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Improving postharvest vase-life and quality of cut gerbera flowers using natural and chemical preservatives

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Abstract

Gerbera is one of ten popular cut flowers in the world which occupies the forth place according to the global trends in floriculture. Hence, this study aimed to investigate the effects of chemical, hormonal and essential oil substances in preservative solutions to improve its postharvest qualitative characteristics. Two pulse treatments including distilled water (pulse1) and 4% CaCl2 + 3% sucrose (pulse2) for 24 hour were applied before long-time treatments. Long-time treatments were comprised of (1) Hormonal treatments: 0, 25, 30 mg*l^{-1} Gibberellic acid, 0, 150, 250 mg*l^{-1} Benzyl adenine and 0, 100, 200 mg*l^{-1} 5-Sulfosalicylic acid (2) Chemical treatments: 0, 0.4, 0.8 mM Silver thiosulphate, 0, 5, 10 mg*l^{-1} Nano-silver particles, 0, 4, 6 mM Aminooxy acetic acid and 0, 200 and 400 mg*l^{-1} 8-hydroxyquinoline sulphate (3) Essential oils: *Thymus* essential oil and *Stevia* essential oils (0, 0.1 and 0.2 mg*l^{-1}). Data were subjected to analysis of variance based on the factorial experiment model in the layout completely randomized design. Mean comparison was performed using the Duncan's multiple range test. Parameters of fresh weight, stem bending, capitulum diameter, carotenoid pigments of petal and vase-life longevity were evaluated during 12 days. The highest fresh weight was obtained when cut flowers were held in a solution containing pulse1 + 250 mg*l^{-1} BA. Among all treatments, 8-HQS treatment showed the best effects on preventing stem bending, increasing capitulum diameter and also on prolonging of vase-life, but nonetheless, the effects of pulse treatments and 8-HQS concentrations were insignificant. To conclude, 200 mg*l^{-1} 8-HQS without pulse treatment has the potential to be used as a commercial preservative solution to improve the keeping quality and vase-life of this important cut flower.

Keywords: cut flower, gerbera, longevity, postharvest, preservative solution

Abbreviations: GA_3, Gibberellic acid; **BA,** Benzyl adenine; **SA,** salicylic acid; **SSA,** 5-Sulfosalicylic acid; **STS,** Silver thiosulphate; **AOA,** Aminooxy acetic acid; **SNPs,** Nano-silver particles; **8-HQS**, 8-hydroxyquinoline sulphate; **ASA**, acetylsalicylic acid;

$CaCl_2$, calcium chloride; **CRD**, completely randomized design; **TSS**, total soluble solids; **PGRs**, plant growth regulators.

Introduction

Gerbera (*Gerbera jamesonii* cv. 'Dune') is an important ornamental flower and is commonly used as a cutting flower belonging to Asteraceae family, classified as a flowering plant and is one of ten popular cut flowers in the world which occupies the forth place according to the global trends in floriculture (Choudhary and Prasad, 2000). As described by Wilberg (1973), flower wilting can be considered as one of the main postharvest disorders which may lead to stem break that occurs 10 cm below capitulum. As well as this, blockage of xylem vessels due to bacterial or microorganisms accumulation is another contributing factor leading to quality loss (Jalili Marandi, et al., 2011). This blockage can be culminated in water uptake deficiency and water loss (Hassan, 2005). Zagory and Reid (1986) showed that some bacteria from vase water could elevate ethylene production. It has been reported that some antibacterial compounds such as SNPs (Morones, et al., 2005) and ASA (Kazemi and Ameri, 2012) can perceptibly extend vase-life of cut gerbera flowers. In addition, the advantage of using 8-HQS and $CaCl_2$ alone or in combination with 4% sucrose as chemical preservative solutions to improve keeping quality of cut gerbera flowers has been demonstrated (Soad, et al., 2011). The efficacy of 8-HQS has been attributed to improving water uptake by overcoming vessel blockage (Reddy, et al., 1996). Similar to STS, benefits of using the AOA; having the effects of inhibiting ethylene production, reducing the rate of ethylene formation and delaying senescence have also been corroborated (Nowak and Rudnicki, 1990).

In the case of hormonal treatment, studies showed that GA_3 treated cut flowers of gerbera at the range of 2.5, 5 and 7.5 $mg*l^{-1}$ significantly delayed petal senescence and abscission which might be attributed to the maintenance of flower turgidity (Emongor, 2004). Moreover, as described by Danaee, et al. (2011), by applying 50 $mg*l^{-1}$ BA and 50 $mg*l^{-1}$ GA, cut flower vase-life, fresh weight, solution uptake, membrane stability and TSS of gerbera increased. As described by Kavosiv (2013), application of thyme oil (*Thymus vulgaris* L.) successfully culminated in increasing cut rose flower (cv. White Naomi) relative fresh weight (RFW). Considering the environmental hazards of some preservatives such as STS, which contains a heavy metal, using natural substances such as plant essential oils and SA is more demanding (Jalili Marandi, et al., 2011).

Therefore, the objective of this study was to investigate the effects of chemical and natural preservatives in holding solutions and determining the best ones in order to both extend vase-life of cut gerbera flower and also to increase its qualitative characteristics.

Materials and Methods

Plant material

Treatments and environmental conditions

The present study was conducted at the laboratory of Department of Horticulture, Faculty of Agriculture, Islamic Azad University, Isfahan (Khorasgan) branch and commenced on June 10, 2012. Firstly, the flowers were harvested in the morning hours by pulling the scapes of 60 cm from the crowns. Instantly after harvest, cut

flowers were transferred vertically under dry condition to the laboratory and the lower 2 cm was cut off under tap water to avoid air embolism. Subsequently, half of the flowers were placed in distilled water and the others were placed in pulse treatments containing 4% $CaCl_2$ + 3% sucrose for 24 hour (these treatments considered as short-time period treatments). After these 24 hour cut flowers were taken off and placed in 3 liters plastic vases containing 500 ml preservative solutions. In order to compare two pulse treatments control treatment containing distilled water was applied as a long time treatment.

