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RESEARCH ARTICLE

Effects of organic acids, chemical treatments and herbal essential oils on the vase life of cut carnation (*Dianthus caryophyllus* L.) flowers

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ABSTRACT

Among the cut flowers, carnations are sensitive to ethylene. Therefore, the vase life is short and a negative situation arises in terms of marketing. Different chemical preservatives, herbal essential oils and growth regulators are used today to extend the post-harvest life of cut flowers. This study was conducted to determines the effects of single-use of some organic acids, chemical acids and essential oils and their combinations with sugar on postharvest life (vase life) of cut carnation flowers. Experiments were conducted in randomized plots design with three replications. Experimental treatments included: Salicylic Acid (100 mg/L), Ascorbic Acid (100 mg/L), Citric Acid (200 mg/L), Benzyl Adenine (50 mg/L), Carvacrol (200 mg/L), Thymol (200 mg/L) and sugar-supplemented (10 g/L) combinations of these treatments. Measured parameters included: Vase life, relative fresh weight, daily vase solution uptake, total vase solution uptake, pH change, bacteria population and diagnosis of dominant bacteria. Among the present treatments, 200 mg/L citric acid treatment decreased the bacteria population, prevented blockage in the plant's stem section, and thus, promoted water uptake. Thus, the best outcomes in terms of vase life, total vase solution uptake and relative fresh weights were achieved with 200 mg/L citric acid treatment. It was also observed that Salicylic Acid (100 mg/L) + Sucrose (10 g/L) and Benzyl Adenine (50 mg/L) + Sucrose (10 g/L) treatments yielded expected outcomes. Besides, non-sugar containing vase solutions yielded better outcomes than sugar-supplemented combinations. In terms of pH changes, decreases were observed in the final pH values of sugar-supplemented solutions compared to the initial pH values. Additionally, in present vase solutions, mostly *Burkholderia cepacia* species were diagnosed and also *Pseudomonas antarctica, Pectobacterium carotovorum, Xanthomonas hortorum, Pseudomonas chlororaphis* bacteria colonies were identified. As a result of the study, it is thought that 200 mg/L citric acid treatment and 100 mg/L salicylic acid + 10 g/L sugar treatment will contribute to cut flower and cut carnation producers and researchers working in this in the post-harvest field.

Keywords: Bacteria; Benzyl adenine; Citric; Environment-friendly; Sugar

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) has first place among the cut flower species grown worldwide (Yagi et al., 2014). Turkey has excellent contributions to carnation export and ranked in 3rd place in world carnation export after the Netherlands and Colombia. In 2018, world carnation export was realized as 227 million Euros (AIPH, 2019). In 2019, 635 million carnations were produced over 5 thousand decares of under-cover area (TUİK, 2020).

The vase life of cut flowers is generally relatively short based on genetic and environmental factors, and such a short vase life limits the development of the cut flower industry (Kumar et al., 2014; Van Meeteren and Aliniaeifard, 2016; Aalifar et al., 2020). Postharvest ageing of cut flowers is an active process including various physiological and biochemical changes (Buchanan-Wollaston and Morris, 2000; Rubinstein, 2000; Battelli et al., 2011). Since carnation is a model flower, researchers mainly focus on mechanisms designating vase life (Sugawara et al., 2002; Tanase et al., 2008; Satoh, 2011; Tanase et al., 2015). Under normal conditions, carnations have a short vase life varying between 5–10 days (Reid et al., 1980). The prolonged vase life of carnation will aid in the wholesale or retail trade of flowers to far regions, preserve flower quality, and extend flower life (Sardoei et al., 2014).

