



Effect of Some Post-Harvest Treatments on Quality and Vase Life of *Moluccella Laevis* Cut Flowers

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ABSTRACT

The present study was carried out in the Post-harvest Lab of Ornamental Plants and Landscape Gardening Res. Dept., Hort. Res. Inst., Giza, Egypt in the two successive seasons of 2017 and 2018. The experiment aim was to evaluate the effect of pulsing in silver thiosulfate (STS) or H₂O with or without the pinching process on cut *Moluccella laevis* flowers at three cold storage periods for 3, 6 and 9 days additionally with a holding solution containing citric acid at 0.2g/l +sucrose at 20g/l and clarify their effect on some postharvest characteristics. The obtained data exhibited that STS treatment enhanced the vase life of cut flowers, raised the water uptake, improved the relative fresh weight, decreased the stem curvature and wilting percentage of cut flowers and maintained the chemical composition like chlorophyll contents as well as increasing reducing sugars in cut flowers compared to pulsing treatment of cut flowers in H₂O. The pinching procedure on cut flowers had a slight effect on almost all traits tested in this study. The previous measurements achieved the best results by reducing cold storage periods whereas, storing cut flowers for 3 days (the shortest period) had an increment in the vase life, increased the water uptake, reduced stem curvature and wilting besides, it maintained the chemical composition of cut flowers. Thus, the interaction between STS as a pulsing treatment and non-pinching procedure of cut flowers under room temperature can be recommended as the superior treatment to achieve the best result and record the good quality of cut flowers.

Keywords: *Moluccella laevis*, Preservatives solution, Silver thiosulphate (STS), Pinching, Vase life, Storage.

1. Introduction

Moluccella laevis L. (shell flower and bells of Ireland are common names). It belongs to the Lamiaceae Family. The flowers are two-lipped and tubular and the actual flower is a small white fragrant flower inside the bells. It can be used as cut flowers in many floral arrangements.

Chemical preservatives (chemical substances) have been applied to inhibit bacterial growth and prolong the vase life of cut flowers. Floral preservative solutions mainly contain carbohydrates (usually sucrose) and several types of biocides. Sucrose is widely used in floral preservatives, it acts as a food source and respiratory substrate that delays the degradation of proteins and plays an important role in improving the water balance of cut flowers by affecting their osmotic potential (Elhindi, 2012). Many agents of preservatives have been used in vase solutions of the cut flowers to extend vase life by inhibiting bacterial growth, improving water uptake and enhancing vase life (Amin, 2017). Silver thiosulfate (STS) is considered a strong agent of chemical preservatives used as a strong ethylene inhibitor (Hassan and Ali, 2014). The longevity of many cut flowers is negatively influenced by the presence of ethylene, which induces a variety of physiological responses, including abscission and wilting of leaves, petals and sepals. Silver thiosulphate (STS) is known to suppress autocatalytic ethylene production by inhibition of ethylene action (Da Silva, 2003) which in the presence of silver ions (Ag⁺) may block ethylene action, perhaps by replacing the metal component in the receptor. It had been reported as an important bactericide in preservative solutions (Figuroa *et al.*, 2005). Several

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authors reported that the application of silver thiosulphate in a pulse way was an effective treatment for promoting the vase life of cut flowers (Abadi *et al.*, 2013). The pulse application of silver thiosulfate led to extend the vase life of cut rose flowers (Liao *et al.*, 2000), carnation and sweet pea (Ichimura *et al.*, 2002), *Polianthes tuberosa* (Hutchinson *et al.*, 2003), and Dendrobium orchids (Uthaichay *et al.*, 2007).

Pinching is a manual technique done by removing the shoot apices for the purpose of removing the apical dominance and inducing the lateral branching in some ornamental plants production and help to reduce plant height and increase the number of flowers in several plants. The apical meristem and young leaves appoint a metabolic sink and auxin source that prevents the outgrowth of lateral buds and the auxin motion from these tissues which may limit concentrations of cytokinins and maintain apical control through a hormonal interaction. So, using the pinching process can regulate the plant architecture and improve the quality of some flowers (Ona *et al.*, 2015). Ehsanullah *et al.* (2021) found that delaying flowering in chrysanthemum by pinching was due to the removal of the physiological mature portion and the new shoots which emerged out from the pinched plants and take more time to become physiologically inductive to produce flowers than non-pinched plants. Similar results have been observed by Rajan *et al.* (2019) on chrysanthemums.

