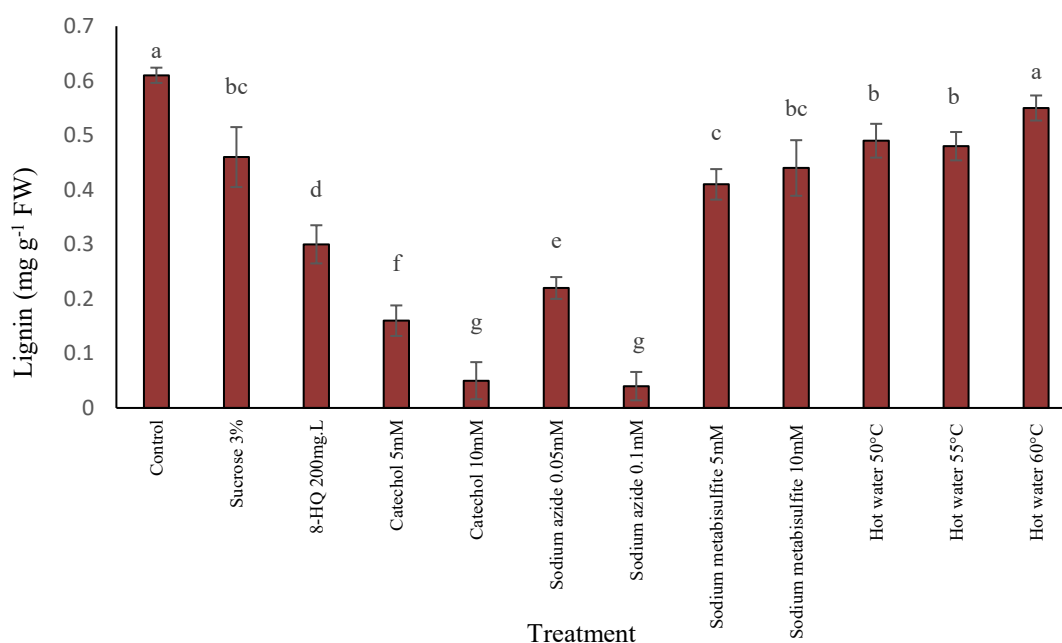


Fig. 2. Effect of different treatments on the lignin content of rose cut flowers cv. Bakara. The same letters indicate no significant difference with Duncan's multiple range test at $P \leq 0.05$.

Our results showed that catechol and sodium azide treatments had a significant effect on reducing the lignin content of rose stem end, which reduces vascular occlusion and preserves the process of water uptake and preservative solution. Laccase enzyme activity has been reported to stop at high temperatures (Palonen et al., 2003). High temperatures may have similar effects on other enzymes involved in the lignin biosynthesis pathway. Because these enzymes have a protein structure and high temperatures can destroy their structure. Therefore, the reduction of lignin observed in the present study due to temperature treatment is probably due to decreased activity of enzymes of the lignin biosynthesis pathway.

Flower diameter

The results showed that in most treatments, until the 6th day, flower diameter increased and then decreased. Among treatments, the 0.1 mM sodium azide and 50 °C hot water treatments had a greater effect (more than % 45) on the increase of flower diameter, on the sixth day (Fig. 3). In control treatment, flower diameter increased until the fourth day and then decreased.

Flower diameter in cut flowers is a measure for the opening rate of flower buds so that the flower diameter also increases until the anthesis stage. At the time of roses' opening, the accumulation of soluble carbohydrates such as fructose and glucose in the vacuole of petal cells reduces the osmotic potential in the symplast, which ultimately facilitate water absorption, cell development and full opening of the flower (Yamada et al., 2009). It seems that the treatments used in this study have a positive role in the process of cell development and bud opening. But this opportunity is not available for the flower bud in the control treatment. In et al. (2010) and Abri et al. (2014) reported the increase in the diameter of the rose in the middle days of the experiment, which is consistent with our results. Ghafouriyan et al. (2019) also showed that the highest diameter of flower in the gerbera cut flower was observed on the ninth day of vase life and in sodium azide treatment, which was consistent with the results of this research. Sodium azide may provide the energy necessary to expand the flower buds by facilitating absorption of the preservative solution. In this study, the vase solution contained 3% sucrose and 8-HQ during the experiment. Therefore, the 8-HQ continues to increase the cellular expansion of the petals by preventing the microbial occlusion of the stem and sucrose by providing the required energy (Norikoshi et al., 2016). In the present study, short-term temperature treatment increased the absorption of the solution by a cut flower, which may be effective in increasing the diameter of the flower.