Essential oils are inherently organic, safe and environment-friendly compounds, and they have strong antimicrobial

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properties against some pathogens (Hashemabadi et al., 2013; Bidarigh, 2015). Phenolics and mono-terpene compounds are the primary components of essential oils (Ghorbanpour, 2015). Essential oils also contain carvacrol, thymol andeugenol-like high-level phenolic compounds (Bayat et al., 2012; Kazemi and Ameri, 2012). Essential oil and compound levels play a significant role in preventing microbial growth in vase solutions (Dejene et al., 2019). Compared to control plants, Alkaç et al. (2020) achieved the best outcomes in terms of daily uptake and total uptake of vase solution in Dahlia plants using essential oils. Vase solutions should contain substances preventing sugar and bacteria formation (Asrar, 2012). Sugars provide a respiration substrate; anti-bactericides control harmful bacteria and prevent blockage of conducting tissues (Gómez-Merino et al., 2020). Sugars also preserve the bulginess of the cut flowers, provide energy for respiration and play an essential role in flower freshness (Reid, 2009). Sucrose treatments may increase the mechanical hardness of flower stem based on the thickening of the cell membrane and lignification of vascular tissues (Gómez-Merino et al., 2020). Mehraj et al. (2016) reported that sugar-containing solutions increased vase life compared to the control plants and yielded positive outcomes. Two primary factors are dominant in the postharvest physiology of cut flowers: carbohydrate sources in stem and water balance (Vehniwal and Abbey, 2019). In recent years, plant extracts and essential oils have emerged as popular preservatives prolonging the vase life of cut flowers (Dejene et al., 2019). They make vase solution acidic, recess ethylene production and prevent the growth of microorganisms (Dishaben et al., 2018). Bayat and Aminifard (2017) reported that salicylic acid (SA) used in vase solution reduced solution pH and bacterial growth. Seman and Rafdi (2019) indicated that sugar supplementation into SA-containing vase solution increased vase life by about 1.6 times compared to the control. It was reported that salicylic acid, citric acid, and sugar-supplemented vase solutions have the best performance in prolonging vase life and preserving the quality of cut flowers (Aziz et al., 2020). In *Narcissus tazetta* plants, 100 ppm citric acid treatments significantly prolonged vase life compared to the control (Gun, 2020).

The effort spent on prolongation of vase life of cut flowers have recently been accelerated; however, active ingredients of plant essential oils, organic acids, and some kinds of chemical acids have limited use with sugar supplementation (Hashemabadi et al., 2013; Bazaz et al., 2015; Bidarigh, 2015). Essential oils, organic acids and sugars are biologically degradable and environment-friendly substances (Bayat et al., 2013). People wish to feed the pleasure of cut flowers for longer durations than their normal vase life; thus, the most crucial thing in cut flower handling is its postharvest treatments to prolong vase life (Hussen and Yassin, 2013). Therefore, this study was conducted with vase solutions of carnation plants, considering the worldwide market size of carnations, to prolong vase life and present the potential positive outcomes for the information and service of consumers.

MATERIALS AND METHODS

Plant material

Carnations (*Dianthus caryophyllus* L.) used in the present study were supplied from a commercial greenhouse dealing with carnation production in Antalya province of Turkey. Standard-type carnations (60-70 cm), healthy and fully-bloomed, were selected in early morning hours (Nichols and Ho, 1975).

Experimental design and treatments

Harvested flowers were brought to the laboratory, in which experiments to be conducted within water-filled containers. Then, flowers were cut in 40 cm length and placed one by one into sugar (Merch, Turkey) supplemented salicylic acid (Tekkim, Turkey), ascorbic acid (Merck, Turkey), citric acid (Tekkim, Turkey), benzyl adenine (Sigma-Aldrich, Turkey), carvacrol (Sigma- Aldrich, Turkey) and thymol (Fisher Scientific) solutions at different concentrations specified in Table 1. Finally, all vase solutions were arranged to 250 ml. Throughout the vase life experiments, the room temperature was kept at 20±2 °C, relative humidity was kept at 50±5% (Hobo Data Logger U12-012, Onset, United States of America), and photoperiods were applied for 12 hours.

Vase life (days)

Wilting level and end of vase life of a flower are identified with the symptoms of contractions in leaves, browning at leaf edges and breaking of stems (Paulin et al., 1986; VBN, 2005). In addition, the vase life of each flower was identified as the time passed from the experimental set upto wilting of the flower (Yamamoto et al., 1992).

