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Evaluation the Effect of Nanosilver with Salicylic Acid and Benzyladenine on Longevity of Gerbera Flowers

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ABSTRACT

In the view of Benzyladenine (BA), salicylic acid (SA) and nanosilver (NS) potential, the present research was conducted to screen various treatments for promoting the vase life and evaluating the possible physiological induced changes during the postharvest life of gerbera cut flowers (*Gerbera jamesonii* Dune). The gerbera cut flowers in sixteen different groups were treated in pulse method (24 h) by the four levels of benzyladenine (0, 50, 100 and 150 mgL^{-1}) or salicylic acid (0, 50, 100 and 200 mgL^{-1}) containing 8% sucrose and then transferred to the solution containing distilled water or nanosilver of 5 mgL^{-1}. As the time went by, the reduction rates of the weight, the soluble solid contents, anthocyanin levels and the membrane stability were declined by the applied preservatives, BA and SA, which the second one was more effective and the effectiveness of the used preservatives were promoted by the nanosilver usage. The activities of phenylalanine ammonia lyase (PAL), catalase (CAT) and superoxide dismutase (SOD) decreased with the passage of time, however, the applied treatments of SA or BA, especially the combined ones with nanosilver, led to the induced activities of the mentioned enzymes. The applied preservatives had promoting effects on the longevity of gerbera cut flowers where the highest used concentration of SA in combination with nanosilver was the most effective treatment. Results indicated that the efficacy of SA or BA on the vase life of gerbera cut flowers significantly promoted by the applied concentration of nanosilver and with increasing the tested levels of BA, the longevity of treated cut flowers reversely affected, most probably due to higher pH, suitable condition for bacterial contamination.

KEYWORDS: Cytokinin*; Gerbera jamesonii*; ornamental; postharvest; preservative

Abbreviations: BA- Benzyladenine; SA- Salicylic acid; NS- Nanosilver; SOD- Superoxide dismutase; PAL- Phenylalanine ammonia lyase; CAT- Catalase

INTRODUCTION

Nowadays with respect to the high competition in the market, there is more concern about postharvest life of fruits and cut flowers. It is obvious that cut flowers are facing wounding, water deficit, microbial contamination and oxidative stresses during the postharvest life. According to the scientific findings, the postharvest life of different ornamental cut flowers could be affected by the application of various chemicals as preservatives [24:3:23].

It has been stated that microbial contamination, the cause of vascular occlusion, is the most limiting factor in longevity of cut flowers [11]. Thus, with the aim of promoting the vase life of cut flowers, chemicals with antimicrobial effects such as silver nitrate, aluminium sulphate and 8-hydroxyquinoline sulphate, have been applied in vase solutions [17].

In recent years, nanosilver (NS), as a novel antiseptic, is being applied to the many industrial process like medical industry, water purification and vegetable disinfection [25]. In addition, nanosilver treatment has been proposed for the aim of modifying the postharvest life of cut flowers [13:14].

The participation of salicylic acid (SA), a natural phenolic secondary metabolite, in various aspects of vital processes like ethylene biosynthesis, stomatal conductance, respiration, senescence and the activation of defense systems against different pathogens is well documented [26:6:20:2:16] and its exogenously application may modify some plant reactions like respiration, transpiration and senescence [2]. It has been stated that acetyl salicylic acid may have potential postharvest application for alleviating chilling injury, maintain quality and improve the health benefits of pomegranate fruit consumption by inducing the antioxidant system [28].

Exogenously application of cytokinine because of its participation in critical process like nutrient mobilization, tissue metabolism, senescence and stress [27] may induce changes led to desirable results in

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horticultural activities. Benzyladenine (BA), a synthetic cytokinine, has been used by many researches mainly because of its possible impacts on crucial process such as cell divisions, senescence processes, biochemical reactions and activities of various enzymes.

These features highlight the significance of BA, SA and NS in agricultural applications. Gerbera, a member of Asteraceae family, greatly considered as valuable ornamental plants [3**]**. As the effects of diferent concentrations of various preservatives on the postharvest life of cut flowers are changeable depending on plant species, the applied chemicals, the method of treatment and interaction of compounds present in vase solution, the determination of the effective economical preservatives as well as the method of application is of importance and concern for modern horticulture. In view of BA, SA and NS potential, the present research was conducted to screen various treatments for promoting the vase life as well as evaluating the possible physiological induced changes by the applied mixture treatments during the postharvest life of gerbera cut flowers.

