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RESEARCH ARTICLE

Physiological response of gladiolus flowers to anti-ethylene treatments and their relation to senescence

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Manuscript Info *Abstract*

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In order to study the physiological response of gladiolus flowers to anti-ethylene treatments, the effects of 1-methylcyclopropene (1-MCP) or silver thiosulphate (STS) on the postharvest quality of gladiolus cut flowers were investigated. 1-MCP was used at 0.2, 0.3 or 0.4 g m^{-3} for 6 hand STS was applied at 0.2 or 0.4 mM for 6 h. The control spikes were kept in distilled water. 1-MCP or STS treatments significantly extended the vase life and minimized the weight loss of gladiolus spikes compared with the control. Both treatments enhanced the relative water content (RWC) of leaves and maintained chlorophyll and carbohydrate contents compared with the control values, which were decreased. Ethylene production was increased in florets of untreated spikes and membrane stability was reduced while 1-MCP or STS treatments minimized ethylene production and retained membrane stability. An increase in floret antioxidant enzyme activities (CAT, SOD and POX) was observed in 1-MCP or STS treated spikes compared with the control. The effects of 1-MCP or STS on floret senescence seemed not entirely limited to their effects on ethylene, but they most likely had a sustainable impact on the above tested physiological parameters.

Introduction

Gladiolus is one of the four famous cut flowers in the world (Bai et al., 2009). The longevity of gladiolus cut flowers is very short. The typical vase life of individual florets is just 4 to6 days and senescent florets remain at the bottom of the spikes after the opening of the upper florets (Yamada et al., 2003).In floriculture, delaying the onset of senescence is the focus of a great deal of research in an effort to extend the useful life of the product

Maintaining the quality of cut flowers is one of the main challenges of florists in the flower trade worldwide. In floriculture, delaying the onset of senescence in order to prolong the vase life of cut flowers is the focus of many researchers. Flower senescence and thus shortening the vase life is influenced by several factors including endogenous ethylene (Seglie et al., 2012). Moreover, unfavorable transport and storage conditions can reduce the flower quality as a result of endogenous ethylene production in response to postharvest stressful environment (Nowak and Rudnicki, 1990).Ethylene induces leaf yellowing, flower or petal drop, irregular opening and premature death (Nowak and Rudnicki, 1990). It also causes loss of cellular turgor, chlorophyll and pigment degradation and hence product quality such as vase life (Serek et al., 2006). Stimulation of ethylene biosynthesis has been reported to involve the generation of reactive oxygen species (Pellinen et al., 1999). It has been reported that flower senescence

is accompanied with increased permeability of petal cells and increased ROS production (Reezi et al., 2009). Ethylene control is; therefore, a critical factor in the flower maintaining quality after harvest.

Since the 1970s, the best "weapon" against ethylene has been silver thiosulfate (STS), which can at least double the vase life of cut flowers (Reid et al., 1999). Silver thiosulphate (STS) is the most widely used substance as ethylene binding inhibitor. The benefits of using STS are so great that it is mandatory to be used with many species of flowers entering the flower auctions. STS appears to be having also further benefits than as a biocide, which makes it an even more popular substance (Bishop 2002).Different authors reported that vase life was extended and therefore the postharvest quality was improved as a result of STS treatment (Beura et al. 2001; Petridau et al., 2001; Song et al., 2001; Celikel and Reid 2002; Ichimura and Goto, 2002;Hassan et al. 2003; Hassan and Schmidt 2004; Hassan et al. 2004; Dole et al. 2005; Sexton et al. 2005;Williamson and Joyce, 2013).Because STS contains silver, recently considered a potential environmental pollutant, there have been some restrictions on its commercial use (Cross, 1996). Therefore, other alternatives to STS should be used.

1-MCP (C_4H_6) is a non-toxic inhibitor of ethylene action, which acts as a competitive and irreversible inhibitor of binding of ethylene to its receptor thereby inhibiting flower senescence (Sisler et al., 1996; Sisler and Serek, 1997).Several reports have indicated that 1-MCP is a very potent inhibitor of ethylene action in different cut flowers (Hassan, 2009; Liou and Miller, 2011; Seglie et al., 2012), through retarding chlorophyll degradation and senescence (Hassan and Mahfouz, 2010). Even in an ethylene free environment, treatment with 1-MCP significantly improved the longevity of some cut flowers (Serek et al, 1995; Sisler and Serek, 1997; Sisler and Serek, 2001). Furthermore, 1-MCP is environmentally friendly, safe to use, and is appropriate for operations of any size (Liou and Miller, 2011). However, the role of 1-MCP to retard ethylene dependent senescence processes is not studied yet and little information is available about its role in mitigating the oxidative stress in gladiolus cut flowers.Most of the early works on the senescence of different cut flowerswere focused on the effect of 1-MCP on the ethylene action (Sisler et al., 1996; Serek and Sisler, 2001; Celikel et al., 2002; Hassan 2009). The present study was therefore, undertaken to investigate whether 1-MCP can affect the postharvest quality of gladiolus as antioxidant rather than anti-ethylene, and to compare its effects with STS as well. In order to achieve that,physiological and biochemical responses of gladiolus cut flowers to 1-MCP or STS were studied.