Relative fresh weight of cut flowers and solution uptake

A comparison of mean of data showed that in most treatments, relative fresh weight decreased significantly over time. In the control treatment, the relative fresh weight of the flower was almost constant until the third day, but then significantly decreased (Fig. 4). On the 4th, 5th and 6th days, all treatments had a significant effect on the increase of this trait compared to the control. In the final days of the experiment (eighth and ninth), treatments of 0.05 mM sodium azide and 5 mM metabisulfite showed higher relative fresh weight than other treatments (Fig. 4).

Solution uptake of the flower until the 4th day had an increasing trend in most treatments and then there was a decrease in absorption. 0.1 mM sodium azide and 10 mM metabisulfite treatments had the greatest effect on increasing solution absorption, especially in the final days of the experiment (Fig. 5). The results of this study showed that hot water treatment, especially on seventh and eighth days, causes the maintenance of water absorption in rose cut flowers.

Flower fresh weight reduction is proportional to the increase in the number of days after the harvest due to water loss by various flower organs (In et al., 2010). Therefore, maintaining water in cut flowers is a fundamental issue for increasing the longevity of these products (Urban et al., 2002). Reducing the physiological occlusion of the stem by reducing the activity of the enzymes of laccase, phenoloxidase and peroxidase facilitates access to water (Sharifzadeh et al., 2014; Wang et al., 2014). Ghafouriyan et al. (2019) showed that the highest fresh weight in gerbera cut flowers in the final day of the experiment was related to 0.2 mM sodium azide treatment. On the other hand, the results showed that short-term temperature treatment increased the relative fresh weight in rose cut flowers. Short term temperature treatment can provide conditions for increasing the absorption of the vase solution due to the positive role in controlling the growth of microorganisms (Hara, 2012).

Moreover, the results indicated that the temperature treatment was effective in preventing an increase in lignin content (Fig. 2). This treatment may have an effect on the synthesis of lignin in the stem end to provide the conditions for water absorption and, consequently, a relative increase in fresh weight in flower. Reducing water absorption is an indication of the senescence of the flower. The water absorption rate in cut flowers depends on the conductivity of the stem water and the potential difference between the water content of the tissue and the preservative solution (Van Meeteren & Van Gelder, 1999). Therefore, factors that can prevent stem vascular occlusion can maintain the potential for absorbing the solution by cut flowers.

Leaf chlorophyll content

Over time, the chlorophyll content of the leaves in most treatments decreased, indicating the degradation of this pigment in the leaves. According to results, the treatments used preserve chlorophyll content compared to the control and reduced its degradation during the experiment. On day 6, the highest chlorophyll content ($10.24 \text{ mg g}^{-1} \text{ FW}$) was observed in 0.1 mM sodium azide and the lowest ($4.15 \text{ mg g}^{-1} \text{ FW}$) in control treatment. The results also showed that the lowest chlorophyll contents were obtained in treatments of 60°C on the final day of the experiment (Fig. 6).