MATERIAL AND METHODS

Cut gerbera flowers, *Gerbera jamesonii* Dune, were obtained from the reliable commercial growers (Banaei greenhouse, Pakdasht, Tehran). The flowers were harvested just before sunrise at the mature stage. The experiments were performed in the postharvest room (22 ± 1 °C, 60 ± 5% relative humidity, and 12h photoperiods). The gerbera cut flowers were treated in pulse method (24 hours) by the four different levels of benzyladenine (0, 50, 100 and 150 mgL^{-1}) or salicylic acid (0, 50, 100 and 200 mgL^{-1}) containing 8% sucrose. These pulse-treated cut flowers were divided in two groups and transferred to the solution containing distilled water or nanosilver of 5 mgL^{-1}. The cut flowers treated in four different groups including distilled water, pulse treated in 8% sucrose transferred to the distilled water or nanosilver (5 mgL^{-1}) and nanosilver in long term method were used as different control groups. The forty-five centimeter long flowers were cut, weighed, placed in 500 mL containers and were grouped into sixteen different treatment groups with three replications and five flowers per each replication which treatments called as follows:

distilled water (control dw), nanosilver (cotrol nano), distilled water- sucrose (control S dw), nanosilver-sucrose (control S nano), salicylic acid of 50 mgL^{-1}- sucrose (SA50 S dw), salicylic acid of 50 mgL^{-1}, sucrose-nanosilver (SA50 S nano), salicylic acid of 100 mgL^{-1}- sucrose (SA100 S dw), salicylic acid of 100 mgL^{-1}– sucrose-nanosilver (SA100 S nano), salicylic acid of 200 mgL^{-1}- sucrose (SA200 S dw), salicylic acid of 200 mgL^{-1}– sucrose- nanosilver (SA200 S nano), banzyladenine of 50 mgL^{-1}- sucrose (BA50 S dw), banzyladenine of 50 mgL^{-1}- sucrose- nanosilver (BA50 S nano), banzyladenine of 100 mgL^{-1}- sucrose (BA100 S dw), banzyladenine of 100 mgL^{-1}- sucrose- nanosilver (BA100 S nano), banzyladenine of 150 mgL^{-1}- sucrose (BA150 S dw) and banzyladenine of 150 mgL^{-1}- sucrose- nanosilver (BA150 S nano).

The relative fresh weight was calculated during the experimental days and expressed in percent.

The total amount of soluble solids of cut flower stems was measured by digital refractometer and expressed in Brix.

The concentration of anthocyanin was determined by absorbance measurement at 530 nm and 657 nm on a UV-visible spectrophotometer (Varian, Model: Cary 50 Scan). The formula $\Delta A=A_{530}-1/4A_{657}$ was used to deduct the absorbance contributed by chlorophyll and its degradation products in the extract. The results were plotted as $\Delta A\ g^{-1}$ FW.

MDA content was determined with thiobarbituric acid (TBA) reaction. 0.2 g tissue sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10000 × g for 5 min. 4 ml of 20% TCA containing 0.5% TBA was added to 1 ml aliquot of the supernatant. The mixture was heated at 95ºc for 15 min and cooled immediately. The non-specific absorbance of the supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for MDA measurement. The results were plotted as $\mu m\ g^{-1}$ FW.

The reaction mixture for PAL activity consisted of 6 µM phenylalanine, Tris-HCl buffer (0.5 M pH 8) and 200µl of enzyme extract. After 60 min at 37 °C, the reaction was terminated by the addition of 50 µl of 5 N HCl. PAL activity was determined as the rate of conversion of L-phenylalanine to trans- cinnamic acid at 290 nm. PAL activity was assessed by measuring the amount of cinnamic acid produced and was expressed as microgram of cinnamate per microgram of protein, according to the procedure described by Beaudoin-Eagan and Thrope (1985).

CAT activity was assayed by the photochemical method described by Aebi (1984). The activity was expressed as Unit E g^{-1} FW.