2. Materials and methods

2.1. Plant materials

Cut flowers used in the experiment were *Gladiolus grandiflorus* cv. "White Friendship". The flowers were obtained from a commercial grower and directly transported to the laboratory of Faculty of Science, Taif University, Saudi Arabia at November 10, 2013. Only the first floret in all spikes was shown its color. Lower leaves were removed and the spikes were trimmed to a uniform length of 75 cm.

2.2. 1-MCP treatments

Pre-treatment with 1-MCP was released from a commercial powdered formulation (Smartfresh™, Rohm and Haas Italy, Inc.) by adding distilled water, according to the manufacturer's instructions. The spikes of each treatment were placed in a glassy chamber and 1-MCP was applied at a concentration of 0.2, 0.3 or 0.4 g $m^{-3}$for 6 h. The treatment of 1-MCP was conducted at 15 °C.

2.3. STS treatments

Another group of gladiolus flowers was pulsed with STS for 6 h at concentrations of 0.2or 0.4 mM. Silver thiosulfate (STS) was prepared as described by Gorinet al., (1985).Control spikes were not treated with 1-MCP or STS and were kept in 500 mL beakers containing distilled water. After 1-MCP and STS treatments the cut flowers were put in glasses containing 500 mL distilled water for vase life evaluation till the end of the experiment. Vase solutions were changed every 24 h for all treatments. Five treatments with four replicates were applied and each replicate consists of five flowers.

2.4. Vase life evaluation

The longevity of gladiolus cut spikes was evaluated at 21 °C, 65 ± 5 % RH and 12 h photoperiod with 10 µmol $m^{-2}s^{-1}$ irradiance from cool-white fluorescence lamps. The longevity was defined as the number of days in vase life required for 50% of the florets of each spike to lose its ornamental value (lost turgor and wilted).

2.5. Number of opened and unopened florets

On each spike, the number of opened and unopened florets was recorded from the beginning of the experiment until day 16.

2.6. Fresh weight measurements

The cut spikes were initially weighed at the beginning of the experiment. The fresh weight was repeated again daily until the end of vase life of control flowers. The change in fresh weight was determined. The spikes were analyzed subsequently for fresh weight changes during vase life.

2.7. Relative water content (RWC)

Flower RWC were measured according to Weatherley (1950) as following:

$(W_{fresh}- W_{dry}) / (W_{turgid}- W_{dry}) \times 100$, where $W_{fresh}$is the sample fresh weight, W_{turgid} is the sample turgid weight after being saturated with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry (at 70 °C for 48 h) weight of the sample. The samples were taken from the third floret at the base of spike on days 1, 2, 3 and 4 from the beginning of the experiment.

2.8. Chlorophyll determination

Randomly samples of fresh leaves on days 1, 3, 5, 7 and 9 from the beginning of the experiment were taken from the third leaf from stem base for chlorophyll determination. Chlorophyll content was determined according to Sadasivam and Manickam (1992) by using spectrophotometer (Pharmacia, LKB-Novaspec II and calculated as (mgg^{-1} FW).

2.9. Carbohydrate content

Total carbohydrate percentages were determined in the third leaf from stem base on days 1, 3, 5, 7 and 9 from the beginning of the experiment. Samples were dried in an electric oven at 70 °C for 24 hours, and then the fine powder was used to determine total carbohydrate percentages according to Herbert et al. (1971).

2.10. Ethylene production determination

The floret samples for ethylene production were taken from the third floret at the base of spike at days 0, 1, 2, 3 and 4. The samples were putin50 mL air tight jars sealed and fitted with gas sampling ports. The jars were kept at 20 °C and 70 - 75 % RH for 2 h. Gas samples (1 mL) was withdrawn from the headspace of jars for ethylene determination. Ethylene content of the samples was quantitatively analyzed by gas chromatography using a Varian GC CP-3800 and MS Saturn 2200 equipped with a Factor Four capillary column (VF-5 ms 30 X 0.25 mm ID and film thickness 0.25 µm). The injector, column, and detector temperatures were 80, 100 and 220 °C, respectively (Heiser et al., 1998). Ethylene values were indicated as (nL g^{-1} h^{-1}FW).