Storage of cut flowers is economically important because it enables producers to distribute and transport the product to its destination and meet the buyers' demand. With an exponential increase in demand, export had to be used to fill these requirements but there was a problem that was the acceleration in respiration rate of cut flowers as the temperature increases (Vehniwal and Abbey, 2019). It was found that refrigeration prior to packaging and transportation reduces metabolic changes such as enzymatic activity, slows down the senescence of flowers, and decreases ethylene production and metabolic processes thereby, it improves the quality and longevity of cut flowers (De *et al.*, 2015). The main objective of this study was to evaluate the efficacy of pulsing solutions and pinching before cold storage on some measurements that show the quality of the flowers such as water relations and vase life of cut flowers of *Moluccella laevis*.

2. Materials and Methods

Cut *Moluccella laevis* flowers were obtained from a well-known commercial farm of Floramix Farm (El-Mansouria, Giza) in Egypt. They were wrapped in groups inside kraft paper and carton boxes then, immediately transported within 1h to the Postharvest Laboratory of Ornamental Plants and Landscape Gardening Res. Dept., Hort. Res. Inst., ARC, Giza, Egypt during two successive seasons of 2017 and 2018. Cut flowers were cooled in cold water for half-hour to reduce high heat arising from the field and then transport. Stem bases of cut flowers were re-cut underwater by using a sharp knife to uniform lengths and to ensure no air blocky of the stem end in cut flowers. The experiments were carried out at $20 \pm 1^\circ\text{C}$, 70 ± 1 RH, and $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, light intensity under a daily light period of 12 h. All flowering stems were cut at similar lengths of 55cm. Thereafter, the flowering stems were divided into similar and equal groups as follows:

- Pinching with pulsing in STS without storage.
- Pinching with pulsing in STS with cold storage for 3 days.
- Pinching with pulsing in STS with cold storage for 6 days.
- Pinching with pulsing in STS with cold storage for 9 days.

- Pinching with pulsing in H₂O without storage.
- Pinching with pulsing in H₂O with cold storage for 3 days.
- Pinching with pulsing in H₂O with cold storage for 6 days.
- Pinching with pulsing in H₂O with cold storage for 9 days.

- Non-pinching with pulsing in STS without storage.
- Non-pinching with pulsing in STS with cold storage for 3 days.
- Non-pinching with pulsing in STS with cold storage for 6 days.
- Non-pinching with pulsing in STS with cold storage for 9 days.
- Non-pinching with pulsing in H₂O without storage.

- Non-pinching with pulsing in H₂O with cold storage for 3 days.
- Non-pinching with pulsing in H₂O with cold storage for 6 days.
- Non-pinching with pulsing in H₂O with cold storage for 9 days.

Pinching procedure was by removing 2cm from the apical stem and pulsing flowers in STS or H₂O solutions for 45 min. Silver thiosulfate (STS) was prepared as described by Gorin *et al.* (1985) as follows:

1. Dissolve 0.079 g AgNO₃ in 500 ml of deionized water.
2. Dissolve 0.462 g Na₂S₂O₃ .5H₂O in 500 ml of deionized water.
3. Pour AgNO₃ solution into Na₂S₂O₃.5H₂O solution while stirring. The concentration of silver thiosulfate is 0.463 mM. Cold storage was at 5° C for 3, 6 and 9 days after storage, the cut flowers were placed individually in glass bottles (500 ml) with 350 ml of the experiment solutions (citric acid at 0.2g/l + sucrose at 20g/l).

2.1. Recorded data

Vase life (days) determined as the time from the start of treatment until the senescence of flowers as described by Amin (2017).

Water uptake (g) was estimated by subtracting the amount of solution at the end of experiment from the beginning according to Amin (2017).

Relative fresh weight (%) was recorded daily during the experiment and calculated using the following formula (He *et al.*, 2006):

$$\text{RFW (\%)} = \frac{\text{Fresh weight of stem in the mentioned day}}{\text{Fresh weight of stem in day zero}} \times 100$$

-Stem curvature (%) according to Khenizy *et al.* (2013).