SOD activity was assayed by the photochemical method described by Giannopolitis and Ries (1977). One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT (nitro blue tetrazolium) reduction measured at 560 nm. The activity was expressed as Unit E g^{-1} FW.

The vase life of cut flowers was completed until petal wilting. The vase life was determined from the number of days to senescence of the cut flowers.

The experimental design was the completely randomized one. Data were analyzed as a factorial experiment by analysis of variance using SPSS software. Mean separation was performed with Duncan's multiple range test at $P < 0.05$.

RESULTS

With the passage of time, the amounts of relative fresh weight gradually decreased although the application of different concentrations of BA or SA had declining effects on the weight loss of the cut flowers (Table 1). The total soluble solids of the cut flowers decreased with time, however reduction rates in SA, BA and/or nanosilver treated samples, especially the first one combined with the nanosilver, were delayed by the utilization of the mentioned preservatives and the effectiveness of BA and SA were increased by the nanosilver as indicated by the best results obtained in SA200 S nano, SA200 S dw, BA50 S nano (Table 2). As it was shown in table 3, in comparison to the control samples, the rates of anthocyanin degradation during the experimental days were reduced by the application of SA or BA which first one were more effective and The effectiveness of the used treatments of SA or BA was promoted by the utilization of nanosilver. compared to the controls, the alleviated lipid peroxidation levels shown by the fewer amounts of malondealdehyde (MDA) contents resulted from the used preservatives which the highest membrane stability were found in SA200 S nano, SA200 S dw and BA50 S nano (Table 4).

As the time went by, the activities of PAL, CAT and SOD diminished, however, the applied treatments of SA or BA, especially the first one, led to the induced activities of the mentioned enzymes compared to the control groups as indicated by the highest amounts recorded in SA200 S nano, SA200 S dw and BA50 S nano (Table 5, 6, 7).

As it was shown in figure , the applied preservatives had promoting effects on the longevity of gerbera cut flowers where the best results were observed in SA200 S nano, BA50 S nano, SA200 S dw, BA100 S nano and SA100 S dw groups, respectively. Results indicated that the efficacy of SA or BA on the vase life of gerbera cut flowers significantly enhanced by the applied concentration of nanosilver. With increasing the tested levels of BA, the longevity of treated cut flowers reversely affected.

Table 1. The effects of different applied preservatives including salicylic acid or banzyladenine and/or nanosiver on relative fresh weights of gerbera cut flowers (expressed as %) over 7 days.

Days treatments	0	1	4	7
control dw	100^{a}	100.96^{g}	56.29^{c}	.
cotrol nano	100^{a}	100.98^{g}	65.42^{b}	.
control S dw	100^{a}	101.50^{efg}	61.89^{bc}	.
control S nano	100^{a}	101.52^{efg}	68.21^{b}	.
SA50 S dw	100^{a}	103.33^{def}	87.97^{a}	69.78^{b}
SA50 S nano	100^{a}	103.53^{de}	90.20^{a}	74.88^{ab}
SA100 S dw	100^{a}	103.36^{de}	87.07^{a}	73.65^{ab}
SA100 S nano	100^{a}	104.26^{bcd}	89.03^{a}	73.91^{ab}
SA200 S dw	100^{a}	106.27^{a}	89.49^{a}	76.87^{ab}
SA200 S nano	100^{a}	106.78^{a}	89.76^{a}	79.82^{a}
BA50 S dw	100^{a}	102.39^{defg}	86.73^{a}	71.19^{ab}
BA50 S nano	100^{a}	105.58^{ab}	89.46^{a}	75.24^{ab}
BA100 S dw	100^{a}	101.66^{efg}	88.09^{a}	73.63^{ab}
BA100 S nano	100^{a}	105.32^{abc}	89.76^{a}	75.27^{ab}
BA150 S dw	100^{a}	$101.30f^{g}$	86.67^{a}	70.18^{b}
BA150 S nano	100^{a}	101.64^{efg}	87.62^{a}	72.61^{ab}

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 2. The effects of different applied preservatives including salicylic acid, banzyladenine and/or nanosiver on total soluble solid of gerbera cut flowers (expressed in Brixes) over 7 days.