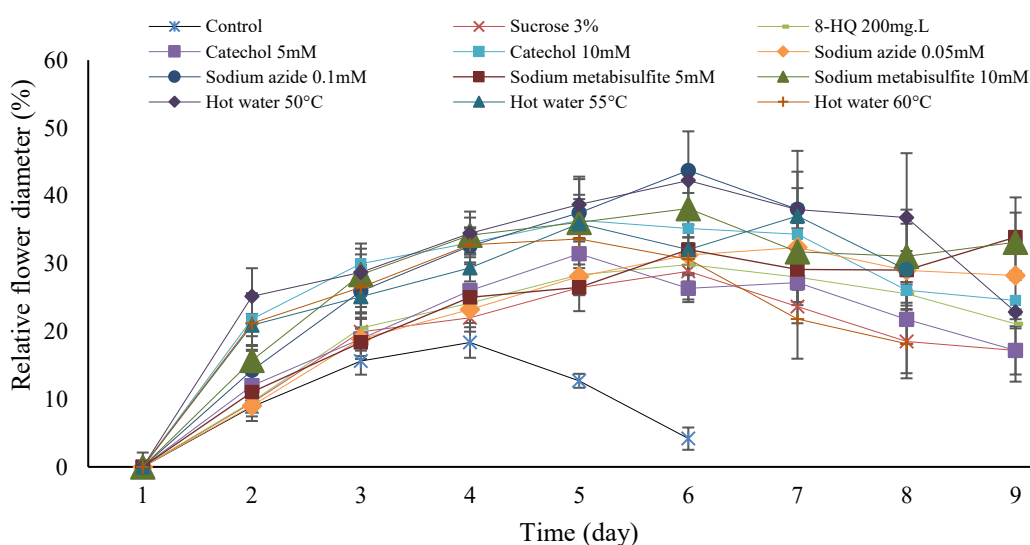


Fig. 3. Changes in relative flower diameter of rose cut flowers cv. Bakara treated with different inhibitors of vascular occlusion. The values are the mean $\pm$ SE of five replicates.

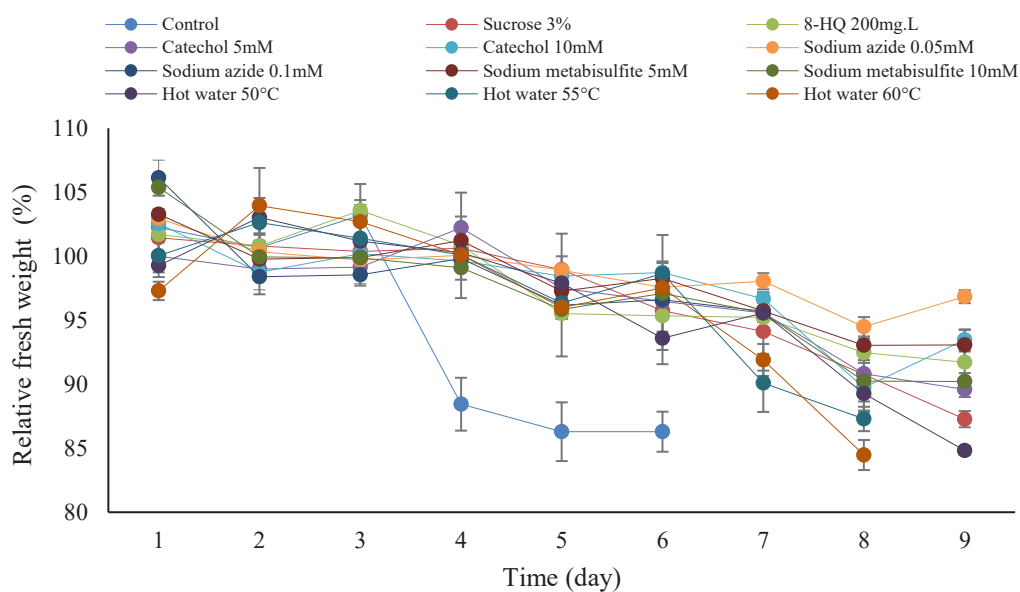


Fig. 4. Changes in relative fresh weight of rose cut flowers cv. Bakara treated with different inhibitors of vascular occlusion. The values are the means $\pm$ SE of five replicates.