-Wilting percentage in cut flowers.

-Chlorophyll a, b and carotenoids (mg/g fw) as described by Saric *et al.* (1967).

Reducing sugars (%) was determined calorimetrically according to the method described by Dubois *et al.* (1956).

-Ca (calcium) % estimated in the leaves and petals according to Asher *et al.* (2001)

The layout of this experiment was in a factorial in completely randomized design (FCRD) and the treatment means were compared least significant difference (L.S.D.) test by Snedecor and Cochran (1994). Statistical analysis was carried out by a special statistical program (MSTAT-C).

3. Results and Discussion

3.1. Vase life

Data presented in Table (1) indicated that pulsing cut flowers (without pinching) in STS solution under room temperature was the best treatment for improving the vase life of cut moluccella flowers, it had a significant influence from the interaction and recorded 19 and 18.33 days compared to those pinched and pulsed in H₂O as gave 13.67 and 13.33 days in the first and second seasons, respectively. The pinching process was done by removing the apical bud thus, releases the lower axillary buds from apical dominance and produces the auxin and may induce the growth of vegetative laterals. The obtained results gave an indication that the pinching procedure is not of high benefit in postharvest treatments of cut flowers.

Treated cut flowers by STS as pulsing solution recorded the highest increase in vase life compared to treatment with H₂O even under pinching or without pinching, it gave 14.92 and 16.17 days (STS treatment) compared to 11.84 and 13.33 days (H₂O treatment) at zero time of storage. Silver thiosulfate (STS) retarded senescence in flowers as the main factor causing shorter vase life and lowed value of quality of cut flowers, it is involved in the degradation of proteins, nucleic acids and cell membranes, and moreover, it increases activities of RNase and other hydrolytic enzymes (Shabaniyan *et al.*, 2018). Also, the short life span of cut flowers was attributed to the growth of microorganisms in the vase solution and ethylene production. The microbial infection enhances the destruction of cut flowers and

increases the deposition of the material in the vessels; this leads to a decrease in the rate of absorption through flower stems, which speeds up the senescence process. STS owing to the fact that it has a strong antibacterial activity could suppress the growth of bacterial population in vase solution as well as in the xylem vessels thus improving water relations which increase fresh weight thereby extending the vase life of the flower. These findings were confirmed by Elhindi (2012) who studied the effects of the STS treatment on the vase-life of cut sweet pea flowers and showed that STS has been shown to be a very effective treatment in increasing the vase-life of sweet pea also, on many cut flowers including *Rosa hybrida* L. cv. 'Diana' (Liao *et al.*, 2000), carnation, delphinium (Ichimura *et al.*, 2002), *Polianthes tuberosa* (Hutchinson *et al.*, 2003), and Dendrobium orchids (Uthaichay *et al.*, 2007). The positive effect of STS due to silver is effective as an anti-ethylene and it reduced the activity of the ACC oxidase enzyme (Liao *et al.*, 2000, Alimoradi *et al.*, 2013). The vase solution contained citric acid at 0.2g/l + sucrose at 20g/l may share in this positive effect of treatment with STS, in agreement with Abdel-Kader *et al.* (2017) who reported that sucrose as a source of energy required for the continuation of the vase life of the cut flowers and may also act as an osmotically active molecule, thereby increases their vase life.

Table 1: Effect of pulsing and pinching treatments on vase life (days) of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments	STS pulsing	H ₂ O pulsing	Mean (A x C)	STS pulsing	H ₂ O pulsing	Mean (A x C)	
	First season			Second season			
Pinching	0	18.00b	13.67f	15.84B	17.67b	13.33e	15.50B
	3	15.33e	12.00hi	13.67E	15.33d	11.33g	13.33D
	6	14.00f	11.67i	12.84F	13.33e	11.33g	12.33E
	9	12.33h	10.00j	11.17G	12.00f	9.33h	10.67F
Mean A x B	14.92B	11.84D		14.58B	11.33D		
Non pinching	0	19.00a	15.67de	17.34A	18.33a	15.67cd	17.00A
	3	16.67c	14.00f	15.34C	16.00c	13.67e	14.84C
	6	16.00d	13.67f	14.84D	15.67cd	13.33e	14.50C
	9	13.00g	10.00j	11.50G	12.33f	9.67h	11.00F
Mean A x B	16.17A	13.33C	Mean (C)	15.58A	13.09C	Mean (C)	
Mean (B x C)		18.50A	14.67C	16.59A	18.00A	14.50C	16.25A
		16.00B	13.00D	14.50B	15.67B	12.50D	14.08B
		15.00C	12.67D	13.84C	14.50C	12.33D	13.42C
Mean of A		12.67D	10.00E	11.33D	12.17D	19.50E	10.83D
Mean of B		13.38B	14.75A		12.96B	14.33A	
Mean of B		15.54A	12.59B		15.08A	12.21B	