In all treatments, cut flowers were kept in the laboratory at ambient temperature of 25 ± 1°C, 25-30% RH and a photosynthetically active photon flux of 15 $\mu mol*m^{-2}*s^{-1}$ (from Philips TDL 36W/84 cool white fluorescent tubes) from 7 a.m. to 7 p.m.

Hormonal treatments

To investigate long-time period treatments, hormonal treatments were comprised of: (1) GA_3 at the range of 0, 25 and 30 $mg*l^{-1}$, (2) BA at the range of 0, 150 and 250 $mg*l^{-1}$, (3) SSA at the range of 0, 100 and 200 $mg*l^{-1}$ (All PGRs were purchased from Merck factory). In order to inhibit hormonal decomposition caused by light, cut flower vases were wrapped using aluminium foils.

Chemical treatments

Chemical treatments considered for long-time period were comprised of: (1) SNPs at the range of 0, 5 and 10 $mg*l^{-1}$, (2) STS at the range of 0, 0.4 and 0.8 mM, (3) AOA at the range of 0, 4 and 6 mM, (4) 8-HQS at the range of 0, 200 and 400 $mg*l^{-1}$. As described by Gorin, et al. (1985) the preparation of the STS solution proceeds as follows:

(1) Dissolve 0.079 g $AgNO_3$ in 500 ml of deionized water = Solution A

(2) Dissolve 0.462 g $Na_2S_2O_3.5H_2O$ in 500 ml of deionized water = Solution B

(3) Pour solution A into solution B while stirring. The concentration of silver was 0.463 mM.

Essential oil treatments

Essential oil treatments considered for long-time period were comprised of: (1) *Thymus vulgaris* alcoholic extract at the range of 0, 0.1 and 0.2 $mg*l^{-1}$ was applied. Thyme used in this research was obtained from the research institution of Isfahan Agricultural Research Center. First of all, 100 g leaves dry powder was placed in conical flask containing 1000 ml hydroalcohol (850 ml alcohol + 250 ml distilled water) and mixed by magnetic blender for 30 min. Afterwards, using shaker, the extract was shaken at 150 rmp for 72 hour. Subsequently, using watman filter paper, the extract was filtered. Using filtered limpid, extraction continued for 12 hour at 80 °C by using Vacuum Rotary Evaporator. Finally, obtained extract solution was placed in oven for 72 hour at 70 °C to prepare alcoholic extract. (2) Extracted *Stevia* leaves with acetone at the range of 0, 0.1 and 0.2 $mg*l^{-1}$ was applied. First of all, 150 g of air-dried powder of *Stevia* leaves was immersed in 100 ml of organic solvent containing 50 mg acetone and 50 mg distilled water. It was incubated at room temperature for 48 hour at 150 rmp in an orbital shaker. After that, using watman filter paper, filtered limpid was placed in hot air oven for 72 hour at 70 °C to prepare *Stevia* dry powder. The extract was dissolved in 0.25% Dimethyl Sulphoxide (DMSO, Merck) to a concentration of 100 $mg*ml^{-1}$ (Jayaraman, et al., 2008).

Determination of gerbera qualitative characteristics

Fresh weight (g*day^{-1}) rate

Evaluation of cut flower fresh weight was considerably important because of showing the amount of cut flower water uptake after harvesting time. The cut flowers were weighed using Sartorius model digital balance to determine the fresh weight after 0, 3, 6, 9 and 12 days of harvesting time. This method was applied to avoid air embolism for fresh weight determination (Solgi, et al., 2009).

(vase plastic weight + vase solution weight + cutting flower weight) – (vase plastic weight + vase solution weight)

Stem bending (degree*day^{-1})

The stem bending in gerberas was determined and classified based on 4 degrees of bending. Scape curvature was measured using a protractor and expressed with respect to the angle. The gerberas stem bending were rated as follows: 0 for bending up to 15°, 1 bending between 15 and 25, 2 for bending between 25° and 65°, 3 for bending between 65° and 90°, 4 for bending more than 90° (Celikel and Reid, 2002).

Capitulum diameter (cm*day^{-1})

The maximum flower diameter was assessed by measuring capitulum diameter from the back of capitulum at a sepal tangent using common ruler.

Vase-life (days after harvesting time)

In regard to absence of petal abscission observation, in order to determine the durability of vase-life, petal wilting and senescence accompanied stem bending (90 <) were considered and reported as the end of cut flower longevity (Gerasopoulos and Chebli, 1999).

Carotenoid pigments of petal (mg*g^{-1} initial fresh weight)

Carotenoid pigments of petal were assessed by Arnon method (1949) using liquid nitrogen. Initially, 0.5 g petal was placed in nitrogen tank (model: MVE 900) then after a few seconds when fresh petal sample dried, petal was transferred to mortar. Subsequently, after petal sample was ground, 5 ml of 80% acetone was added and then thoroughly mixed with sample. Later, the upper part of the solution was transferred to laboratory tubes and placed in centrifuge (Kokusan, model H-11N) with 4000 rmp for 20 min. After that, the upper part of limpid solution was filtered using watman filter paper and a limpid liquid was extracted. Ultimately, for determination of carotenoid uptake existed in extract, two ray spectrophotometer (Uvikon, model 922) within the wave length of 480-510 was used. Results were put in following formula:

$7.6\ (A_{480}) - 1.49\ (A_{510}) \times V/1000 \times 10 =$ mg carotenoid *g^{-1} sample

Statistical Analysis

In each treatment 4 flowers were placed in preservative solution and replicated 3 times. In each replication, 3 cut flowers were used for the measurement of qualitative characteristics and one flower was used to determine the amount of petal carotenoid pigment. Statistical analyses of the data were performed using SPSS statistical software. Factorial experiment in the layout completely randomized design (CRD) was used for analysis of variance (ANOVA) and mean comparison was established using Duncan's multiple range test ($P \leq 0.05$).