Days treatments	0	1	4	7
control dw	5.27[a]	5.20[c]	3.97[g]	.
cotrol nano	5.27[a]	5.00[c]	4.40[g]	.
control S dw	5.27[a]	6.00[b]	4.73[ef]	.
control S nano	5.27[a]	6.13[b]	5.07[de]	.
SA50 S dw	5.27[a]	6.17[b]	5.53[bcd]	4.63[de]
SA50 S nano	5.27[a]	6.23[ab]	5.47[cd]	4.80[cde]
SA100 S dw	5.27[a]	6.13[b]	5.50[bcd]	5.00[abc]
SA100 S nano	5.27[a]	6.23[ab]	5.73[bc]	5.13[bc]
SA200 S dw	5.27[a]	6.57[ab]	6.03[ab]	5.37[ab]
SA200 S nano	5.27[a]	6.73[a]	6.33[ab]	5.70[a]
BA50 S dw	5.27[a]	6.07[b]	5.43[cd]	4.77[cde]
BA50 S nano	5.27[a]	6.33[ab]	5.90[abc]	5.40[ab]
BA100 S dw	5.27[a]	6.20[ab]	5.60[bcd]	4.67[de]
BA100 S nano	5.27[a]	6.20[ab]	5.70[bc]	5.13[bc]
BA150 S dw	5.27[a]	6.00[b]	5.43[cd]	4.53[e]
BA150 S nano	5.27[a]	6.10[b]	5.47[cd]	4.77[cde]

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 3. The effects of different applied preservatives including salicylic acid, banzyladenine and/or nanosiver on anthocyanin contents of gerbera cut flowers (expressed in $\Delta A\ g^{-1}$ FW) over 7 days.

Days treatments	0	1	4	7
control dw	0.8255[a]	0.6085[d]	0.4808[b]	.
cotrol nano	0.8255[a]	0.6728[cd]	0.5223[b]	.
control S dw	0.8255[a]	0.6295[cd]	0.4998[b]	.
control S nano	0.8255[a]	0.7005[bc]	0.5310[b]	.
SA50 S dw	0.8255[a]	0.7779[ab]	0.6901[a]	0.5968[a]
SA50 S nano	0.8255[a]	0.8004[a]	0.7157[a]	0.6074[a]
SA100 S dw	0.8255[a]	0.7761[ab]	0.7181[a]	0.6375[a]
SA100 S nano	0.8255[a]	0.7972[a]	0.7307[a]	0.6362[a]
SA200 S dw	0.8255[a]	0.8028[a]	0.7288[a]	0.6502[a]
SA200 S nano	0.8255[a]	0.816[a]	0.7713[a]	0.6697[a]
BA50 S dw	0.8255[a]	0.8011[a]	0.7199[a]	0.6350[a]
BA50 S nano	0.8255[a]	0.8132[a]	0.7631[a]	0.6471[a]
BA100 S dw	0.8255[a]	0.7853[ab]	0.7205[a]	0.5996[a]
BA100 S nano	0.8255[a]	0.7934[a]	0.7382[a]	0.6407[a]
BA150 S dw	0.8255[a]	0.7747[ab]	0.6833[a]	0.5994[a]
BA150 S nano	0.8255[a]	0.7933[a]	0.7243[a]	0.6377[a]

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 4. The effects of different applied preservatives including salicylic acid, banzyladenine and/or nanosiver on MDA contents of gerbera cut flowers (expressed in $\mu m\ g^{-1}$ FW) over seven days.

Days treatments	0	1	4	7
control dw	12.8[a]	26.9[h]	35.73[h]	.
cotrol nano	12.8[a]	23.67[gh]	32.63[g]	.
control S dw	12.8[a]	25.5gh	34[gh]	.
control S nano	12.8[a]	22.63[fg]	31.77[g]	.
SA50 S dw	12.8[a]	19.97[ef]	24.43[f]	28.43[h]
SA50 S nano	12.8[a]	17.8[bcde]	22.17[ef]	24.83[ef]
SA100 S dw	12.8[a]	16.2[abcd]	19.2[bcd]	23.37[cde]
SA100 S nano	12.8[a]	15.43[abc]	18.87[abcd]	21.90[bc]
SA200 S dw	12.8[a]	14.67[ab]	17.63[abc]	20.53[ab]
SA200 S nano	12.8[a]	13.53[a]	16.13[a]	19.37[a]
BA50 S dw	12.8[a]	17.17[abcde]	21.6d[ef]	24.63[def]
BA50 S nano	12.8[a]	13.7[a]	16.8[bc]	19.87[a]
BA100 S dw	12.8[a]	19.3[def]	23.77[f]	27.13[gh]
BA100 S nano	12.8[a]	15.53[abc]	18.8[abcd]	23.03[cd]
BA150 S dw	12.8[a]	18.57[cde]	22.87[ef]	25.80[fg]
BA150 S nano	12.8[a]	16.6[abcde]	20.27[cde]	24.10[de]