Means followed by the same letter/s in a column or raw do not differ significantly according to Duncan's New Multiple Range test

3.2. Relative fresh weight

It is one of the main factors affecting quality of cut flowers. The changes in fresh weight during vase life depend on the ratio between the water uptake and the transpiration rate. From data averaged in Table (2) it is clear that the most effective treatment in improving the percentage of relative fresh weight was obtained from treated cut flowers by pulsing in STS without pinching and then placing it in a holding solution containing citric acid and sucrose under room temperature which gave 82.71 and 81.00 % compared to those in the same conditions but with a pinching process which gave 81.88 and 81.30 % in the first and second seasons, respectively. The decrease in relative fresh weight may be due to the time of pinching which was after harvesting from the mother plant where it is difficult to grow new side branches on the main stem of the cut flower unlike those on the mother plants. Singh *et al.* (2017) found that pinching of apical bud suppresses the bud initiation process by inhibiting cell division in the lateral meristem resulting in the prevention of flower primordial development which might be due to the fact that the photosynthate and translocate of protein to the growing branches and leaves of

pinched plant overweight the photosynthetic energy consumed by general growth. Pinched plant energy was shared by the growing side branches, while the energy of non-pinched plants was limited to the flower growing in the main branch only and parallel results were also obtained by Khobragade *et al.* (2012) on China aster and Nain *et al.*, (2017) on marigold. Gaidhani *et al.*, (2020) observed that the pinching process delayed flower bud formation in China aster compared to the plants no pinching. Jena *et al.* (2021) confirmed that the flowers had a maximum diameter produced from untreated plants by a pinching process and the diameter of *Chrysanthemum coronarium* was decreased with pinching. On the other side, cut flowers that were treated under room temperature had a superior effect in improving the fresh weight of cut flowers compared to those under cold storage at 5°C at different periods during the vase life, especially the pulse treatment in STS which gave the best results than the pulse treatment in H₂O as this may be attributed to detracting the carbohydrate and oxidative stress caused by cold storage suggesting a mechanism for reducing the fresh weight of the stored flowers and for rapid flower senescence, as described by Ranwala and Miller (2005) on hybrid lilies. Also, Karimi and Asil (2008) suggested that cold storage of *Lilium longiflorum* cut flowers raised the production of ethylene and increased tissue sensitivity after cold storage. The great influence of silver thiosulphate on the fresh weight of cut flowers was agreed upon by Bhaskar *et al.* (2003) as they stated that STS is effective in increasing the permeability of the cell membrane and keeping the peroxidative changes at a minimum rate in rose flowers. Zencirkiran (2010) concluded that the loss of fresh weight decreased with the application of silver thiosulphate in cut freesia flowers. Moreover, silver thiosulphate prevents flower abscission (maintain fresh weight). Analogous observations were also elicited by Doi and Reid (1996), Ichimura and Hismatsu (1999), Hayat *et al.*, (2012) and Ha *et al.*, (2017). In relation to storage periods, it was observed that it was associated with continued loss of water from the tissues of cut flowers through transpiration and decreased capability of flowers to absorb water from vase solution during storage periods thereby decreasing relative fresh weight. Decreasing in fresh weight at senescence was reported by Jain *et al.* (2007) in cut flowers of rose and Varu and Barad (2008) in tuberose.