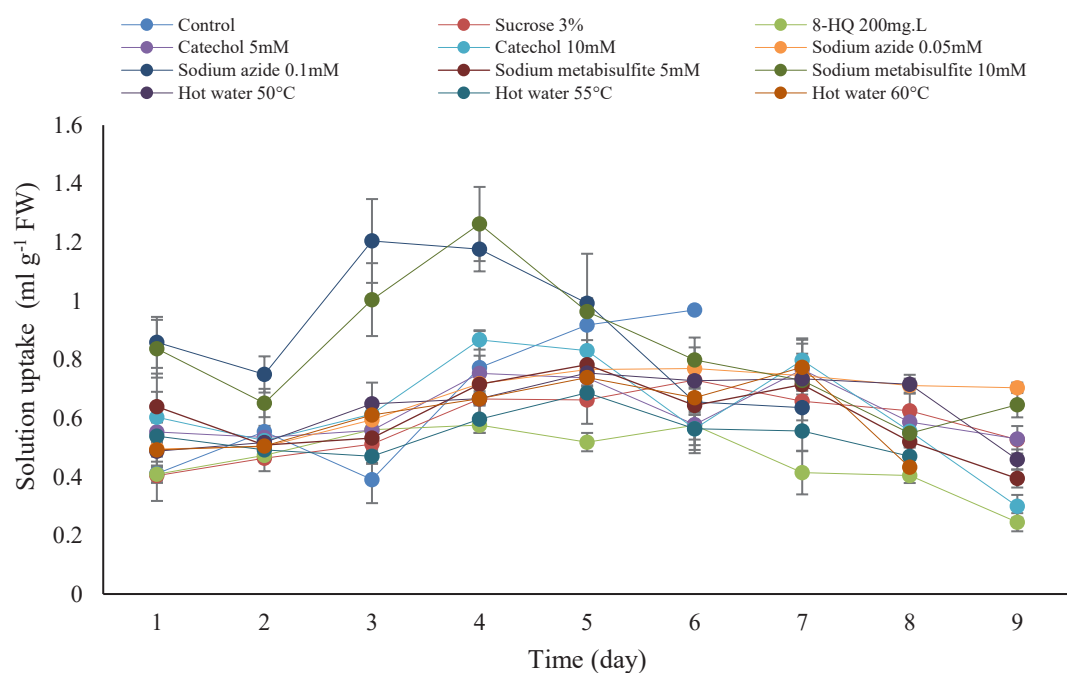


Fig. 5. Changes in solution uptake of rose cut flowers cv. Bakara treated with different inhibitors of vascular occlusion. The values are the mean $\pm$ SE of five replicates.