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 5. The effects of different applied preservatives including salicylic acid, banzyladenine and/or nanosiver on PAL activities of gerbera cut flowers (expressed in microgram of cinnamate per microgram of protein) over seven days.

Days treatments	0	1	4	7
control dw	16.200^{a}	13.9^{c}	8.87^{c}	.
cotrol nano	16.200^{a}	14.1b^{c}	9.1^{c}	.
control S dw	16.200^{a}	13.97^{c}	9^{c}	.
control S nano	16.200^{a}	14.23^{abc}	9.1^{c}	.
SA50 S dw	16.200^{a}	14.4^{abc}	12.27^{b}	10.07^{f}
SA50 S nano	16.200^{a}	14.67^{abc}	12.9ab	10.57^{def}
SA100 S dw	16.200^{a}	15.1^{abc}	13.47^{ab}	11.17^{bcde}
SA100 S nano	16.200^{a}	15.5^{abc}	13.9ab	11.67^{abc}
SA200 S dw	16.200^{a}	15.77^{ab}	14^{ab}	11.97^{ab}
SA200 S nano	16.200^{a}	15.93^{a}	14.4a	12.43^{a}
BA50 S dw	16.200^{a}	14.77^{abc}	13.1^{ab}	10.77^{cdef}
BA50 S nano	16.200^{a}	15.87^{a}	14.27^{ab}	12.3^{a}
BA100 S dw	16.200^{a}	14.5^{abc}	12.37^{b}	10.1^{f}
BA100 S nano	16.200^{a}	15.3^{abc}	13.67^{ab}	11.53^{abcd}
BA150 S dw	16.200^{a}	14.53^{abc}	12.63^{a}	10.47^{ef}
BA150 S nano	16.200^{a}	14.93^{abc}	13.3^{ab}	11.07^{bcdef}

*Mean values followed by different letters are significantly different at P < 0.05 according to Duncan's multiple range test.

Table 6. The effects of different applied preservatives including salicylic acid, banzyladenine and/or nanosiver on CAT activities of gerbera cut flowers (expressed in Unit E g^{-1} FW) over seven days.