Table 2: Effect of pulsing and pinching treatments on relative fresh weight (%) of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments		STS	H ₂ O	Mean (A x C)	STS	H ₂ O	Mean (A x C)
		pulsing	pulsing		pulsing	pulsing	
		First season			Second season		
Pinching	0	81.88b	79.33d	80.61B	81.30a	76.78c	79.04B
	3	76.92e	73.87g	75.40D	72.12e	69.41f	70.77D
	6	73.79g	69.54i	71.67F	71.99e	67.89g	69.94E
	9	69.93i	65.77j	67.85H	69.38f	64.16i	66.77G
	Mean A x B	75.63B	72.13D		73.70B	69.56D	
Non pinching	0	82.71a	80.67c	81.69A	81.00a	79.44b	80.22A
	3	77.66e	75.49f	76.58C	73.93d	71.55e	72.74C
	6	74.63g	71.86h	73.25E	71.65e	70.12f	70.89D
	9	71.88h	69.82i	70.85G	70.02f	66.23h	68.13F
	Mean A x B	76.72A	74.46C	Mean (C)	74.15A	71.83C	Mean (C)
Mean (B x C)		82.30A	80.00B	81.15A	81.15A	78.11B	79.63A
		77.29C	74.68D	75.99B	73.03C	70.48E	71.75B
		74.21D	70.70E	72.46C	71.82D	69.01G	70.41C
		70.91E	67.80F	69.35D	69.70F	65.20H	67.45D
Mean of A	73.88B	75.59A		71.63B	72.99A		
Mean of B	76.18A	73.29B		73.92A	70.70B		

Means followed by the same letter/s in a column or raw do not differ significantly according to Duncan's New Multiple Range test

3.3. Water uptake

As shown in Table (3) data cleared that cut stems of flowers held in the preservative solution containing citric acid plus sucrose after pulsing in STS absorbed a higher amount of solution (151.90

and 149.60g) compared to those pulsed in H₂O (145.60 and 144.30 g) in the first and second seasons, respectively and that may be due to the protracted impact of STS as an antimicrobial agent and prevented the blockage in vessel xylem in the stem of flowers and improved water uptake by controlling the population of microorganisms in vase solution (Reid *et al.*, 2002). It also, reduced water loss and led to better water balance. Moreover, ethylene is a key limiting event in the vase life of cut flowers by inhibiting cell expansion through the regulation of aquaporins (Ma *et al.*, 2008) which are integral membrane proteins that serve as channels in the transfer of water, and in some cases, small solutes across the membrane. They are conserved in bacteria and plants and the structural analyses of the molecules have revealed the presence of a pore in the center of each aquaporin molecule (Xue *et al.*, 2020) and ethylene causes subsequently a negative water balance so reducing the vase life (Van Meeteren and Aliniaiefard, 2016). Ahmad *et al.* (2016) found that, the use of STS has been a common practice for many cut flower crops during post-harvest handling to inhibit ethylene synthesis and to counteract the harmful effects of ethylene or as a biocide to control bacterial populations in floral solutions. In the same line, Ha *et al.*, (2017) demonstrated that, STS enhanced the water uptake rate of cut rose due to decreased stem plugging. This agrees with what the scientist Gani *et al.* (2018) discovered in cut carnation. In relation to the effect of storage periods, data revealed that uptake of the solution gradually decreased with an increase in the periods of storage where it recorded 165.7, 159.80, 135.60 and 133.8g in the first season while in the second season it recorded 163.8, 157.4, 134.4 and 132.30g at different periods (zero, 3, 6 and 9 days) of storage. These findings are in the same line with Santos *et al.* (2012) who studied the effect of storage on *Epidendrum ibaguense* flowers and found that the rate of water uptake decreased and the transpiration rate increased. This decline is due to the tissues being damaged caused by lack of cell hydration and the transpiration rate surpassing the absorption rate. The interaction between pulsing and pinching of cut flowers, cleared that the treatment with STS with pinching gave a fewer value than pulsed in the same preservatives solution but with a non-pinching procedure, as well as pulsed cut pinched flowers in H₂O gave a fewer value than pulsed them in H₂O with non-pinching.