Table 1: Vase solutions and concentrations.

Treatments	Concentrations
(T1) Distilled Water (DW) (Control) (ml)	250
(T2) Salicylic Acid (mg/l)	100
(T3) Ascorbic Acid (mg/L)	100
(T4) Citric Acid (mg/L)	200
(T5) Benzyl Adenine (mg/L)	10
(T6) Carvacrol (mg/L)	200
(T7) Thymol (mg/L)	200
(T8) Salicylic Acid (mg/L) + Sucrose (g/L)	100+10
(T9) Ascorbic Acid (mg/L) + Sucrose (g/L)	100+10
(T10) Citric Acid (mg/L) + Sucrose (g/L)	200+10
(T11) Benzyl Adenine (mg/L) + Sucrose (g/L)	10+10
(T12) Carvacrol (mg/L) + Sucrose (g/L)	200+10
(T13) Thymol (mg/L) + Sucrose (g/L)	200+10

Relative fresh weight (RFW)

Flower weight was measured at the beginning of the experiment's day 0, 2, 4, 6, 8, 10, 12 and 15^{th}days (Zeng et al., 2011). Relative fresh weight was then calculated with the use of the following equation:

RWF (% of initial FW)= (At/At = 0) x100

At: weight of stem (g), At = day 2 (eg. 2, 4, 6 etc.)

At=0: weight of the same stem (g), day 0 (He et al., 2006).

Daily water uptake (DWU)

The following equation was used to calculate the daily uptake of vase solution:

DWU= $(S_t\text{-}1)\text{-}(S_t)$

St-1 = The weight of the vase solution for the previous day,

S_t = The weight of the vase solution on day t (eg. 2, 4, 6, etc.) (He et al., 2006).

Total water uptake (TWU)

The following equation was used to calculate the total uptake of the vase solution:

TWU=A-B-(C-D)

A: The initial weight of vase solution.

B: The final weight of vase solution at the end of vase life.

C: The weight of evaporation bottles at day 0.

D: The weight of evaporation bottles at the end of vase life (He et al., 2006).

pH

The pH values of 13 different vase solutions were measured at the beginning of experiments (day 0) and the end of the vase life with a pH meter (Hanna HI 2211, Hanna Instruments, United States of America).

Vase solution bacteria counts

For bacteria counts of vase solutions, samples were taken from vase solutions at the end of vase life. The dilution series method was used to determine the bacteria population. About 1 mL of vase solution were placed into 9 mL physiological saline water (0.85% NaCl solution -saline buffer) containing tubes and diluted six times to get dilution series. About 100 μl samples were taken from the series's last two tubes and inoculated in 90 mm Petri dishes containing Nutrient Agar (NA) with a sterile glass rod. Petri dishes were then incubated at 37°C for 24 hours. At the end of the incubation period, bacteria colonies were counted to determine the bacteria population of the vase solution (Liu et al., 2009).

Bacteria composition of vase solution

After identifying the bacteria population of vase solutions, selections were made from bacteria colonies developed in nutrient agar and classified these colonies. The bacteria isolates grown in NA at 37°C for 24 hours were subjected to gram reaction with 3% potassium hydroxide (KOH), oxidase and catalase tests (Janse, 2005). Following the biochemical tests, the diagnosis of bacteria isolates was performed using the MALDI-TOF MS method.

Statistical analysis

Experiments were conducted in randomized plots design with three replications with three plants in each repetition. Vase life measurements were made daily, and the other parameters were measured every other day. Resultant data were subjected to variance analysis (One-way ANOVA) using SPSS 17.0 (IBM) software. Significant means were compared using Duncan's multiple range tests at $p<0.05$.

RESULTS AND DISCUSSION

Vase life

Effects of experimental treatments on vase life of the flowers and differences between the treatments are presented in Fig. 1. The longest vase life was measured in the T4 treatment (16 days) and increased by 17% compared to the control. The shortest vase life (8.33 days) was measured in the T11 treatment. By the 8^{th}day, the effects of 13 different treatments on flower petals are presented in Fig. 2.