2.11. Membrane stability index (MSI)

Floret samples from each treatment were taken from the third floret at the base of spike on days 0, 1, 2, 3 and 4 for determining ions leakage by using the method of Sairam et al. (1997). Two floret samples (0.2 g) were taken and placed in 20 mL of double distilled water in two different 50 ml flasks. The first one was kept at 40 °C for 30 min while the second one was kept at 100 °C in boiling water bath for 15 min. The electric conductivity of the first (C_1) and second (C_2) samples were measured with a conductivity meter. The leakage of ions was expressed as the membrane stability index according to the following formula, $MSI = [1- (C_1/C_2)] X 100$

2.12. Antioxidant enzyme assays

Antioxidant enzyme activities were determined in the third floret from the base of spike on days 0, 1, 2, 3 and 4. Soluble protein contents of the enzyme extract were assayed according to the method of Bradford (1976).One gram of floret tissue was homogenized with 5 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 1 M KCl, 0.5 % polyvinylpyrrolidone, 0.1 mM EDTA and 2 mM dithiothreitol by using a chilled pestle and mortar. The homogenate was filtered and the extract was centrifuged at 20000*g* at 4 °C for 15 min. The obtained supernant was used as an enzyme extract to determine catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) activities.

CAT (Ec 1. 11. 1.6) activity was spectrophotometrically measured according to the method described by Clairbone (1985), following the disappearance of H_2O_2 at 240 nm. The level of enzyme activity was expressed as µmol min^{-1} mg^{-1} protein.

SOD (Ec 1. 15. 1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT). SOD activity was expressed as SOD units $min^{-1}mg^{-1}$ protein. SOD was measured by spectrophotometer at 560 nm and one unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50% according to Giannopolitis and Ries (1977).

POX (Ec 1. 11. 1.7) activity was estimated as mentioned by Shanon et al. (1966). Sodium acetate buffer (0.1 M) and 0.5% guaiacol were added to the enzyme extract. The reaction started with 0.1% H_2O_2. The change in level of absorbance was measured at 470 nm using spectrophotometer, and the level of enzyme activity was expressed as µmol min^{-1} mg^{-1} protein.

2.13. Statistical analysis

In all experiments, flowering spikes were arranged in a complete randomized design. The experiment was performed three times and had qualitative and quantitative results. Where indicated, the results were expressed as

mean values (± SD) from the three experiments (n = 12). The results of three experiments were pooled and the analysis of variance (ANOVA) was performed using MSTAT program, USA. Means were separated using LSD at a significance level of 0.05.

3. Results

3.1. Vase life

Various concentrations of 1-MCP or STS significantly extended the vase life of gladiolus cut spikes compared to with the control, more so with higher concentration of STS treatment (Fig. 1A). Among all treatments, applying STS at 0.4 mM recorded the highest vase life however; there were no significant differences between this treatment and 1-MCP at 0.3 gm^{-3}

3.2. Number of opened and unopened florets

Data presented in Fig. (1B) clearly indicate that the number of fully opened florets was increased while the number of unopened florets was decreased as a result of treating gladiolus spikes with 1-MCP or STS, the effect was more pronounced with higher concentrations of 1-MCP or STS. The average of opened florets of the control was 37.78 %, while it was 56.82, 67.23, 75.56, 70.45 and 80.28 % for 1-MCP at 0.2, 0.3 and 0.4 gm^{-3} and STS at 0.2 and 0.4mM, respectively.