Table 3: Effect of pulsing and pinching treatments on water uptake (g) of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments	STS pulsing	H ₂ O pulsing	Mean (A x C)	STS pulsing	H ₂ O pulsing	Mean (A x C)
pinching	0	163.3d	156.4f	159.9C	158.9d	157.9C
	3	153.1g	155.4f	154.3D	148.7g	151.2D
	6	132.6ij	132.2j	132.4G	130.5k	130.3G
	9	132.3j	129.6k	131.0H	130.0k	128.9H
	Mean A x B	145.3C	143.4D		142.0C	142.1C
Non pinching	0	177.9a	165.2c	171.6A	176.1a	169.8A
	3	170.1b	160.7e	165.4B	168.5b	163.7B
	6	143.5h	134.2i	138.9E	143.1h	138.4E
	9	142.0h	131.3j	136.7F	140.9i	135.7F
	Mean A x B	158.4A	147.8B	Mean (C)	157.2A	146.6B
Mean (B x C)		170.6A	160.8B	165.7A	167.5A	163.8A
		161.6B	158.1C	159.8B	158.6C	157.4B
		138.1D	133.2E	135.6C	136.8E	134.4C
		137.2D	130.5F	133.8D	135.5F	132.3D
Mean of A	144.4B	153.1A		142.1B	151.9A	
Mean of B	151.9A	145.6B		149.6A	144.3B	

Means followed by the same letter/s in a column or raw do not differ significantly according to Duncan's New Multiple Range test

3.4. Stem curvature (%)

Recent studies have demonstrated that physiological senescence of cut flowers, such as petal discoloration or stem bending reduces the ornamental value, as was mentioned by Mochizuki-Kawai *et al.* (2014) in the tulip. Data illustrated in Table (4) cleared that, the gravitropic or binding response of cut flowers inhibited by STS was a superior application either by pinching or without pinching process than the percentage of curvature recorded from H₂O application under the same conditions. This may be attributed to the STS pulsing is an antibacterial compound that led to minimizing the microorganisms present in the holding solutions of cut flowers and it maximizes curvature depending on the concentration of microorganisms that clog the xylem channels which lower inflow through the stem of cut flowers thereby increase stem binding or another reason that the strong influence of STS inhibits the ethylene production and indicated the fact that is the silver thiosulfate is an ethylene retardant and bactericidal. These results are in accordance with those of Philosoph-Hadas *et al.* (1996) they showed that silver thiosulfate (STS) was effective in reducing cut flowers of snapdragon bending by increasing the ACC level only in the upper segment. Another assumption to explain curvature in flowers is that gene expression analysis of genes involved in ethylene and lignin biosynthesis pathways also supported the importance of lignin and ethylene in the leg bending mechanism. Stem bending in cut flowers usually occurs due to the low content of lignin in the stems, which leads to the inability to strengthen the stiffness of the stem and vascular tissues. This will reduce the mechanical resistance of the spikes of the entire flower, as well as disrupt the transfer of water and minerals to the flowers, which in turn leads to rapid bending of the stem (Naing *et al.*, 2021). Concerning the effect of storage periods, the same Table (4) declared that, as the storage period increases, the percentage of curvature increases. Moreover, the bending rate was the highest among the pinched flowers compared to the non-pinched cut ones.

Table 4: Effect of pulsing and pinching treatments on curvature (%) of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments	STS pulsing	H ₂ O pulsing	Mean (A x C)	STS pulsing	H ₂ O pulsing	Mean (A x C)	
	First season			Second season			
Pinching	0	30.36l	35.38i	32.87F	34.25k	36.25i	35.25F
	3	33.56j	36.22h	34.89E	36.19i	38.54g	37.37E
	6	38.35g	43.11d	40.73C	40.60f	45.04d	42.82C
	9	44.23c	49.59a	46.91A	46.43c	52.39a	49.41A
Mean A x B	36.63C	41.08A		39.37C	43.06A		
Non pinching	0	28.29m	33.26	30.78G	29.37m	35.39j	32.38G
	3	31.48k	35.33	33.41F	33.20l	37.40h	35.30F
	6	35.19i	40.48	37.84D	38.41g	42.18e	40.30D
	9	41.40	48.26	44.83B	44.55d	50.74b	47.65B
Mean A x B	34.09D	39.33B	Mean (C)	36.38D	41.43B	Mean (C)	
Mean (B x C)		29.33H	34.32F	31.82D	31.81K	35.82F	33.82D
		32.52G	35.78E	34.15C	34.70G	37.97E	36.33C
		36.77D	41.80C	39.28B	39.51D	43.61C	41.56B
		42.82B	48.93A	45.87A	45.49B	51.57A	48.53A
Mean of A	38.85A	36.71B		41.21A	38.91B		
Mean of B	35.36B	40.20A		37.88B	42.24A		