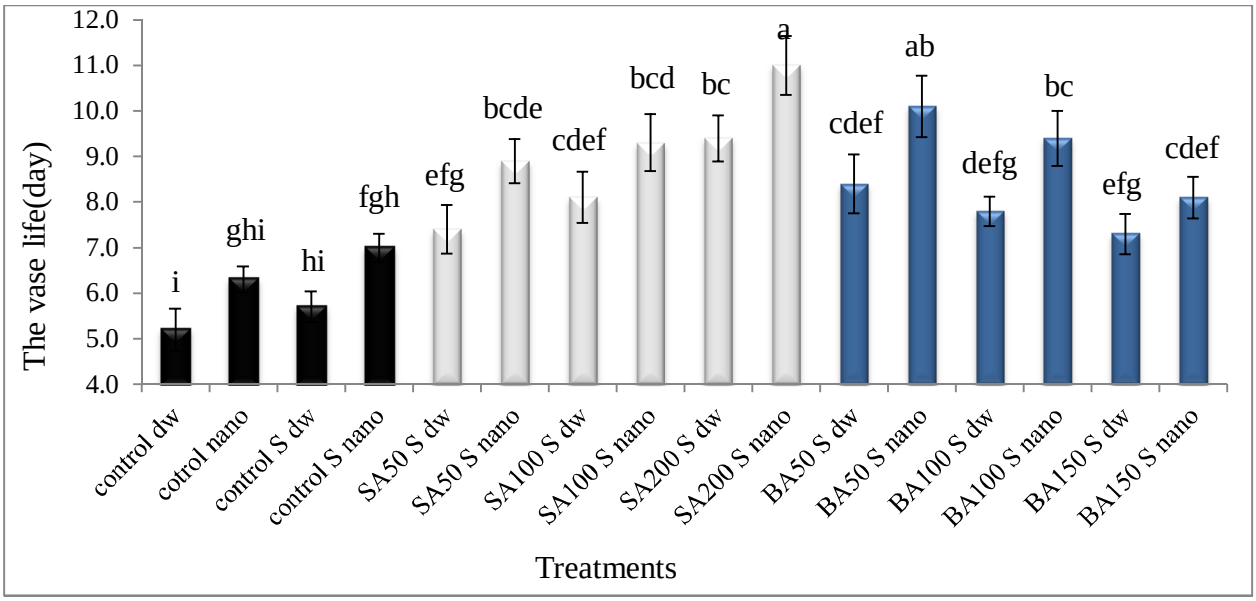
Days treatments	0	1	4	7
control dw	6.70^{a}	5.37^{f}	2.37^{c}	.
cotrol nano	6.70^{a}	5.43^{ef}	2.53^{c}	.
control S dw	6.70^{a}	5.50^{def}	2.63^{c}	.
control S nano	6.70^{a}	5.67^{cdef}	2.70^{c}	.
SA50 S dw	6.70^{a}	5.77^{bcdef}	4.63^{ab}	3.63^{ab}
SA50 S nano	6.70^{a}	5.83^{abcdef}	5.07^{ab}	3.80^{ab}
SA100 S dw	6.70^{a}	6.17^{abcde}	5.13^{ab}	4.20^{ab}
SA100 S nano	6.70^{a}	6.30^{abc}	5.30^{ab}	4.37^{ab}
SA200 S dw	6.70^{a}	6.40^{abc}	5.37^{ab}	4.47^{ab}
SA200 S nano	6.70^{a}	6.60^{a}	5.70^{a}	4.73^{a}
BA50 S dw	6.70^{a}	6.00^{abcdef}	5.10^{ab}	3.90^{ab}
BA50 S nano	6.70^{a}	6.50^{ab}	5.50^{ab}	4.57^{ab}
BA100 S dw	6.70^{a}	5.83^{abcdef}	4.83^{ab}	3.60^{b}
BA100 S nano	6.70^{a}	6.27^{abcd}	5.33^{ab}	4.17^{ab}
BA150 S dw	6.70^{a}	5.83^{abcdef}	4.87^{b}	3.80^{ab}
BA150 S nano	6.70^{a}	6.17^{abcde}	5.17^{ab}	3.97^{ab}

*Mean values followed by different letters are significantly different at P < 0.05 according to Duncan's multiple range test.

Table 7. The effects of different applied preservatives including salicylic acid, banzyladenine and/or nanosiver on SOD activities of gerbera cut flowers (expressed in Unit E g^{-1} FW) over 7 days.

Days treatments	0	1	4	7
control dw	6.1^{a}	4.77^{e}	1.7^{b}	.
cotrol nano	6.1^{a}	4.83^{de}	1.8^{b}	.
control S dw	6.1^{a}	4.90^{cde}	2.03^{b}	.
control S nano	6.1^{a}	5.00^{bcde}	2.1^{b}	.
SA50 S dw	6.1^{a}	5.17^{abcde}	4.23^{a}	3.20^{a}
SA50 S nano	6.1^{a}	5.33^{abcde}	4.53^{a}	3.43^{a}
SA100 S dw	6.1^{a}	5.57^{abcd}	4.63^{a}	3.60^{a}
SA100 S nano	6.1^{a}	5.70^{ab}	4.7^{a}	3.77^{a}
SA200 S dw	6.1^{a}	5.80^{a}	4.77^{a}	3.87^{a}
SA200 S nano	6.1^{a}	5.87^{a}	4.97^{a}	4.03^{a}
BA50 S dw	6.1^{a}	5.50^{abcde}	4.53^{a}	3.43^{a}
BA50 S nano	6.1^{a}	5.87^{a}	4.9^{a}	3.97^{a}
BA100 S dw	6.1^{a}	5.30^{abcde}	4.33^{a}	3.30^{a}
BA100 S nano	6.1^{a}	5.67^{abc}	4.73^{a}	3.63^{a}
BA150 S dw	6.1^{a}	5.27^{abcde}	4.47^{a}	3.37^{a}
BA150 S nano	6.1^{a}	5.57^{abcd}	4.6^{a}	3.50^{a}

*Mean values followed by different letters are significantly different at P < 0.05 according to Duncan's multiple range test.