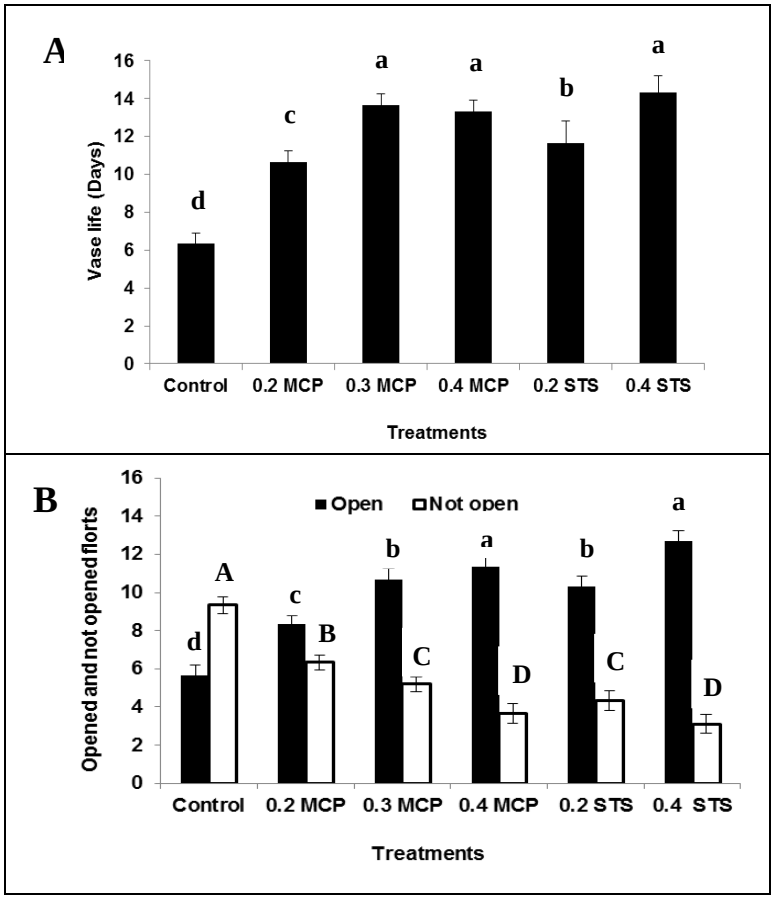

Fig.1. Effect of 1-methylcyclopropene (1-MCP) or silver thiosulphate (STS) on vase life (A) and opened and not opened gladiolus flowers (B). The values (mean ± S.D.) are the average of three independent experiments (n = 12 replicates of 5 spikes each). Bars had different letters significantly differ from each other according to Duncan's multiple range test at P = 0.05.

3.3. Fresh weight changes (%)

The fresh weight of flowers kept in water (control) was slightly increased till day 7 and decreased thereafter (Fig. 2A). The fresh weight of flowers treated with 1-MCP or STS, especially with higher levels, remained higher than the initial weight until day 11 during the postharvest days. Meanwhile, on day 11, the untreated spikes lost 19.46 % from their initial weight.

3.4. Relative water content (RWC)

During postharvest life, RWC was gradually reduced with the progressive development in both treated and non-treated spikes (Fig. 2B). However, 1-MCP or STS treatments significantly decreased this decline in treated leaves compared with the control. This effect was clear after the third day, 1-MCP at 0.4 gm^{-3} or STS at 0.4mMhad the best effect.

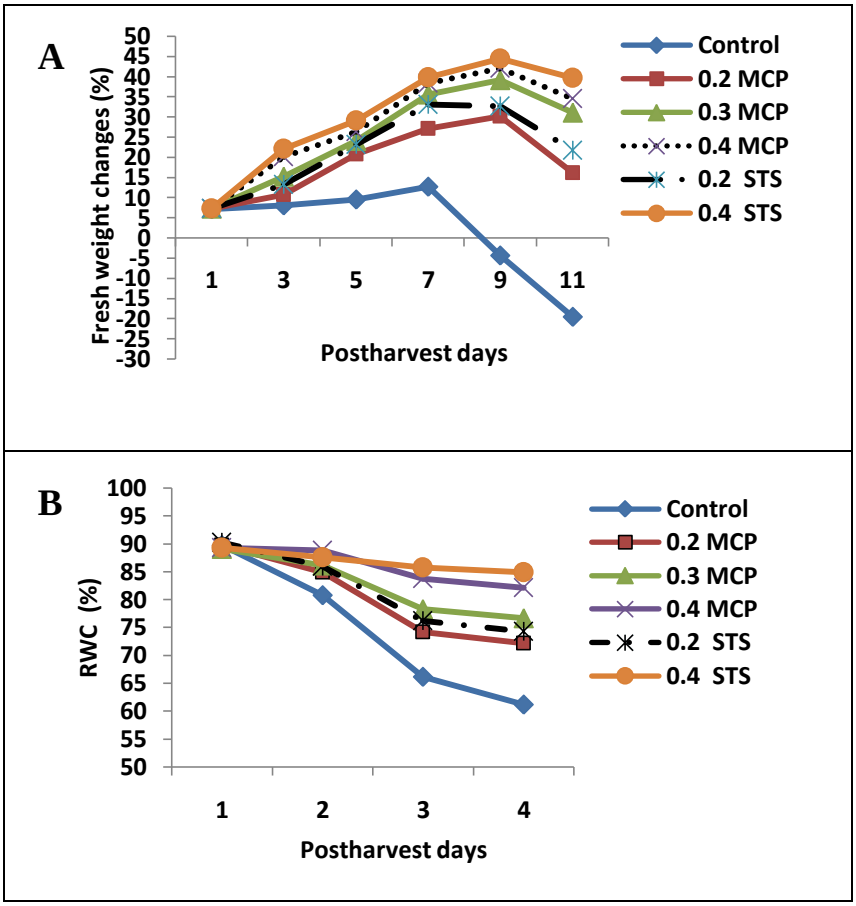

Fig.2. Effect of 1-methylcyclopropene (1-MCP) or silver thiosulphate (STS) on fresh weight changes (A) and relative water content (RWC) of gladiolus flowers. The values (mean ± S.D.) are the average of three independent experiments (*n* = 12 replicates of 5 spikes each).