Means followed by the same letter/s in a column or raw do not differ significantly according to Duncan's New Multiple Range test

3.5. Wilting percentage in cut flowers

According to data presented in Table (5) it can be concluded that the wilting rate was high in cut flowers treated by immersion in water, while those treated by immersion in STS gave the lowest wilting rate in both seasons. Similar results reported the positive effect of STS that reduced carbohydrate depletion and consequently inhibited the wilting rate (Meir *et al.*, 2013). STS inhibit ethylene which

involved with flower abscission and wilting by triggering abscission zone formation and by oxidative stress promoted by ROS, including the overproduction of superoxide anion and hydrogen peroxide (Costa *et al.*, 2020). Wongjunta *et al.* (2021) reported that the ethylene inhibitor (STS) delayed the percentage of open buds increasing and senescent florets in both ‘Moo-deang’ and ‘Dao-lai’ hybrids, as compared to control (inflorescences pulsed in distilled water). Furthermore, with an increase in the period of storage, the wilting rate will increase which explicates the mechanisms of decreasing the fresh weight of stored flowers and flower senescence which may be due to depletion of carbohydrate reserves and oxidative stress caused by cold storage. In this concern, Van Doorn and Han (2011) noted that cold storage accelerates petal wilting, increases leaf yellowing and enhances bud abscission. Similar results were also revealed on Asiatic lily hybrids (Ranwala and Miller, 2002, Sindhu and Pathania, 2003, Abd El-Aal and Mohamed, 2017). STS has antimicrobial activity that inhibits microbial blockage of xylem vessels so, it can enhance water uptake and maintains turgidity in petals and guard cells of stomata resulting in closure stomata and decreasing water loss that minimizes wilting rate (Gani *et al.*, 2018).

Table 5: Effect of pulsing and pinching treatments on wilting (%) of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments	STS pulsing	H ₂ O pulsing	Mean (A x C)	STS pulsing	H ₂ O pulsing	Mean (A x C)	
	First season			Second season			
Pinching	0	29.31k	35.79g	32.55G	28.71j	34.34g	31.53G
	3	34.42h	41.93e	38.18E	32.92h	42.03d	37.48E
	6	43.43d	49.67b	46.55B	41.10e	50.67a	45.89B
	9	46.46c	51.35a	48.91A	45.27b	50.81a	48.04A
Mean A x B	38.41C	44.69A		37.00C	44.46A		
Non pinching	0	26.65l	31.28j	28.97H	26.08k	31.95i	29.02H
	3	32.23i	39.60f	35.92F	32.84h	40.01f	36.43F
	6	41.03e	44.02d	42.53D	40.81ef	43.73c	42.27D
	9	43.62d	45.58c	44.60C	43.59i	45.33b	44.46C
Mean A x B	35.88D	40.12B	Mean (C)	35.83D	40.26B	Mean (C)	
Mean (B x C)		27.98G	33.54F	30.76D	27.40F	33.15E	30.27D
		33.33F	40.77E	37.05C	32.88E	41.02D	36.95C
		42.23D	46.85B	44.54B	40.96D	47.20B	44.08B
		45.04C	48.47A	46.75A	44.43C	48.07A	46.25A
Mean of A	41.55A	38.00B		40.73A	38.04B		
Mean of B	37.14B	42.40A		36.42B	42.36A		

Means followed by the same letter/s in a column or raw do not differ significantly according to Duncan's New Multiple Range test