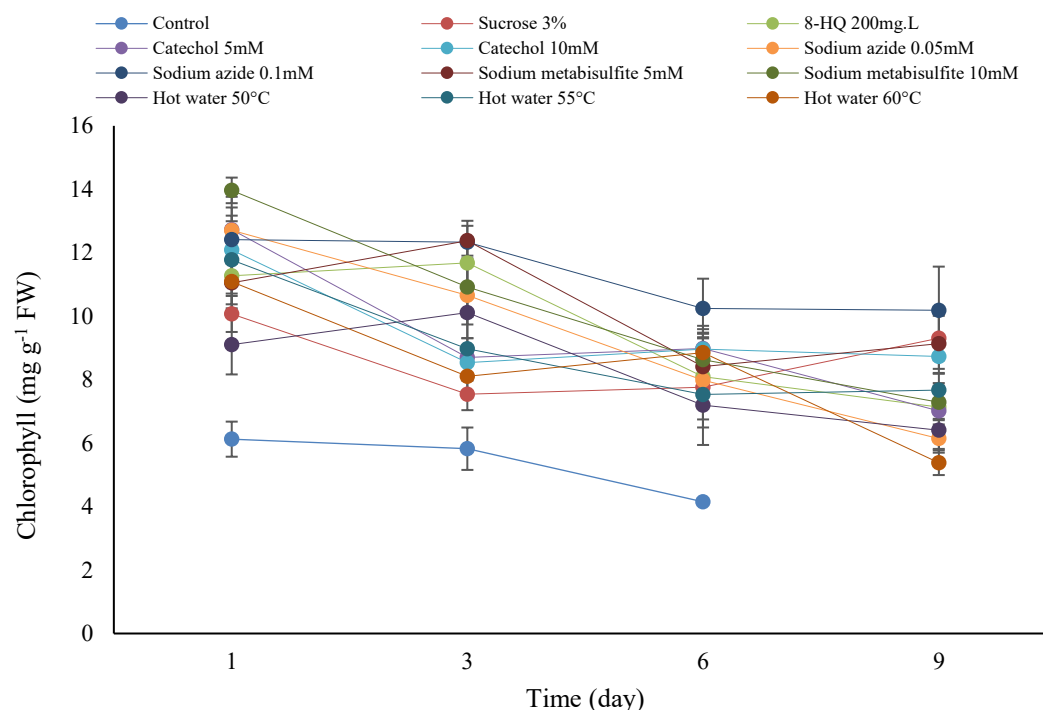


Fig. 6. Effect of different inhibitors treatments on the leaf chlorophyll content of rose cut flowers cv. Bakara. The values are the mean $\pm$ SE of five replicates.

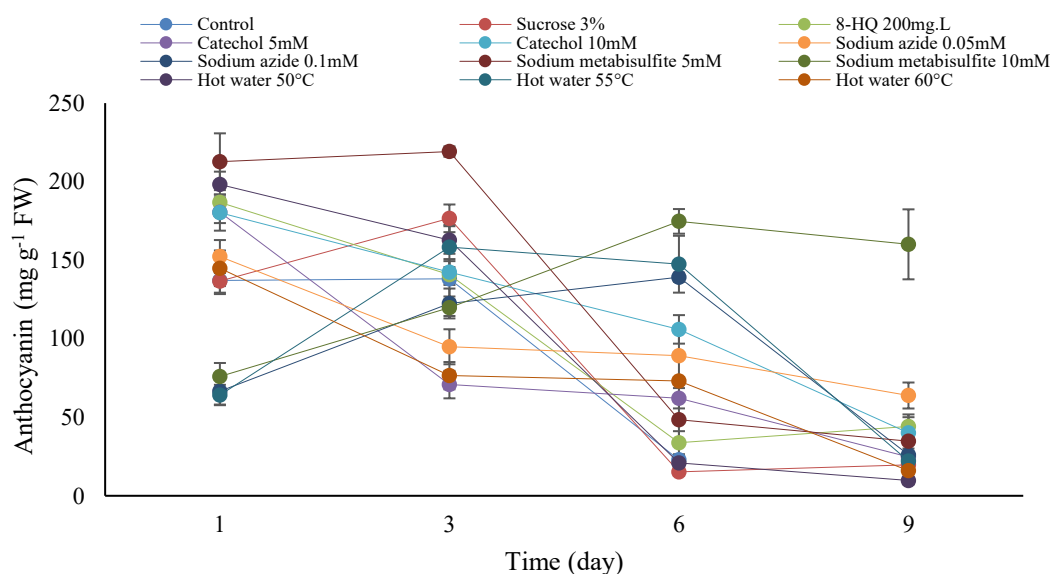


Fig. 7. Effect of different inhibitors treatments on the petal anthocyanin content of rose cut flowers cv. Bakara. The values are the mean $\pm$ SE of five replicates.

Generally, aging in plants is an oxidative process including biological, physiological, hormonal and structural changes which causes the destruction of macromolecules such as proteins, nucleic acids, and lipids. Increasing reactive oxygen species (ROS) causes chlorophyll degradation. Production of ethylene during aging results in the transfer of auxin

and the reduction of chlorophyll content and the death of tissues (Iqbal et al., 2017). Maintaining the content of chlorophyll is a critical factor in increasing the vase life and market quality of the cut roses. The results of other researchers showed that treatments such as silver nanoparticles, by improving the water absorption preserve the chlorophyll content of the rose (Jowkar et al., 2013; Hassan et al., 2014). Delay of chlorophyll degradation in the present study may be due to better absorption of vase solution by used treatments and to prevent water stress.