3.5. Chlorophyll content

The total chlorophyll content of gladiolus leaves was gradually decreased during the days of vase life evaluation in treated and non treated leaves; the decrease in chlorophyll of the control was significantly sharp compared with treated leaves (Fig. 3A). 1-MCP or STS significantly retarded the chlorophyll reduction, more so with higher levels. By the 9^{th} day 32.29 % of initial chlorophyll content was reduced in control leaves.

3.6. Carbohydrate percentage

Data presented in Fig. (3B) show that carbohydrate percentage in gladiolus leaves was gradually decreased with the progressive in vase life days after harvest. This decrease was sharp in control flowers however treatment of 1-MCP or STS retarded the decline in carbohydrate percentage during vase life. The best effect in this concern was obtained by using 1-MCP at 0.4 gm^{-3} or STS at 0.4mM treatments.

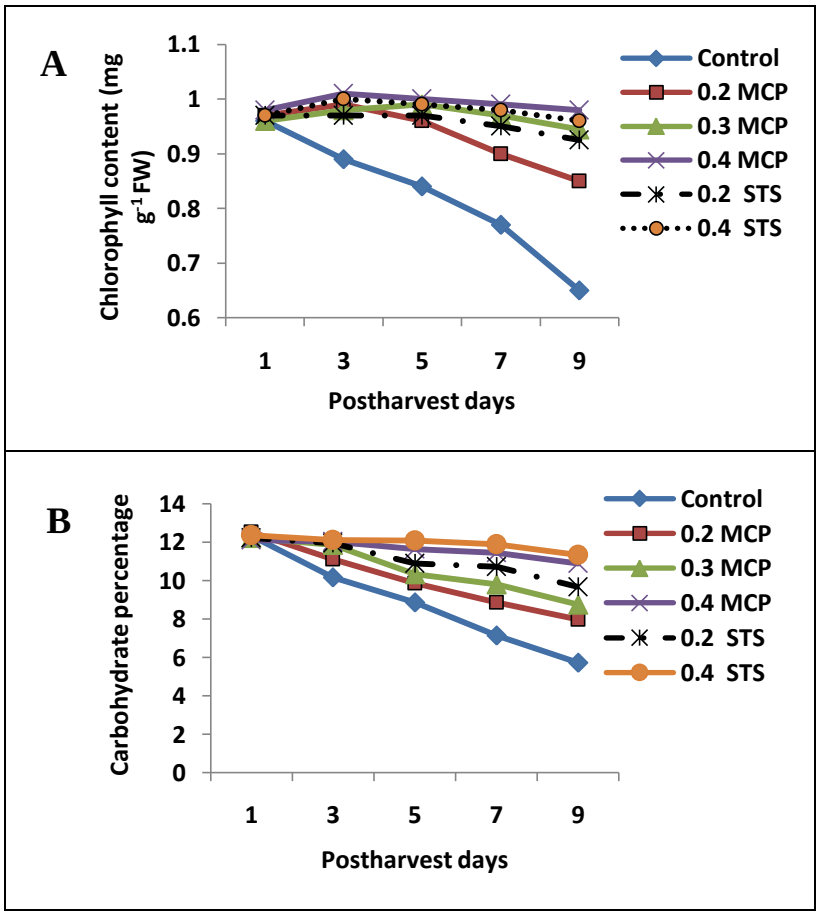

Fig.3. Effect of 1-methylcyclopropene (1-MCP) or silver thiosulphate (STS) on chlorophyll content (A) and carbohydrate percentage of gladiolus flowers. The values (mean ± S.D.) are the average of three independent experiments (n = 12 replicates of 5 spikes each).

3.7. Membrane stability index (MSI)

Control florets lost their MSI upon the progression of their senescence during its life days(Fig. 4A). However, when the spikes were treated with 1-MCP or STS, MSI was retained compared with the control. At day 4, the MSI for control florets was 69.22 % compared with 77.14, 85.06, 93.80, 88.85 and 97.13% for 1-MCP at 0.2, 0.3 and 0.4 gm^{-3} or STS at 0.2 and 0.4mM treatments, respectively.