Results and Discussion

Fresh weight (g*day^{-1}) rate

Having exposed to 150 mg*l^{-1} BA, cut flowers fresh weight maintained at its highest level during 12 days. Also, in the case of BA application the impact of pulse treatments were insignificant (Table 1). Jalili Marandi, et al. (2011) announced that among various preservative solutions including SA, STS and ajowan oil, application of SA were the most efficient treatment for keeping cut gladiolus flowers fresh weight at optimum level. The efficacy of using SA was also reinforced when it was combined with 500 ppm ajowan oil and 4% sucrose. Concerning fresh weight in the presented experiment, after BA, GA_3 and SSA were the most efficient treatments respectively (Table 1). As well as this, pulse2 + SNPs (10 mg*l^{-1}) and pulse1 + *Thymus* essential oil (0.1 mg*l^{-1}) were more efficient than 8-HQS, which was perceived more beneficial for other qualitative measurements (Table 1, 2, 3 and 4). Moreover, *Thymus* essential oil (0.1 mg*l^{-1}) showed a desirable effect on extending vase-life (Table 4). Likewise, Solgi, et al. (2009) showed that in comparison to control treatment, 100 mg*l^{-1} carvacrol essential oil and 1 or 2 mg*l^{-1} SNPs effectively increased relative fresh weight and solution uptake of gerbera. However, in contrast to the results of this study, application of 6% sucrose pulse treatment combined with 5 mg*l^{-1} SNPs was considered more effective than 8-HQS on increasing flowers vase-life. As delineated by Hasan Abadi, et al. (2013) among chemical treatments comprised of silver nitrate, nano-silver and STS, preservative solutions containing silver nitrate followed by nano-silver were the most efficient treatments to hinder the fresh weight reduction of cut gerbera flowers respectively. After 12 days, only silver nitrate, especially at the concentration of 300 mg*l^{-1}, succeeded in maintaining cut flowers quality. Soad, et al. (2011) also described that $CaCl_2$ (1000 ppm) and 8-HQS (200 ppm) only when they were supplemented with 4% sucrose showed desirable effect on cut gerbera flower fresh weight. On the other hand, taking the fresh weight maintenance at high level into consideration, this study indicated that the positive effects of hormonal treatments were considerably and statistically more than chemical and essential oil treatments (Table 1).

Besides, having combined these hormonal treatments with pulse treatments, the differences were insignificant (Table 1). In agreement with the results of this survey, the benefit of using GA_3 and BA has been corroborated by Danaee, et al. (2011). As proclaimed by Danaee, et al. (2011), both BA and GA_3 at the concentration of 50 mg*l^{-1} along with 2.5% ethanol and 3% sucrose were considered as the most efficient treatments to improve the quality of vase-life, fresh weight, solution uptake, membrane stability and cut gerbera flowers TSS. However, in the present study, application of a higher concentration of 150 mg*l^{-1} BA culminated in maximizing fresh weight (Table 1). The effects of BA and GA_3 on membrane stability index improvement have also been reported (Danaee, et al., 2011); this measure shows the comparative electrolyte leakage by tissue, which reaches its lowest point by passing time from the first stage of postharvest prior to senescence. Studies revealed that preservative solution supplemented with sucrose increased stem mechanical rigidity by instigating cell wall thickening and lignifications of vascular tissues (Steinitz, 1982). Besides, it antagonizes the effect of ABA, which promotes senescence (Halevy and Mayak, 1979). Considering the aforementioned explanations, the efficacy of BA and GA_3 on cut gerbera flower fresh weight is likely owing to its antagonistic property toward ABA and senescence delaying as well.

Table 1. Interaction effects of long-time treatments combined with pulse treatments on cut gerbera flower fresh weight (g*day^{-1}) at different times.