It was reported in previous studies that 1.5 mM salicylic acid concentration prolonged vase life of gladiola cut flowers by up to three days (Jalili Marandi et al., 2011; Hasanpour et al., 2012) and herbal essential oils prolonged the vase life of flowers as compared to the control (Hashemabadi et al., 2013). It is reported that the most active monoterpenoid phenols among essential oils are thyme and lavender oil (Dhifi et al., 2016). Studies have reported that the effectiveness of these essential oils is due to their role as antimicrobial agents (Memar et al., 2017). It was reported in another study conducted on Chrysanthemum cv. 'White' that essential oils were found to be 30% effective in extending the vase life of cut flowers (Avar et al., 2015). In another study, it was reported that essential oils cause premature flowering and aging in cut roses (Salehi Salmi et al., 2018). However, in the present study, herbal essential oils and salicylic acid treatments shortened the vase life of carnation flowers compared to the control. Similar to the present findings, Celikel et al., (2020) indicated that

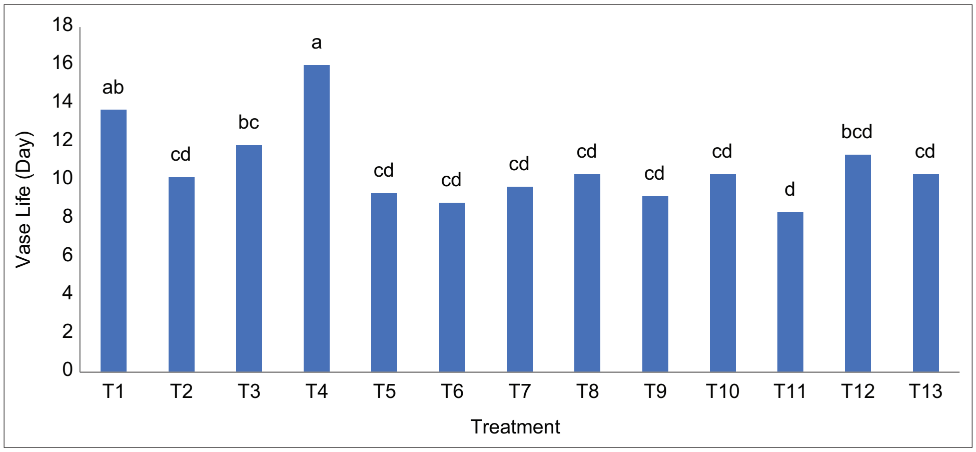

Fig 1. Effect of different solutions on vase life of carnation flowers (day)

Fig 2. Effects of different treatments on carnation flowers in the 8th day

9.5 mg/L BA and 200 mg/L citric acid treatments increased the vase life of the cut flowers compared to the control. Citric acid treatments were also reported to positively affect the vase life of various flower species (Hasanpour and Hasani, 2012; Vahdati et al., 2012; Sheikh et al., 2014). Gun (2020) reported in his study that 100 ppm citric acid application increased the vase life of the daffodil plant compared to the control. Similarly, citric acid, cut rose, lisianthus (Azizi and Onsinejad, 2015), lilies (Darandeh and Hadavi, 2012), 'ABC Blue' lisianthus (*Eustoma grandiflorum*), 'Maryland Plumblossom' snapdragon (*Antirrhinum majus*), 'Mid Cheerful Yellow' ' stock (*Matthiola incana*), and 'Deep Red' Benary's zinnia (*Zinnia violacea*) (Ahmad and Dole, 2014) and *Alstroemeria hybrida* cv of 'Summer Sky' (Ershad Langroudi et al., 2020) it has been reported in studies that it significantly increases shelf life in cut flower varieties. It has been reported that this situation emerged in the investigation of the effect of salicylic acid on ethylene, inhibiting the formation of ethylene from the ACC by inactivating the ACC oxidase enzyme, and thus delaying ageing in the plant, increasing vase life (Leslie and Romani, 1988). In addition, it is thought that the positive effect of the citric acid application on vase life, as Van Doorn (2012) reported in his study, reduces bacterial growth in the cut flower xylem. As a result, it has similar results by promoting water conduction.