Figure– The effects of nanosilver on efficacy of SA or BA as preservatives on the vase life of gerbera cut flowers.

DISCUSSION

As the time went by, the reductions of the weight, soluble solid contents and membrane stability were observed which is natural and expectable; however, their rates were significantly declined by the applied preservatives, BA and SA (especially the second one). the presented results indicated that the rates of anthocyanin degradation were reduced by the application of SA or BA which the first one were more effective and the effectiveness of the used preservatives were promoted by the nanosilver usage. It is possible that the inhibited microbial contamination, increased solution uptake, induced antioxidant enzymes and reduced production of reactive oxygen species and ethylene led to the fewer stress level, thereby declining lipid peroxidation levels and antocyanin degradation rates in BA or SA treated cut flowers in comparison to the controls. The declined degradation rates of compounds like anthocyanin and the alleviated senescence observed in nanosilver treated samples could be resulted from the decreased microbial infection and silver inhibited action of ethylene.

Salicylic acid (SA) is known as an inhibitor of ethylene biosynthesis thereby delaying the senescence process. In addition to involvement of SA in local and systemic resistance to pathogens [36:10], it has been stated that SA suppress the conversion of ACC into ethylene by inhibiting the ACC oxidase activity [4].

Bacteria and their decay products caused stem blockage and water deficit [15:33] as well as secreted pectic enzymes and toxic compounds by bacteria, and/or produced ethylene resulting in the accelerated senescence [32] are main limiting factors during the postharvest life of cut flowers. As the restricted solution uptake by the stem blockage caused by the microbial contamination and/or air emboli is one of the most limiting factors in postharvest life of cut flowers, acidic solutions (unsuitable condition for bacterial growth) are usually considered to have desirable effects for the most cut flowers. The microbial contamination, air emboli and physiological wound induced have been mentioned as typical reason for stem blockage [15:7:35]. The application of a germicide and lowering the pH of the vase solution may extend the longevity of Acacia cut flowers [8].

It is clear that petal carbohydrate contents are one of the most crucial factors influencing the postharvest life of cut flowers. It is proposed that there is positive correlation between the levels of endogenous sugars and the time to petal wilting [34]. In addition, the senescence process of cut flowers is regulated by phytohormones and correlated with the carbohydrate status of the petals [29:34]. Based on some other studies in this field, it has been stated that the senescence process and longevity of cut flowers is closely correlated with the petal carbohydrate contents and solution uptake [29:34:3:22:23]. Cytokinine involvements in wide ranges of critical process such as affecting nutrient mobilization, forming sink tissues and delaying senescence have been well documented [27]. The cytokinin benzyladenine (BA) delayed petal blackening in cut lotus flowers (*Nelumbo nucifera* Gaertn. cv. Saddabutra) [9]. BA treatments improved postproduction quality of *Tulipa gesneriana* improved [19]. The obtained results from the present research indicated that the efficacy of the BA utilization with increasing the used concentrations reduced, in contrast to SA. In addition, SA treatments are more effective than BA and the efficacy of

SA and BA were increased by the applied levels of nanosilver. The presented results indicated that the individual application of nanosilver did not have desirable economical benefits but the combined utilization with BA or SA resulted in the promoted efficacy of them to extend the vase life of gerbera cut flowers. It seems that the application of nanosilver may raise the BA functioning probably via reducing the microbial growth rates.