Treatments			1st Day	3rd Day	6th Day	9th Day	12th Day
Pulse1 + Control	0	mg*l^{-1}	34.776^{c-j}	27.776^{h-n}	23.566^{k-o}	19.800^{e-i}	14.833^{de}
Pulse1 + *Thymus*	0.1	mg*l^{-1}	29.133^{ijk}	26.400^{mn}	20.933^{mno}	18.033^{j-l}	13.600^{d-g}
Pulse1 + *Thymus*	0.2	mg*l^{-1}	35.600^{b-h}	27.133^{k-n}	21.033^{mno}	19.166^{f-k}	15.800^{cd}
Pulse1 + *Stevia*	0.1	mg*l^{-1}	34.500^{c-j}	26.833^{k-n}	23.700^{l-o}	20.600^{e-h}	11.466^{d-k}
Pulse1 + *Stevia*	0.2	mg*l^{-1}	33.733^{d-k}	26.266^{n}	22.133^{mno}	16.966^{g-n}	11.433^{d-k}
Pulse1 + Control	0	mg*l^{-1}	34.666^{c-j}	33.620^{a-g}	31.100^{a-g}	28.596^{abc}	24.113^{ab}
Pulse1 + GA_3	25	mg*l^{-1}	32.233^{f-k}	31.643^{a-n}	28.600^{e-l}	26.670^{a-d}	22.113^{ab}
Pulse1 + GA_3	30	mg*l^{-1}	32.666^{e-k}	31.996^{a-l}	29.866^{b-i}	27.450^{abc}	23.313^{ab}
Pulse1 + BA	150	mg*l^{-1}	35.666^{b-h}	35.066^{a-e}	32.900^{a-e}	29.003^{abc}	22.503^{ab}
Pulse1 + BA	250	mg*l^{-1}	37.666^{b-f}	**36.866^{a}**	34.966^{ab}	31.113^{ab}	**25.803^{a}**
Pulse1 + SSA	100	mg*l^{-1}	35.000^{c-i}	34.00^{a-f}	31.966^{a-f}	25.893^{bcd}	20.923^{ab}
Pulse1 + SSA	200	mg*l^{-1}	33.666^{d-k}	32.666^{a-j}	30.166^{b-i}	26.670^{a-d}	22.623^{ab}
Pulse1 + Control	0	mg*l^{-1}	32.650^{e-k}	29.710^{e-n}	25.986^{g-m}	15.976^{h-n}	7.833^{i-l}
Pulse1 + STS	0.4	mM	38.376^{a-e}	32.070^{a-k}	24.653^{j-o}	17.976^{g-l}	9.000^{g-l}
Pulse1 + STS	0.8	mM	38.873^{a-d}	35.086^{a-e}	24.256^{k-o}	16.476^{h-n}	8.033^{h-l}
Pulse1 + SNPs	5	mg*l^{-1}	41.432^{ab}	35.066^{a-e}	29.443^{c-j}	15.383^{h-n}	8.000^{h-l}
Pulse1 + SNPs	10	mg*l^{-1}	37.086^{a-g}	31.900^{a-m}	25.553^{h-m}	15.956^{h-n}	8.443^{h-l}
Pulse1 + AOA	4	mM	31.800^{f-k}	30.733^{b-n}	30.106^{b-i}	13.233^{lmn}	5.693^{l}
Pulse1 + AOA	6	mM	33.540^{d-k}	31.486^{a-n}	29.110^{d-k}	17.336^{g-m}	8.243^{h-l}
Pulse1 + 8-HQS	200	mg*l^{-1}	37.153^{b-g}	33.146^{a-h}	27.333^{f-l}	16.993^{g-n}	8.930^{g-l}
Pulse1 + 8-HQS	400	mg*l^{-1}	36.764^{b-g}	34.400^{a-f}	29.753^{c-i}	13.663^{k-n}	6.706^{kl}
Pulse2 + Control	0	mg*l^{-1}	30.033^{h-k}	26.466^{lmn}	19.933^{o}	17.266^{g-m}	14.566^{de}
Pulse2 + *Thymus*	0.1	mg*l^{-1}	28.366^{k}	27.833^{h-n}	24.253^{k-o}	17.233^{g-m}	12.133^{d-i}
Pulse2 + *Thymus*	0.2	mg*l^{-1}	28.933^{jk}	28.066^{g-n}	23.666^{l-o}	17.066^{g-m}	9.133^{f-l}
Pulse2 + *Stevia*	0.1	mg*l^{-1}	31.233^{g-k}	30.033^{d-n}	24.600^{j-o}	14.466^{i-n}	9.466^{f-l}
Pulse2 + *Stevia*	0.2	mg*l^{-1}	29.800^{h-k}	28.800^{f-n}	22.166^{mno}	16.566^{h-n}	12.000^{d-j}
Pulse2 + Control	0	mg*l^{-1}	28.266^{k}	27.433^{i-n}	25.066^{i-n}	22.116^{d-g}	12.926^{d-h}
Pulse2 + GA_3	25	mg*l^{-1}	32.933^{d-k}	32.400^{a-k}	30.766^{a-g}	27.673^{abc}	23.870^{ab}
Pulse2 + GA_3	30	mg*l^{-1}	34.466^{c-j}	33.733^{a-f}	31.533^{a-f}	28.226^{abc}	24.476^{ab}
Pulse2 + BA	150	mg*l^{-1}	37.600^{b-f}	**36.633^{a}**	34.300^{abc}	**31.336^{a}**	**25.630^{a}**
Pulse2 + BA	250	mg*l^{-1}	32.966^{d-k}	32.000^{a-l}	29.966^{b-i}	27.780^{abc}	23.686^{ab}
Pulse2 + SSA	100	mg*l^{-1}	36.333^{b-j}	35.366^{a-e}	32.966^{a-e}	27.226^{a-d}	23.083^{ab}
Pulse2 + SSA	200	mg*l^{-1}	35.300^{c-i}	34.300^{a-f}	30.433^{a-h}	24.560^{cde}	19.793^{bc}
Pulse2 + Control	0	mg*l^{-1}	32.761^{e-k}	30.433^{c-n}	20.220^{no}	12.920^{lmn}	6.693^{kl}
Pulse2 + STS	0.4	mM	37.296^{b-g}	32.070^{a-k}	28.776^{e-k}	14.073^{j-n}	8.203^{h-l}
Pulse2 + STS	0.8	mM	40.030^{abc}	36.233^{ab}	29.440^{c-j}	14.680^{i-n}	9.233^{f-l}
Pulse2 + SNPs	5	mg*l^{-1}	35.710^{b-h}	32.953^{a-i}	28.326^{e-l}	15.176^{h-n}	8.163^{h-l}
Pulse2 + SNPs	10	mg*l^{-1}	38.376^{a-e}	**37.066^{a}**	30.266^{b-h}	23.996^{c-f}	13.976^{def}
Pulse2 + AOA	4	mM	**43.570^{a}**	**36.860^{a}**	34.330^{abc}	12.160^{mn}	7.913^{h-l}
Pulse2 + AOA	6	mM	37.863^{b-f}	35.366^{a-e}	**35.443^{a}**	11.573^{n}	7.000^{jkl}
Pulse2 + 8-HQS	200	mg*l^{-1}	37.030^{b-g}	36.066^{abc}	33.996^{a-d}	19.223^{f-j}	10.320^{e-l}
Pulse2 + 8-HQS	400	mg*l^{-1}	36.816^{b-g}	34.326^{a-f}	32.433^{a-f}	16.023^{h-n}	8.086^{h-l}

Pulse1 = distilled water for 24 h; Pulse2 = 4% CaCl2 + 3% sucrose for 24 h; Means sharing the same letters are not significantly different by Duncan's Multiple range test at P < 0.05.

Stem bending (degree*day^{-1})

The lowest stem bending was observed when cut flowers were exposed to 8-HQS (Table 2). In order to inhibit stem bending the concentrations of both 200 and 400 mg*l^{-1} 8-HQS were found considerably effective. On the other hand, these observations were not affected by pulse treatments perceptively.

Compared to control treatment, application of 8-HQS in preservative solution, led to significant reduction of stem bending. After 8-HQS + pulse1 application, the lowest stem bending was achieved when cut flowers were treated by 10 mg*l^{-1} SNPs and 0.4 mM STS, both in combination with 4% $CaCl_2$ + 3% sucrose for 24 hour. Apart from the cut flowers treated with these chemical preservatives, gerbera stem bending

was escalated vigorously by hormonal and essential oil preservative solutions (Table 2).

Table 2. Interaction effects of long-time treatments combined with pulse treatments on cut gerbera flowers stem bending (degree*day^{-1}) at different times.