3.8. Ethylene production

Treated gladiolus spikes with 1-MCP or STS significantly inhibited ethylene production by florets compared with the control (Fig. 4B). A sharp increase in ethylene production was observed in untreated florets from the beginning of the experiment and reached the highest value in the second day, followed by a gradual reduction was recorded thereafter (Fig. 4B).However, all treatments delayed and reduced the ethylene peak and the lowest ethylene production was obtained by the treatment of STS at 0.4 mM.

3.9. Antioxidant enzyme activities

Florets in spikes treated with 1-MCP or STS showed higher activities of CAT, SOD and POX compared with the control (Figs. 5A, B and C). The greatest activities of these enzymes were obtained from spikes kept in 0.4 mM STS or 0.4 g m^{-3} 1-MCP at the 3rd day. Although a slight decrease was observed after day 3, the activities of enzymes were still higher than that of the control until the4th day.

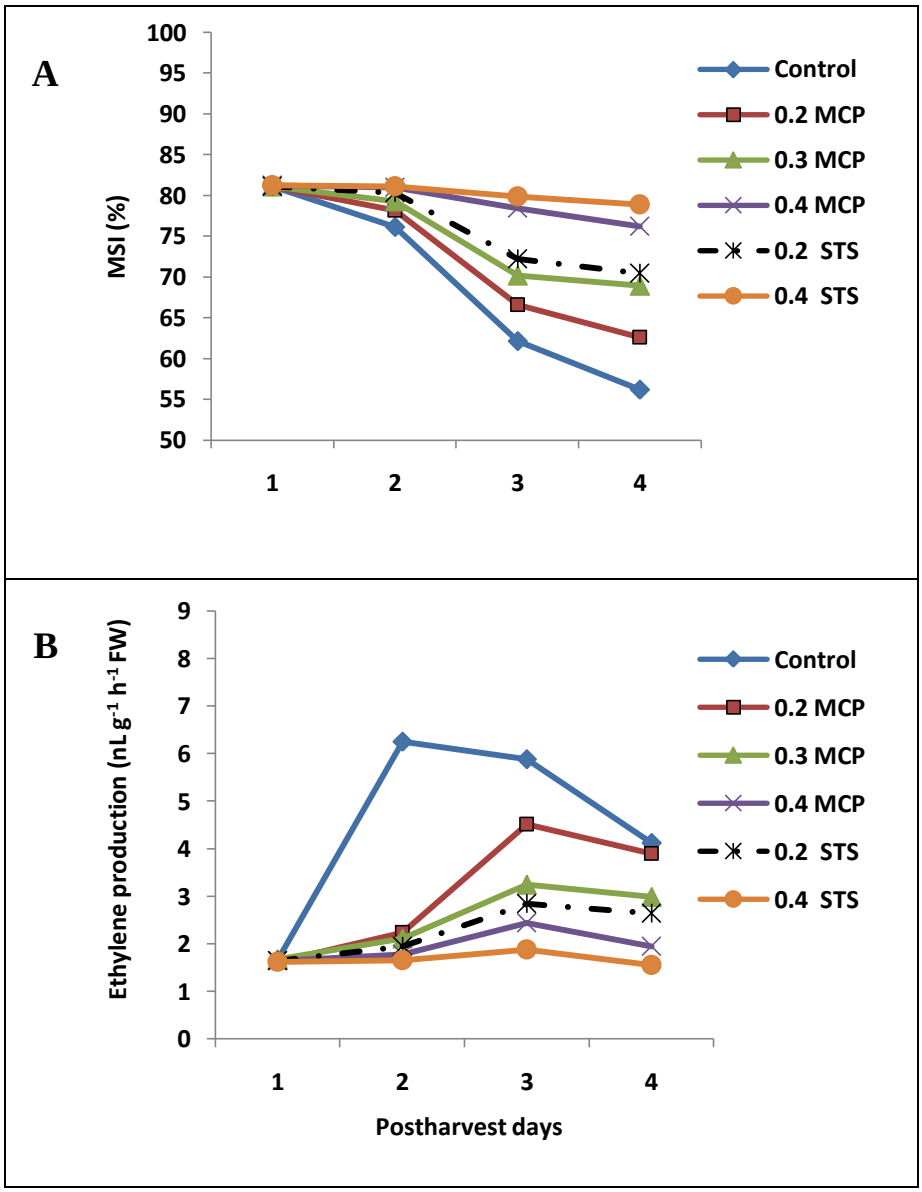

Fig.4. Effect of 1-methylcyclopropene (1-MCP) or silver thiosulphate (STS) on membrane stability index (A) and ethylene production of gladiolus flowers. The values (mean ± S.D.) are the average of three independent experiments (n = 12 replicates of 5 spikes each).