Relative fresh weight (RFW)

Effects of vase solutions on the relative fresh weight of carnation flowers throughout the vase life are presented in Fig. 3. Compared to the other treatments, in the T6 treatment, increases were observed until the 4th day, then decreases were observed. Except for T11 and T6 treatments, relative fresh weights increased from the 4th day. The greatest relative fresh weight (113.21%) was obtained from T8 treatment followed by T4 treatment (108.33%). By the 10th day, the best outcomes were obtained from T4 treatment. In this treatment, the relative fresh weight did not go below the initial weight throughout the vase life, and better results were achieved than the control group (T1). The greatest weight loss was observed throughout the vase life in T11 treatment (Fig. 3).

Maintaining relative fresh weight during the vase period is vital to improve vase life. During post-harvest, this factor is expected to decrease gradually, but lower amounts of weight reduction are an effective indicator for better effect of the applied treatments (Dehestani-Ardakani et al., 2022). It was reported that different citric acid doses (100, 200 and 300 mg/L) increased fresh weight and water uptake of daffodil (Bayat and Aminifard, 2018) and rose (Kazaz et al., 2017). Thusly, similar findings were obtained from the present experiments. Organic acids like salicylic acid and citric acid retarded blockage of plant cut sections in vase solution and thus increased water uptake and relative fresh weights of cut flowers. Krause et al., (2021) reported that 100 ppm citric acid and 20 g/L sugar applications in *Lilium pumilum* cut flower provided a decrease in fresh weight loss. It is seen that similar results emerged in our study. It is seen that organic acids such as salicylic acid and citric acid delay the clogging of the cutting points of the plants in the vase solution, reduce the proliferation of the bacterial population, and thus increase the proportional fresh weight with the water intake. Therefore, increasing the vase solution intake and decreasing the transpiration rate may have affected the fresh weight positively by preventing water loss of cut flowers. Previous studies have also reported that there is a relationship between solution intake and fresh weight (Alaey et al., 2011; Amin, 2017).

Daily water uptake (DWU)

Significant differences were observed in the daily water uptake of vase solutions. In general, decreases were observed in daily water uptake of all treatments in 0-2nd day and except for T6, T10, T11 and T13 treatments decreased water uptakes continued in 2-4th day of the other treatments. In T4 treatment, a linear increase was observed in daily water uptake of vase solution. The greatest daily water uptakes were respectively observed in 8-10th day of T8 treatment (2.55 g/stem day^{-1}), and 6-8th day of T11 treatment (2.32 g/stem day^{-1}). By the 10th day, stem weight did not go below the initial weight (Fig. 4).

Increasing daily vase solution uptakes were reported with citric acid treatments as compared to the control (Gun, 2020). Parallel findings were observed especially in T4 treatment, and a daily vase solution uptake was observed. Amin (2017) reported that citric acid reduces the proliferation of the bacterial population in the solution and increases the water conductivity in the cut flower xylem, so it is estimated that the uptake of vase solution is higher in citric acid applications. Studies have also reported that sugar-containing vase solutions have the highest vase solution intake and are more effective than sugar-free solutions (MohdRafdi et al., 2018). It has been reported that sugar-containing solutions provide water balance in plants. Sugar accumulates in flowers, increasing the concentration of osmotically active solutes and maintaining petal swelling (Pivetta et al., 2019). As a result, it is seen that sugar-containing solutions provide more solution intake in daily vase solution intake (Kumar and Deen, 2017). Aryal et al., (2019) and Kshirsagar et al., (2021) reported that sucrose-containing solutions promote water uptake in roses