Treatments			3rd Day	6th Day	9th Day	12th Day
Pulse1 + Control	0	mg*l^{-1}	32.666^{ab}	65.533^{d-i}	92.766^{g-p}	180.000^{k}
Pulse1 + *Thymus*	0.1	mg*l^{-1}	50.333^{bc}	58.866^{c-h}	78.333^{e-n}	101.133^{c-i}
Pulse1 + *Thymus*	0.2	mg*l^{-1}	32.666^{ab}	94.266^{g-l}	133.800^{o-r}	138.800^{g-k}
Pulse1 + *Stevia*	0.1	mg*l^{-1}	68.333^{cd}	91.066^{f-l}	145.000^{pqr}	180.000^{k}
Pulse1 + *Stevia*	0.2	mg*l^{-1}	77.833^{cd}	100.600^{h-l}	106.133^{j-q}	173.333^{jk}
Pulse1 + Control	0	mg*l^{-1}	71.633^{cd}	90.000^{e-l}	94.000^{h-p}	101.666^{c-i}
Pulse1 + GA_3	25	mg*l^{-1}	98.333^{de}	111.066^{i-l}	111.000^{k-q}	112.200^{d-j}
Pulse1 + GA_3	30	mg*l^{-1}	111.066^{e}	116.100^{jkl}	116.333^{l-q}	143.333^{h-k}
Pulse1 + BA	150	mg*l^{-1}	22.166^{ab}	68.866^{d-j}	68.866^{c-m}	107.200^{c-i}
Pulse1 + BA	250	mg*l^{-1}	23.966^{ab}	60.000^{c-h}	60.000^{a-k}	69.433^{a-f}
Pulse1 + SSA	100	mg*l^{-1}	31.420^{ab}	99.966^{h-l}	100.100^{i-p}	135.000^{g-k}
Pulse1 + SSA	200	mg*l^{-1}	12.300^{a}	66.086^{d-i}	66.220^{a-k}	115.000^{e-k}
Pulse1 + Control	0	mg*l^{-1}	6.110^{a}	47.776^{a-g}	62.766^{a-l}	88.333^{b-h}
Pulse1 + STS	0.4	mM	3.886^{a}	66.653^{d-i}	82.216^{d-m}	115.220^{e-k}
Pulse1 + STS	0.8	mM	3.216^{a}	82.996^{d-l}	92.766^{g-p}	123.886^{f-k}
Pulse1 + SNPs	5	mg*l^{-1}	1.165^{a}	14.440^{abc}	18.883^{a-d}	107.776^{c-i}
Pulse1 + SNPs	10	mg*l^{-1}	4.110^{a}	6.996^{ab}	38.883^{a-g}	62.766^{a-f}
Pulse1 + AOA	4	mM	2.530^{a}	12.550^{abc}	78.330 $^{e-n}$	160.000^{ijk}
Pulse1 + AOA	6	mM	**1.662^{a}**	6.420^{ab}	48.883^{a-i}	135.000^{g-k}
Pulse1 + 8-HQS	200	mg*l^{-1}	3.886^{a}	12.220^{ab}	12.220^{ab}	**25.000^{a}**
Pulse1 + 8-HQS	400	mg*l^{-1}	13.876^{a}	14.106^{abc}	37.220^{a-f}	48.553^{a-d}
Pulse2 + Control	0	mg*l^{-1}	122.800^{ef}	180.000^{m}	180.000^{r}	180.000^{k}
Pulse2 + *Thymus*	0.1	mg*l^{-1}	55.600^{bc}	83.366^{d-l}	130.000^{n-r}	180.000^{k}
Pulse2 + *Thymus*	0.2	mg*l^{-1}	21.166^{ab}	53.933^{b-h}	95.566^{h-p}	180.000^{k}
Pulse2 + *Stevia*	0.1	mg*l^{-1}	21.700^{ab}	98.366^{h-l}	175.000^{r}	180.000^{k}
Pulse2 + *Stevia*	0.2	mg*l^{-1}	34.466^{ab}	41.133^{a-e}	155.000^{qr}	180.000^{k}
Pulse2 + Control	0	mg*l^{-1}	150.000^{f}	180.000^{m}	180.000^{r}	180.000^{k}
Pulse2 + GA_3	25	mg*l^{-1}	22.733^{ab}	58.876^{c-h}	61.666^{a-k}	120.866^{f-k}
Pulse2 + GA_3	30	mg*l^{-1}	7.853^{a}	73.876^{d-k}	73.876^{d-m}	102.766^{c-i}
Pulse2 + BA	150	mg*l^{-1}	7.743^{a}	42.766^{a-f}	42.766^{a-h}	112.766^{d-j}
Pulse2 + BA	250	mg*l^{-1}	7.543^{a}	37.733^{a-d}	37.733^{a-e}	105.000^{c-i}
Pulse2 + SSA	100	mg*l^{-1}	6.600^{a}	37.186^{a-d}	37.666^{a-f}	150.000^{e-k}
Pulse2 + SSA	200	mg*l^{-1}	10.520^{a}	118.333^{kl}	118.333^{m-q}	160.000^{ijk}
Pulse2 + Control	0	mg*l^{-1}	120.553^{ef}	125.000^{l}	180.000^{r}	180.000^{k}
Pulse2 + STS	0.4	mM	4.553^{a}	4.660^{ab}	14.993^{abc}	54.440^{a-e}
Pulse2 + STS	0.8	mM	6.000^{a}	10.553^{abc}	22.773^{a-d}	105.776^{c-i}
Pulse2 + SNPs	5	mg*l^{-1}	9.333^{a}	11.216^{abc}	38.330^{a-g}	91.110^{b-h}
Pulse2 + SNPs	10	mg*l^{-1}	4.876^{ab}	5.110^{ab}	26.110^{a-e}	48.333^{a-d}
Pulse2 + AOA	4	mM	3.876^{a}	4.366^{ab}	55.553^{a-j}	160.000^{ijk}
Pulse2 + AOA	6	mM	4.973^{a}	8.553^{ab}	38.886^{a-g}	180.000^{k}
Pulse2 + 8-HQS	200	mg*l^{-1}	2.553^{a}	**2.643^{a}**	24.996^{a-e}	46.220^{abc}
Pulse2 + 8-HQS	400	mg*l^{-1}	3.886^{a}	6.886^{ab}	**6.886^{a}**	38.000^{ab}

Pulse1 = distilled water for 24 h; Pulse2 = 4% CaCl2 + 3% sucrose for 24 h; Means sharing the same letters are not significantly different by Duncan's Multiple range test at $P < 0.05$.