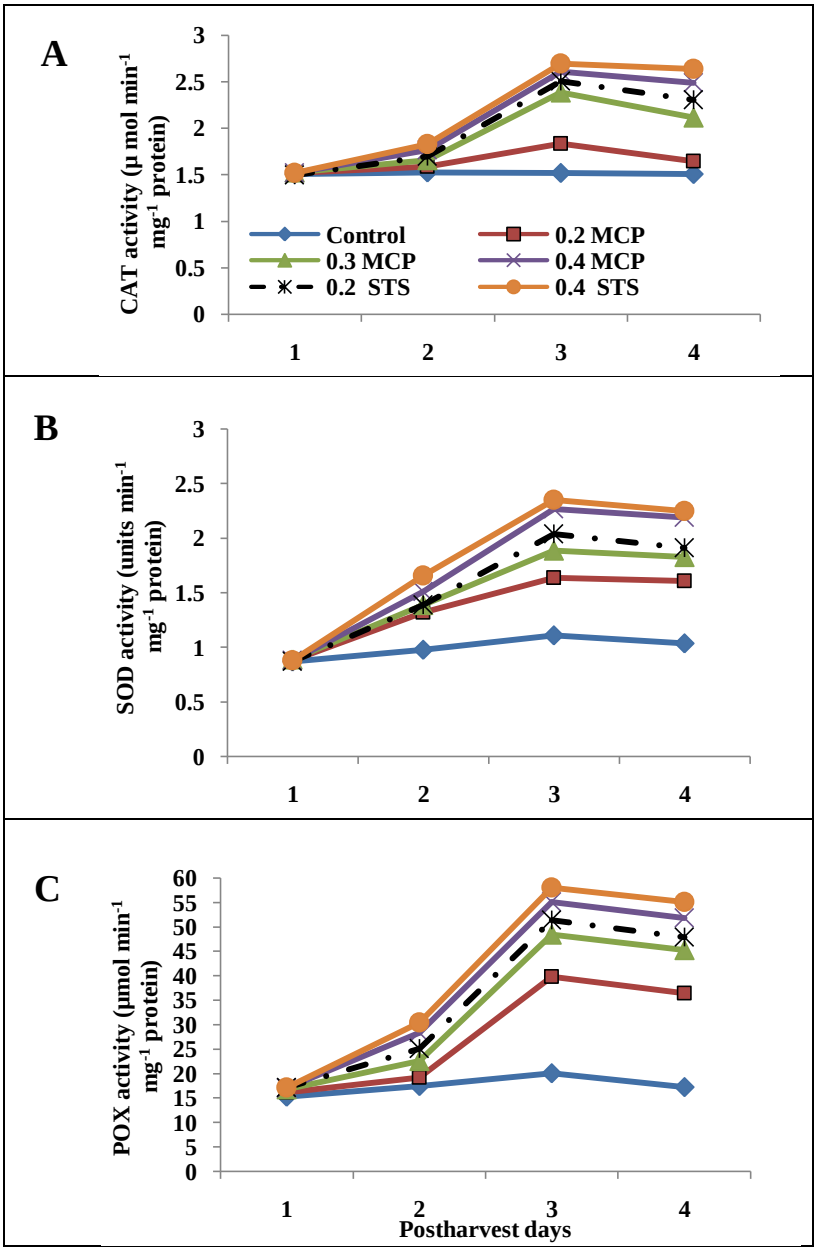

Fig.5. Effect of 1-methylcyclopropene (1-MCP) or silver thiosulphate (STS) on antioxidant enzyme activities: Catalase (A), superoxide dismutase (B) and peroxidase (C) of gladiolus flowers. The values (mean ± S.D.) are the average of three independent experiments (*n* = 12 replicates of 5 spikes each).

4. Discussion

In this study, the effects of 1-MCP or STS on the postharvest quality of cut gladiolus spikes were assessed. All treatments of 1-MCP or STS significantly increased the vase life of cut gladiolus spikes compared with the control. Higher number of opened florets produced from treated spikes may explain the higher longevity resulted from 1-MCP or STS treatments. The obtained results are in agreement with the results of Hassan et al. (2004),

Uthaichay et al. (2007), Hatamzadeh et al. (2012), Seglie et al. (2012), Shimizu-Yumoto and Ichimura, (2013) and Hassan and Ali (2014)who reported that the longevity of cut flowers was increased as a result of using 1-MCP or STS treatment. However, Serek et al. (1994**)** reported that using STS as an ethylene inhibitor in gladiolus improved the floret opening but not the life of individual florets.