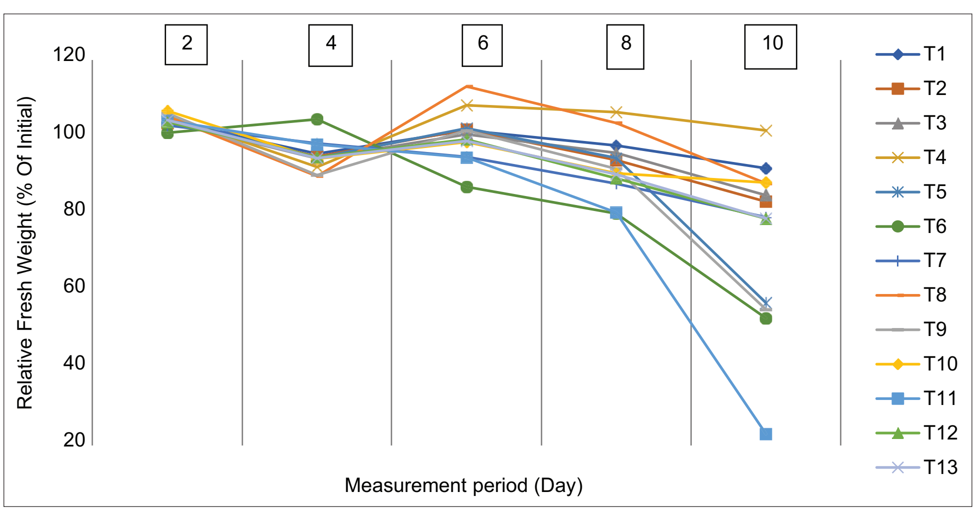

Fig 3. Change in relative fresh weight of carnation plant throughout the vase life

and decrease respiration and transpiration rate. In the study, it is thought that the excessive daily vase solution intake is evident in T8 and T11 applications and that similar results are obtained in studies on this subject due to the presence of sugar in the solutions.

Total water uptake (TWU)

There were significant differences in total vase solution uptake of experimental treatments. Throughout the vase life, the greatest total water uptake was observed in T4 (citric acid 200 mg/l) treatment (38.53 g/stem) and the lowest total water uptake was observed in t11 (benzyl adenine + sucrose 10 mg/L + 10 g/L) treatment (18.99 g/stem) (Fig. 5).

Similar to the present findings, citric acid-like acidic components were reported to prevent bacteria growth and accumulation in vase solutions and thus increase the water uptake of cut flowers (Alaey et al., 2011; Mansouri, 2012). It was also reported that citric acid treatments promoted stem water uptake from vase solution in *Acacia holosericea* plants (MohdRafdi et al., 2018).

pH

The pH values of vase solutions were measured at the beginning of the experiments (initial) and the end of vase life (final). Initial pH values varied between 2.97-5.07, and the last pH values ranged between 2.91-6.79. Among the sugar-supplemented solutions, except for T8 treatment, pH values decreased in all treatments (T9, T10, T11, T12 and T13) as compared to the initial pH values. On the other hand, pH values increased in non-sugar-containing solutions (T1, T2, T3, T4, T5, T6 and T7) as compared to the initial values (Fig. 6).

Salicylic acid, ascorbic acid, citric acid and sugar added combinations have a lower pH compared to other preservative solutions as they release more H^+ ions in the