In regards with table 2, the chemical preservatives with higher antibacterial properties suppressed stem bending strikingly more. It has been cited that "bent neck is caused by insufficient flower stem hardening, maturation of the stem tissue below the harvest flower or level of dry matters and water content of flowers" (Danaee, et al., 2011). They reported that gerbera stem bending was hindered by applying 50 mg*l^{-1} BA and GA_3, whereas in this study hormonal treatments failed to hinder the increase of stem bending (Table 2). Studies unfolded that the presence of bacterial contamination in

preservative solution can trigger both vessel blockage on the cut surface (Nowak and Rudnicki, 1990) and ethylene production (Zagory and Reid, 1986) that consequently decrees cut flower water conductivity.

Considering the overall trend presented in table 2, apart from chemical preservatives, essential oil and hormonal treatment combined with pulse 1 resulted in lower stem bending. However, when these treatments were combined with pulse 2, the stem bending disorder increased. This result may be attributed to the capability of enhancing bacterial contamination by applying sucrose and efficiency of antibacterial preservatives. In addition, compared to *Stevia*, *Thymus* essential oil with higher antibacterial property resulted in lower stem bending (Table 2). Kazemi and Ameri (2012) disclosed that application of salicylic acid and nano-silver affected MDA content, ACO activity and anthocyanin leakage. Application of 5 $mg*l^{-1}$ nano-silver improved the permeability of membrane by reducing both ACO (ACC- oxidase) activity and MDA accumulation, which consequently prohibited the conversion of ACC into ethylene.

The positive effects of 8-HQS at 100 and 200 ppm supplemented with 4% sucrose on flower fresh and dry weight, longevity, soluble sugars and anthocyanin pigment in petal of gerbera (Soad, et al., 2011). Likewise, the present study implied that the efficacy of 8-HQS on stem bending (Table 2), vase-life longevity (Table 4) and capitulum diameter (Table 3) was noticeably more than all other preservatives.

Capitulum diameter ($cm*day^{-1}$)

As described by Ansari, et al. (2011) preservative solution supplemented with 5 $mg*l^{-1}$ SNPs + 4% sucrose effectively increased flower diameter of cut gerbera flower. As shown in table 3, apart from essential oils treatments, other treatments resulted in a desirable amount of capitulum diameter.

The highest length of capitulum diameter after 12 days was observed in 8-HQS treatments. In addition, considering the effects of 8-HQS on capitulum diameter, using pulse 1 and 2, capitulum diameter results did not vary significantly analogous to the results of other qualitative parameters. The differences between 200 and 400 $mg*l^{-1}$ of 8-HQS concentrations were not statistically significant in all qualitative parameters as well (Table 1, 2, 3, 4 and 5).

It can be inferred that 8-HQS was more beneficial than other preservatives likely mediated by higher antibacterial activity. High antibacterial efficiency can hinder the increase of bacterial accumulation at the end of stem, which leads to vessels plugging (Kazemi, et al., 2010) and ethylene synthesis (Zagory and Reid, 1986) that links to water conductivity disorder, wilting and decreasing capitulum diameter.

Vase-life (days after harvesting time)

As denoted in table 4, compared to all preservatives, cut gerbera flower vase-life was radically extended by applying preservative solutions supplemented with 8-HQS (200 and 400 $mg*l^{-1}$). However, short-time treatments failed to improve the efficacy of 8-HQS.

Table 3. Interaction effects of long-time treatments combined with pulse treatments on cut gerbera flowers capitulum diameter (cm*day^{-1}) at different times.