The spikes fresh weight treated with 1-MCP or STS was greater than those kept in distilled water (Fig. 2A). The decrease of fresh weight may be due to a reduction in water uptake or an increase in water loss. Other studies demonstrated that the fresh weight of cut flowers was positively affected by 1-MCP treatment (Sisler and Serek, 2001; Hassan and Schmidt, 2004), and even maintain the fresh weight of cut leaves resulting in increased shelf-life (Hassan and Mahfouz, 2012). The positive effect of STS on retaining the fresh weight could be attributed to increased water uptake, thus preventing fresh weight loss (Uthaichay et al., 2007; Hassan and Ali, 2014). RWC refers to the amount of water in the plant organs and their ability to keep the water. Therefore, it is likely that the positive effects of

1-MCP or STS on fresh weight may be due to the higher values of RWC obtained when spikes were treated with them. That is, control spikes were under stress and could not uptake or maintain water properly, meanwhile spikes treated with 1-MCP or STS was in suitable conditions for uptaking and maintaining water. Increasing RWC as a result of 1-MCP or STS application has been previously reported (Hassan and Mahfouz, 2012; Hassan and Ali, 2014).

The current results showed that all treatments decreased chlorophyll and carbohydrate contents; however that reduction of control spikes was significantly retarded when spikes were treated with 1-MCP or STS. These positive results were in accordance with other results on different cut flowers (Terek et al., 2010) or even on detached leaves (Hassan and Mahfouz, 2010). Cheng et al. (2012) suggested that 1-MCP can delay chlorophyll degradation by inhibiting ethylene production and suppressing some genes expression, which are closely associated with chlorophyll catabolic pathway. STS similarly enhanced chlorophyll and carbohydrate contents of gladiolus leaves. It has been reported that STS increased the longevity and maintained the chlorophyll and carbohydrate contents of different cut flowers (Celikeland Reid, 2002; Picchioniet al., 2002; Hassan and Ali, 2014).

The ethylene production was significantly inhibited as a result of 1-MCP or STS treatments compared with the control. The results were in accordance with the finding of Uthaichay et al. (2007) who suggested that 1-MCP not only inhibited ethylene action, but also decreased ethylene production. Reduced ethylene production by 1-MCP might be adequate to explain the effect of 1-MCP on the vase life of gladiolus spikes. It is hypothesized that 1-MCP competes with ethylene for receptor bending, and that 1-MCP has much affinity to the receptor than ethylene (Sisler and Serek, 1997). Although published reports indicated that flower undergoes senescence independent of the ethylene effect and gladiolus has been classified as ethylene insensitive (Woltering and van Doorn, 1988), our results suggest that ethylene play a role in gladiolus senescence. This is because we observed a climacteric like peak of ethylene production. It is likely that the involvement of ethylene in senescence of the flowers is cultivar dependent (Serek et al., 1994; Hunter et al., 2004), and that, the effect of 1-MCP, as an ethylene action inhibitor, will differ according to the cultivar. Ethylene production was also inhibited by STS treatment. These results are in accordance with the results of Uthaichay et al. (2007) who mentioned that, STS not only inhibited ethylene action but also inhibited ethylene production. Similar observations have been previously reported in some cut flowers (Hunter et al., 2004; Chamaniet al., 2005; Valenzuela-Vazquez et al., 2007; Hassan and Ali, 2014).

It is interesting to report that 1-MCP and STS can regulate gladiolus floret senescence not only through its effect on ethylene action, but also by other mechanisms including maintaining membrane stability and increasing the antioxidant enzyme activity. During the floret life, 1-MCP or STS treated spikes increased MSI as well as CAT, SOD and POX activities relative to untreated spikes. The results indicate that both treatments alleviated the oxidative stress induced in cut flowers after harvest. The activities of these enzymes are considered an adaptative response to defend cells against oxidative stress (Zhou et al., 2014). 1-MCP or STS as anti-ethylene compounds may play role in scavenging the ROS and preventing floret senescence (Ezhilmathi et al., 2007; Hatamzadeh et al., 2012). Therefore, both treatments retained the membrane stability as our data indicated. Such effects of 1-MCP or STS on maintained cell stability and increasing the activity of antioxidant enzymes were previously reported (Ezhilmathi et al., 2007; Hassan and Mahfouz, 2012; Hatamzadeh et al., 2012; Li et al., 2013; Hassan and Ali, 2014).

As a conclusion, the study was an attempt to investigate the potential roles of 1-MCP or STS in maintaining the quality of cut gladiolus spikes. Both 1-MCP or STSwere able to prolong the vase life and delay floret senescence by regulating the flower water content, maintaining chlorophyll, carbohydrates and membrane stability, decreasing ethylene production and increasing the antioxidant enzyme activities. The effects of both treatments on retarding floret senescence arenot only due to reduced ethylene production, but also to increased antioxidant enzyme activities and thus suggest to reduced lipid peroxidation and therefore maintained membrane stability.

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