The benefits of NS on the postharvest life of cut flowers are attributed to its antimicrobial effects as well as affecting ethylene production and transpiration rates. Despite of the proposed benefits of nanosilver usage, the high levels of nanosilver may be detrimental. The applications of nanosilver in pulse way were effective to extend the vase life of cut rose flowers and suppress the reduction in fresh weight during the vase period whereas a high level was phytotoxic [13:14]. The applied level of nanosilver in this study did not have damaging effects; however, its individual application was not effective as much as BA or SA.

Silver ion (Ag+) is known as an efficient growth inhibitor of microorganisms [25]. NSs function properly as effective germicides mainly due to their high surface area to volume ratio among other unique chemical and physical properties thereby providing good contact with microorganisms [25] and therein, they may prevent critical processes such as respiration and cell division and subsequently leading to cell death [13:14]. Nanosilver utilization in pulse way was effective to improve the longevity of cut gerbera via preventing the growth of bacteria [13:14]. The nanosilver improved water relations prolonged the vase life of rose cut flowers [17:18]. The NS efficacy in alleviating bacterial related blockage in the stem ends of cut roses using scanning electron microscopy was estimated to be about 80%-100% against the four tested bacterial strains [17:18]. *Invitro* and microscopy analysis showed that NS applied in pulse way inhibited bacterial growth in the vase solution and at cut stem ends during the first two tested days [13:14]. In addition to antiseptic effects, NS may act as an anti ethylene agent. NS reduced stomatal conductance in rose cut flowers resulted in inhibited leaf transpiration. Also, the expression of the aquaporin gene, *Rh-PIP2*, in rose cut flowers was affected by the application of NS treatments [13:14]. the desirable effectiveness of NS in post harvest life of various cut flowers have been attributed to its antimicrobial properties [30:17:14], functioning as an ethylene inhibitor [12] and/or modified stomatal conductance [12:13:14]. The efficacy of NS in extending the vase life of some cut flowers, including cut gerberas [29:17] and roses have been reported.

Considering that antioxidative enzymes are directly involved in scavenging of reactive oxygen species, measurements of their activities may provide information about status to which tissue is exposed to them. The activities of PAL (the key enzyme of phenylpropanoid metabolism), CAT and SOD (as the components of antioxidative system) declined with the passage of time, however, the applied treatments of SA or BA, especially the combined ones with nanosilver, led to the induced activities of the mentioned enzymes in comparison to the control groups. The production of reactive oxygen species suggested as a main signal for alerting and modifying metabolism and gene expression [5]. Based on the available evidences it is obvious that free oxygen radicals are involved in the senescence process via inducing oxidative stress. Salicylic acid treated peach fruit during postharvest life showed higher free radical scavenging activities, activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) as well as acceptability [31]. PAL and antioxidant enzymes like SOD and CAT may involve in postharvest life [28]. The phenylpropanoid pathway produces diverse phenolic compounds many of which are implicated in plant defense reactions and scavenging of oxygen active species [19]. In addition, there are close correlation between phenol content and PAL activities. It has been stated that PAL activity is influenced by a variety of factors such as growth regulators and wounding [21].

The applied preservatives had promoting effects on the longevity of gerbera cut flowers where SA treatments, especially at highest used concentration and combined with nanosilver, were the most effective one, as it was indicated with the highest longevity observed in SA200 S nano. Results indicated that the efficacy of SA or BA on the vase life of gerbera cut flowers significantly enhanced by the applied concentration of nanosilver and with increasing the tested levels of BA, longevity of treated cut flowers reversely affected.

The presented research indicated that SA treatments, especially at highest used concentration, were effective to affect postharvest life of cut flowers probably via the declined bacterial growth, reduced vascular blockage, higher soluble solid contents, reduced transpiration, prevented ethylene formation and induced antioxidant system in treated cut flowers thereby delaying the senescence process.

In conclusion, based on our results it could be stated that the utilization of SA or BA, especially the first one in combination with nanosilver, may promote the vase life of gerbera cut flowers probably via inhibited microbial contamination, increased solution uptake, induced antioxidant enzymes, reduced oxidative stress, induced phenylpropanoid metabolism, the declined lipid peroxidation levels, elevated soluble solid contents, prevented ethylene formation and decreased transpiration rates thereby delaying the senescence process. In addition, the application of nanosilver in combination with BA or SA may enhance efficacy of them to extend the vase life of gerbera cut flowers.

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