Treatments			1st Day	3rd Day	6th Day	9th Day	12th Day
Pulse1 + Control	0	mg*l^{-1}	11.083^{c-j}	10.986^{f-l}	7.100^{jk}	6.800^{k-o}	3.346^{l}
Pulse1 + *Thymus*	0.1	mg*l^{-1}	10.473^{k-p}	10.696^{g-n}	6.496^{k}	6.333^{k-o}	3.700^{l}
Pulse1 + *Thymus*	0.2	mg*l^{-1}	10.620^{i-o}	11.286^{b-g}	7.106^{jk}	5.166^{o}	3.010^{l}
Pulse1 + *Stevia*	0.1	mg*l^{-1}	11.086^{c-j}	11.010^{d-j}	6.240^{k}	5.933^{mno}	3.666^{l}
Pulse1 + *Stevia*	0.2	mg*l^{-1}	11.100^{c-j}	10.966^{f-k}	8.066^{ij}	5.620^{no}	2.986^{l}
Pulse1 + Control	0	mg*l^{-1}	11.653^{a-d}	11.653^{a-d}	11.343^{ab}	11.263^{a}	10.250^{ab}
Pulse1 + GA_3	25	mg*l^{-1}	11.363^{b-g}	11.363^{b-f}	11.343^{ab}	11.020^{ab}	9.383^{a-d}
Pulse1 + GA_3	30	mg*l^{-1}	11.650^{a-d}	11.650^{a-d}	**11.553^{a}**	**11.463^{a}**	10.273^{ab}
Pulse1 + BA	150	mg*l^{-1}	11.640^{a-e}	11.640^{a-e}	11.243^{abc}	9.916^{a-g}	8.296^{d-g}
Pulse1 + BA	250	mg*l^{-1}	**12.0733^{a}**	**12.073^{a}**	11.530^{a}	9.840^{a-g}	8.240^{d-g}
Pulse1 + SSA	100	mg*l^{-1}	11.686^{abc}	11.686^{abc}	11.263^{abc}	10.403^{a-f}	9.353^{a-d}
Pulse1 + SSA	200	mg*l^{-1}	11.876^{ab}	11.876^{ab}	10.830^{a-d}	10.586^{a-e}	10.310^{ab}
Pulse1 + Control	0	mg*l^{-1}	10.916^{f-l}	10.916^{f-l}	10.473^{a-e}	9.230^{c-h}	8.440^{c-g}
Pulse1 + STS	0.4	mM	10.986^{f-k}	10.986^{e-j}	9.563^{d-h}	9.553^{b-g}	9.396^{a-d}
Pulse1 + STS	0.8	mM	10.700^{h-n}	10.700^{g-n}	8.610^{g-i}	9.163^{d-h}	8.720^{b-f}
Pulse1 + SNPs	5	mg*l^{-1}	10.986^{f-k}	10.986^{f-j}	10.796^{a-d}	7.883^{h-k}	7.283^{e-k}
Pulse1 + SNPs	10	mg*l^{-1}	10.876^{f-l}	10.876^{f-m}	10.683^{a-e}	7.386^{i-m}	7.796^{d-j}
Pulse1 + AOA	4	mM	11.220^{c-i}	11.220^{c-h}	11.496^{a}	7.830^{h-l}	7.276^{e-k}
Pulse1 + AOA	6	mM	11.053^{d-k}	11.053^{c-i}	11.010^{a-d}	8.830^{h-l}	7.160^{f-k}
Pulse1 + 8-HQS	200	mg*l^{-1}	10.596^{j-o}	10.596^{h-n}	10.906^{a-d}	9.553^{b-g}	9.250^{a-e}
Pulse1 + 8-HQS	400	mg*l^{-1}	10.240^{mp}	10.240^{mno}	10.010^{a-d}	10.330^{a-f}	9.986^{abc}
Pulse2 + Control	0	mg*l^{-1}	10.376^{l-p}	10.453^{i-o}	9.286^{e-i}	7.900^{h-k}	6.510^{ijk}
Pulse2 + *Thymus*	0.1	mg*l^{-1}	10.030^{op}	10.293^{l-o}	9.866^{c-g}	7.066^{j-n}	6.310^{jk}
Pulse2 + *Thymus*	0.2	mg*l^{-1}	10.173^{nop}	10.143^{c-i}	8.986^{f-i}	6.733^{k-n}	5.686^{k}
Pulse2 + *Stevia*	0.1	mg*l^{-1}	10.460^{k-o}	10.400^{i-o}	8.266^{hij}	6.433^{k-o}	5.643^{k}
Pulse2 + *Stevia*	0.2	mg*l^{-1}	10.176^{m-p}	9.850^{o}	8.113^{ij}	6.266^{l-o}	5.833^{k}
Pulse2 + Control	0	mg*l^{-1}	9.853^{p}	9.853^{o}	8.110^{ij}	7.663^{h-l}	6.953^{g-k}
Pulse2 + GA_3	25	mg*l^{-1}	11.120^{c-j}	11.120^{c-h}	10.816^{a-d}	10.863^{abc}	9.096^{a-e}
Pulse2 + GA_3	30	mg*l^{-1}	11.040^{e-k}	11.040^{c-j}	10.786^{a-d}	10.630^{a-e}	9.183^{a-e}
Pulse2 + BA	150	mg*l^{-1}	11.296^{b-h}	11.296^{b-g}	11.133^{abc}	10.796^{a-d}	9.163^{a-e}
Pulse2 + BA	250	mg*l^{-1}	11.150^{c-j}	11.150^{c-h}	10.786^{a-d}	10.673^{a-e}	8.073^{d-i}
Pulse2 + SSA	100	mg*l^{-1}	11.396^{b-f}	11.396^{b-f}	11.163^{abc}	10.750^{a-e}	9.086^{a-e}
Pulse2 + SSA	200	mg*l^{-1}	11.230^{c-i}	11.230^{c-h}	10.410^{a-d}	10.410^{a-f}	7.910^{d-j}
Pulse2 + Control	0	mg*l^{-1}	10.106^{nop}	10.106^{nop}	8.953^{f-i}	7.273^{i-m}	6.553^{h-k}
Pulse2 + STS	0.4	mM	10.270^{mp}	10.270^{l-o}	10.260^{a-f}	9.930^{a-g}	9.183^{a-e}
Pulse2 + STS	0.8	mM	10.093^{nop}	10.093^{nop}	10.263^{a-f}	8.553^{g-j}	8.206^{d-h}
Pulse2 + SNPs	5	mg*l^{-1}	10.106^{nop}	10.106^{no}	10.283^{a-f}	9.493^{b-g}	8.716^{b-f}
Pulse2 + SNPs	10	mg*l^{-1}	10.330^{l-p}	10.330^{k-o}	10.616^{a-e}	9.106^{e-h}	8.886^{a-e}
Pulse2 + AOA	4	mM	11.000^{f-k}	11.000^{d-j}	10.906^{a-d}	9.443^{b-g}	7.773^{d-j}
Pulse2 + AOA	6	mM	11.040^{e-k}	11.140^{c-h}	11.296^{abc}	10.886^{ab}	7.110^{f-k}
Pulse2 + 8-HQS	200	mg*l^{-1}	10.763^{g-m}	10.696^{g-n}	10.553^{a-e}	10.553^{a-e}	10.073^{abc}
Pulse2 + 8-HQS	400	mg*l^{-1}	10.386^{l-p}	10.386^{j-o}	10.920^{a-d}	10.606^{a-e}	**10.563^{a}**

Pulse1 = distilled water for 24 h; Pulse2 = 4% CaCl2 + 3% sucrose for 24 h; Means sharing the same letters are not significantly different by Duncan's Multiple range test at P < 0.05.

The highest longevity was then followed by pulse2 + STS (0.4 mM) and pulse2 + SNPs (10 mg*l^{-1}). Another survey revealed that application of 200 ppm citric acid, 200 ppm AgNO3, 5% sucrose and 0.02% Tween-20 exerted the most positive effects on enhancing postharvest quality and longevity of gladiolus and *China aster* cultivars (Tiwari et al., 2010). Moreover, considering application of natural preservatives, *Thymus* essential oil with distilled water as a pulse treatment had the most desirable result (Table 4). This is in agreement with obtained results of cut gerbera flowers, where application of 200 mg*l^{-1} 8-HQS + 4% sucrose or 2000 mg*l^{-1} CaCl2 + 4% sucrose resulted in the highest longevity (Soad, et al., 2011). In the same way, in another research Oraee, et al. (2011) showed that thyme essential oil at the concentrations of 100 mg*l^{-1} and 6 mg*l^{-1} nano-silver was considered as the best

treatment to prolong gerbera vase-life. On the other hand, studies revealed that when nano-silver concentration rose by 6 to 8 and 10 mg*l^{-1}, toxic effect was detected without more antibacterial efficacy. Conversely, in consistent with our results, Safa, et al. (2012) described that the maximum vase-life and optimum flower diameter were achieved when cut gerbera flowers were treated by 10 mg*l^{-1} SNPs and fresh weight peaked at its highest point by applying 20 mg*l^{-1} nano-silver in preservative solution.