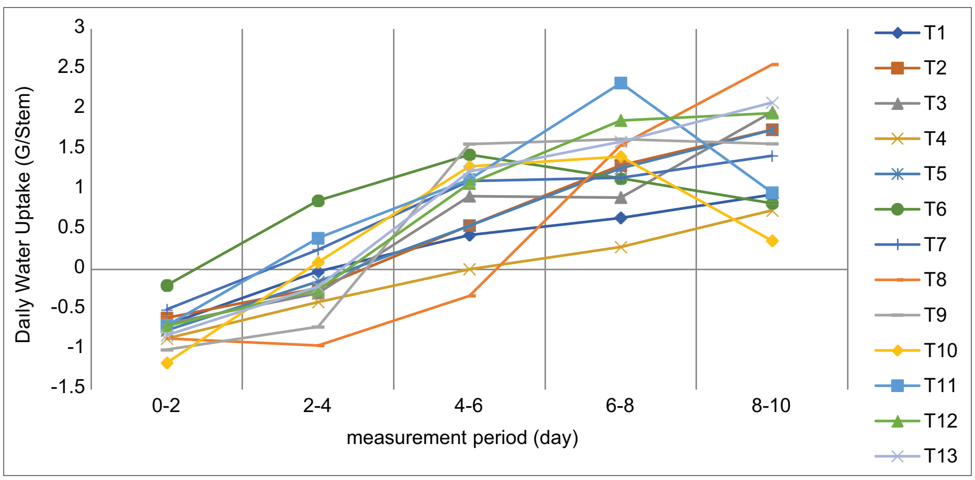

Fig 4. Daily vase solution uptake of experimental treatments throughout the experiments

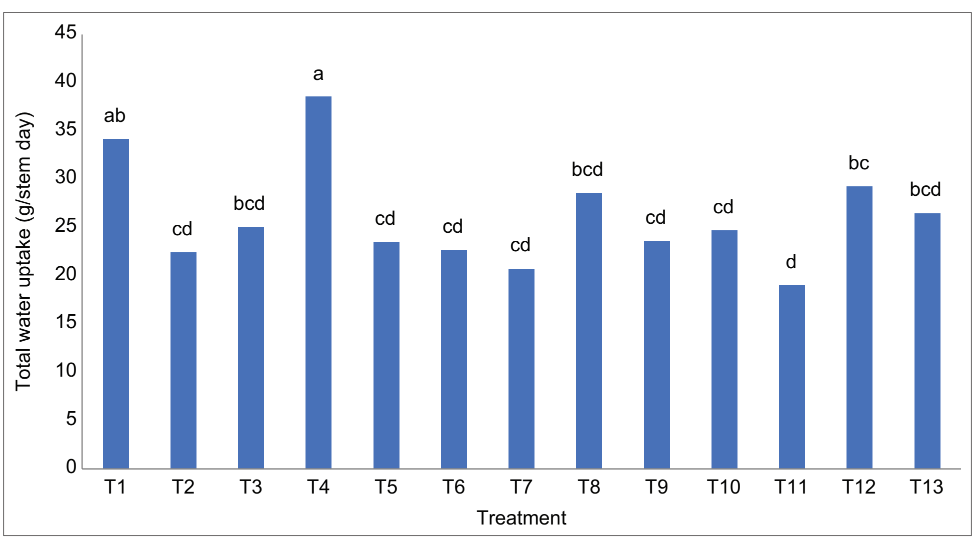

Fig 5. Total vase solution uptake of experimental treatments throughout the experiments

solution. During the study period, the solutions without added sugar approached neutral, while the pH of the ones with added sugar was lower than at the beginning. This variability in pH over the life of the vase may be related to the solutions in the vase, the transport physiology of the plant, and the amount of microorganisms in its metabolism. Similar results were found by Shanan (2017) and Paul et al., (2021) is also seen in their study.

Vase solution bacteria counts

As compared to the control treatment, the lowest bacteria population in vase solutions was observed in T10 treatment (0.8 x 106 CFU mL^{-1}), and the greatest bacteria population was measured in T3 treatment (2.0 x 1010 CFU mL^{-1}) (Table 2).

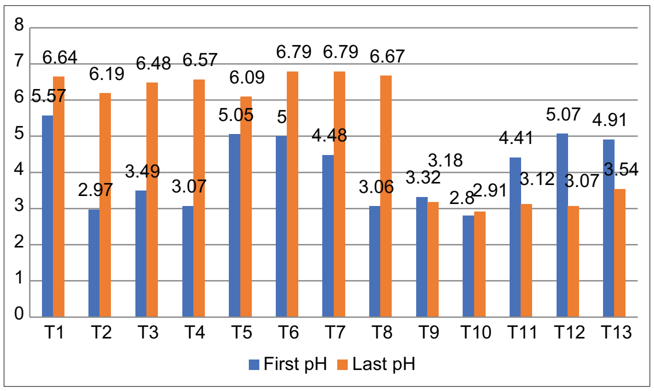

Fig 6. Change in pH values of vase solutions

Table 2: The effect of different solutions on bacteria population of vase solutions