Petal anthocyanin content

Based on the results, treatments had a different effect on petal anthocyanin content at different times. By increasing the preservation time of cut flowers and reaching the ninth day, the anthocyanin content of petals decreased gradually in most treatments (Fig. 7). On the sixth day, anthocyanin was the lowest in the control treatment. The treatments significantly preserved the anthocyanin and prevented its decrease over time. Also, the results showed that the highest anthocyanin content on day 9 was observed in 10 mM metabisulfite treatment, and unlike other treatments, petal anthocyanin content increased. It should be noted that hot water treatments did not have a positive effect on the maintenance of anthocyanin content in the last day of the experiment compared to the mentioned treatments (Fig. 7).

Anthocyanins as water-soluble pigments accumulate in vacuoles of epidermal cells of plants. The destruction of anthocyanins during the aging process in cut flowers may be due to oxidative processes (Iqbal et al., 2017). The anthocyanin content in rose petals significantly decreases during the senescence (Schmitzer et al., 2010). The use of chemicals had a positive effect on the maintenance of petal anthocyanin. Enzymes such as β -glucosidase, polyphenol oxidase, and peroxidase are responsible for anthocyanin degradation (Oren-Shamir, 2009). It has been suggested that anthocyanins are first hydrolyzed by β -glucosidase, then anthocyanidins are formed which are oxidized by polyphenol oxidase or peroxidase. Polyphenol oxidase can accelerate the anthocyanin degradation process in the presence of oxygen (Oren-Shamir, 2009; Marszałek et al., 2017). The results of this study showed that the use of peroxidase and polyphenol oxidase inhibitors enzymes maintains the anthocyanin content of roses at the end of the experiment period, which might be due to inhibitors of the activity of the enzymes involved in the anthocyanin degradation. Similarly, Ghafouriyan et al. (2019) showed that inhibitors of lignin synthesis in Gerbera cut flowers preserved water absorption and prevented the reduction of anthocyanin over time, which is consistent with the results of this study. Holzwarth et al. (2013) reported that the anthocyanin stability in strawberry fruit may increase with the use of polyphenol oxidase inhibitors. They showed that the use of inhibitors such as cysteine and citric acid at appropriate concentrations reduced the activity of polyphenol oxidase and improved the anthocyanin and the color stability of the fruit. In the present study, the use of metabisulfite and sodium azide treatments, especially on the final day, was more effective in preserving anthocyanin in rose petals. By increasing the activity of oxidative enzymes such as peroxidase, the anthocyanin degradation in *Brunfelsia calycina* significantly increased (Vaknin et al., 2005), which may explain the effect of catechol, sodium azide and metabisulfite treatments on the preservation of anthocyanin in rose flowers.

CONCLUSION

In general, the results of our research showed that chemical treatments such as sodium azide, metabisulfite, and catechol, which have inhibiting effect on the physiological occlusion of vessels and also, treatments at temperatures of 50 and 55 °C increased the vase life of roses.

The highest vase life with an average of 9.9 and 9.7 days was observed in the sodium azide 0.5 mM and catechol 10 mM. The positive effects of catechol, sodium azide, and metabisulfite were more than thermal treatments. These chemical compounds preserved petal anthocyanins and leaf chlorophyll contents and prevented the increase of lignin at the stem end, which had a significant effect on increasing the uptake of the solution, relative fresh weight, and the relative diameter of the flower at the end of the experiment. Given the importance of roses and the need to improve its vase life, the use of inhibitors of enzymes involved in the biosynthesis of lignin in combination with sugars and antimicrobial agents is recommended to increase the longevity of roses.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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