Table 4. Interaction effects of long-time treatments combined with pulse treatments on vase-life extension of cut gerbera flowers.

Treatments			Days	Treatments			Days
Pulse1 + Control	0	mg*l^{-1}	11.333^{c-i}	Pulse2 + Control	0	mg*l^{-1}	7.666^{klm}
Pulse1 + *Thymus*	0.1	mg*l^{-1}	13.106b^{cd}	Pulse2 + *Thymus*	0.1	mg*l^{-1}	10.776^{d-k}
Pulse1 + *Thymus*	0.2	mg*l^{-1}	10.886^{d-k}	Pulse2 + *Thymus*	0.2	mg*l^{-1}	10.886^{d-k}
Pulse1 + *Stevia*	0.1	mg*l^{-1}	10.886^{d-k}	Pulse2 + *Stevia*	0.1	mg*l^{-1}	8.000^{j-m}
Pulse1 + *Stevia*	0.2	mg*l^{-1}	10.886^{d-k}	Pulse2 + *Stevia*	0.2	mg*l^{-1}	9.440^{f-k}
Pulse1 + Control	0	mg*l^{-1}	11.773^{b-i}	Pulse2 + Control	0	mg*l^{-1}	6.000^{lm}
Pulse1 + GA_3	25	mg*l^{-1}	9.330^{f-k}	Pulse2 + GA_3	25	mg*l^{-1}	10.640^{d-k}
Pulse1 + GA_3	30	mg*l^{-1}	10.553^{d-k}	Pulse2 + GA_3	30	mg*l^{-1}	11.443^{c-i}
Pulse1 + BA	150	mg*l^{-1}	11.355^{c-i}	Pulse2 + BA	150	mg*l^{-1}	12.443^{b-f}
Pulse1 + BA	250	mg*l^{-1}	11.996^{b-h}	Pulse2 + BA	250	mg*l^{-1}	11.996^{b-h}
Pulse1 + SSA	100	mg*l^{-1}	9.553^{e-k}	Pulse2 + SSA	100	mg*l^{-1}	11.110^{c-j}
Pulse1 + SSA	200	mg*l^{-1}	11.430^{c-i}	Pulse2 + SSA	200	mg*l^{-1}	8.996^{h-k}
Pulse1 + Control	0	mg*l^{-1}	13.443^{bcd}	Pulse2 + Control	0	mg*l^{-1}	5.666^{m}
Pulse1 + STS	0.4	mM	11.663^{b-i}	Pulse2 + STS	0.4	mM	14.663^{b}
Pulse1 + STS	0.8	mM	9.110^{g-k}	Pulse2 + STS	0.8	mM	12.220^{b-h}
Pulse1 + SNPs	5	mg*l^{-1}	13.000^{bcd}	Pulse2 + SNPs	5	mg*l^{-1}	12.776^{b-e}
Pulse1 + SNPs	10	mg*l^{-1}	12.333^{b-g}	Pulse2 + SNPs	10	mg*l^{-1}	14.333^{bc}
Pulse1 + AOA	4	mM	9.666^{e-k}	Pulse2 + AOA	4	mM	8.666^{i-l}
Pulse1 + AOA	6	mM	9.440^{f-k}	Pulse2 + AOA	6	mM	9.553^{e-k}
Pulse1 + 8-HQS	200	mg*l^{-1}	**18.993^{a}**	Pulse2 + 8-HQS	200	mg*l^{-1}	**17.553^{a}**
Pulse1 + 8-HQS	400	mg*l^{-1}	**18.286^{a}**	Pulse2 + 8-HQS	400	mg*l^{-1}	**19.420^{a}**

Pulse1 = distilled water for 24 h; Pulse2 = 4% CaCl2 + 3% sucrose for 24 h; Means sharing the same letters are not significantly different by Duncan's Multiple range test at P < 0.05.

Carotenoid pigments of petal (mg*g^{-1} initial fresh weight)

The results of preservative solutions in this investigation had insignificant impact on the amount of carotenoid pigments. According to prior observations 8-HQS treatment can induced many positive effects on keeping the quality of cut gerbera flowers. For instance, pulse treatment with sucrose + 8-HQS effectively delayed chlorophyll degradation and preserved the soluble carbohydrates of *Antirrhinum majus* L. petals (Asrar, 2012). These functions might be attributed to the potential of ethylene inhibition by 8-HQS (Bartoli, et al., 1997).

Conclusion

It can be concluded that among all preservatives application of 8-HQS successfully has improved qualitative characteristics of cut gerbera flower. Considering the fact that keeping quality of this cut flower has not changed significantly by both pulse treatments and also 200 and 400 mg*l^{-1} concentration of 8-HQS, application of 200 mg*l^{-1} 8-HQS without pulse treatment of $CaCl_2$ and sucrose can be suggested. Keeping quality of cut gerbera flower, namely fresh weight, stem bending and vase-life as measured by number of days and capitulum diameter have been improved noticeably by 8-HQS application. However, compared to 8-HQS, SNPs, *Thymus*

essential oil and BA showed a superior effect on decreasing the fresh weight loss after harvesting time. In addition to decreasing fresh weight loss, *Thymus* essential oil gained credence to extend the vase-life of cut gerbera flowers. Considering the aforementioned results, it can be also concluded that combination of BA and thyme essential oil together or with 8-HQS might be useful for future investigations. All in all, 200 $mg*l^{-1}$ 8-HQS without pulse treatment is recognized as the most suitable component in preservative solution for prolonging the vase-life and improving the postharvest quality of cut gerbera flowers.

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