Treatments	Bacteria population (CFU mL^{-1})
T1	0.5 x 10^6
T2	8.3 x 10^6
T3	2.0 x 10^{10}
T4	4.8 x 10^7
T5	2.4 x 10^6
T6	1.6 x 10^6
T7	1.0 x 10^6
T8	2.0 x 10^7
T9	2.8 x 10^6
T10	0.8 x 10^6
T11	1.9 x 10^7
T12	4.8 x 10^6
T13	1.7 x 10^6

The bacteria growing and propagating in vase solution result in blockage of conducting tissue and thus reduce water uptake and vase life of cut flowers. Therefore, cut carnation flowers are quite sensitive to bacteria growing in vase solutions (Van Doorn, 1998). It was identified that higher bacteria populations in T2, T3, and T11 treatments remarkably prevented total vase solution uptake (Fig. 5); besides, these treatments had shorter vase life than the control (Fig. 1).

Bacteria composition of vase solution

Biochemical and MALDI-TOF MS diagnosis tests were conducted on vase solutions, and five different bacteria species were identified. While mostly *Burkholderia cepacia* were identified in cut carnation vase solutions, the other bacteria identified included *Pseudomonas antarctica, Pectobacterium carotovorum, Xanthomonas hortorum, Pseudomonas chlororaphis*. It was observed accordingly that mostly similar bacteria species were observed in vase solutions of carnations (Table 3).

Present findings on bacteria populations and species of vase solutions comply with the results of the previous studies. For example, Van Doorn and De Witte (1997) indicated dominant bacteria species in stems of cut rose "Sonia" flowers as *Pseudomonas* and *Enterobacteria*. Similarly, *Bacillus* spp., *Staphylococcus* spp. and *Actinomycetes* spp. bacteria were identified as the dominant species in vase solutions of daffodils (Jowkar, 2006). Additionally, gram-negative non-fermentative *Pseudomonas* species and various gram-positive bacteria species, including *Staphylococcusaureus, Saprophethicus, Streptococcus, Bacillus cereus* and *Actinomyces* were reported in vase solutions of cut flowers (Langroudi et al., 2020).

CONCLUSION

In this study, vase life, relative fresh weight, daily vase solution uptake, total vase solution uptake, determination of the density of bacteria in the vase and making their diagnosis are important within the scope of the study. It has been determined that especially organic acids have significant positive effects on these properties and contribute to the improvement of the post-harvest life of cut carnation flowers. The present study used organic acids, plant essential oils, and sugar-like environment-friendly compounds to prepare 13 different vase solutions. Among these vase

Table 3: Biochemical and MALDITOF-MS reaction patterns in primary tests for bacteria.

Tests	B.cepacia	P. antarctica	P. carotovorum	X. hortorum	P. chlororaphis
Gram	-	-	-	-	-
Oxidase	+	+	-	-	+
Catalaze	+	+	+	+	+

solutions, 200 mg/L citric acid treatments reduced the bacteria population, prevented blockage of vascular tissues, and promoted water uptake. Thus, the best outcomes in terms of vase life, total vase solution uptake, and relative fresh weights were achieved with this treatment. Additionally, plain vase solutions yielded better outcomes than sugar-supplemented combinations. It was observed that salicylic acid (100 mg/L) + sucrose (10 g/L) and benzyl adenine (50 mg/l) + Sucrose (10 g/L) treatments yielded expected outcomes in terms of relative fresh weight and daily vase solution uptake. In terms of pH changes, decreases were observed in the final pH values of sugar-supplemented solutions compared to the initial pH values. It was concluded based on present findings that 200 mg/L citric acid treatments could be recommended for vase solutions of cut flowers and especially for vase solutions of carnations.

AUTHOR CONTRIBUTIONS

Onur Sefa Alkaç experiment idea, designed the study, field analysis, data collection analysis and wrote manuscript. Sabriye Belgüzar performend the bacterial analysis parts of the study. Mehmet Güneş revised the manuscript. The final version was approved by all authors.

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