3.6. Effect of pulsing and pinching on chemical composition

Data presented in Table (6) cleared that, pinched and pulsed the cut *Moluccella laevis* flowers in STS gave the highest amount of chlorophyll (a) under room temperature, it gave 0.46 and 0.45 mg/g fw in the first and second seasons, respectively compared those treated with pinching and pulsing under cold storage for 9 days were 0.30 and 0.29mg/g fw in the first and second seasons, respectively. The amount of chlorophyll (b) and carotenoids in the flowers which pinched and pulsed in STS and put under cold storage in a holding solution containing 0.2g/l citric acid + sucrose at 20g/l gave the least value (0.13 and 0.11 in the first season and 0.12 and 0.10mg/ g fw in the second season) while pinched and pulsed cut flowers in H₂O gave the highest amount of chlorophyll (a) under room temperature in comparing with that under several cold storage periods (3, 6 and 9days). The same trend was observed in non-pinched and pulsed in STS and H₂O in different storage periods where the non-pinched cut flowers and pulsed in STS gave the highest amount of chlorophyll (a) under room temperature, it gave 0.49 and 0.48 mg/g fw in the first and second seasons, respectively compared those treated with non-pinching and pulsing under cold storage for 9 days were 0.38 and 0.35 mg/g fw in the first and second seasons, respectively. The highest amount of carotenoids was obtained from cut flowers which non-

pinched and pulsed in H₂O under room temperature compared to that stored in cold storage for the three periods with the little difference between the amount in cut flowers stored in cold storage for 3 days and those without storage (0.19 and 0.20 mg/g fw in the first season and 0.17 and 0.18 mg/g fw in the second season, respectively). The previous result cleared the role of STS in reducing the degradation of chlorophyll content. This finding was demonstrated by Alimoradi *et al.* (2013) who concluded that STS increased the durability and chlorophyll content is appearing in alstroemeria cut flowers also, on cut roses (Ha *et al.*, 2017). However the non-pinching cut flowers gave higher content of chlorophyll than the pinching flowers may be due to the incomplete accumulated assimilates used up by the axillary meristems which were stimulated to grow in non-pinched cut flowers. These results were agreed upon and confirmed by some studies Abd El-Aal and Mohamed (2017) who found that the pinching procedure on *Pelargonium zonale* plants decreased chlorophyll content and delayed flowers. Moreover, Sharaf-Eldien *et al.* (2017) reported that the pinching procedure in *Zinnia elegans* reduced chlorophyll (a) by 22.99 and 17.19%, chlorophyll (b) by 22.22 and 30.30% and chlorophyll (a+b) contents by 22.72 and 21.65% in the first and second seasons, respectively compared to unpinched plants.

Table 6: Effect of pulsing and pinching treatments on chlorophyll a, b and carotenoids (mg/g fw) and reducing sugars (%) of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments	Pinching											
	First season						Second season					
	STS pulsing			H ₂ O pulsing			STS pulsing			H ₂ O pulsing		
	Chl.a	Chlb	Car.	Chl.a	Chlb	Car.	Chl.a	Chlb	Car.	Chl.a	Chlb	Car.
No storage	0.46	0.25	0.19	0.42	0.23	0.18	0.45	0.24	0.17	0.41	0.21	0.17
Storage for 3 days	0.44	0.24	0.18	0.41	0.21	0.16	0.42	0.22	0.16	0.40	0.19	0.16
Storage for 6 days	0.39	0.18	0.14	0.37	0.17	0.13	0.38	0.16	0.13	0.35	0.16	0.12
Storage for 9 days	0.30	0.13	0.11	0.25	0.13	0.12	0.29	0.12	0.10	0.24	0.12	0.11

Treatments	Non pinching											
	First season						Second season					
	STS pulsing			H ₂ O pulsing			STS pulsing			H ₂ O pulsing		
	Chl.a	chl b	Car.	Chl.a	chl b	Car.	Chl.a	chl b	Car.	Chl.a	chl b	Car.
No storage	0.49	0.28	0.22	0.45	0.26	0.20	0.48	0.26	0.20	0.43	0.23	0.18
Storage for 3 days	0.47	0.27	0.21	0.44	0.23	0.19	0.46	0.23	0.18	0.42	0.21	0.17
Storage for 6 days	0.42	0.25	0.18	0.39	0.19	0.16	0.40	0.20	0.16	0.37	0.18	0.14
Storage for 9 days	0.38	0.19	0.15	0.29	0.15	0.14	0.35	0.18	0.14	0.26	0.14	0.12

Data illustrated in Table (7) showed that stored cut flowers at 5° C for 3 days maintained reducing sugars content compared to all the rest treatments which may be attributed to the low temperatures reduce respiration and transpiration and decrease sugars reserves degradation and reducing ethylene production. The highest reduction of reducing sugars concentration was verified on pinching and pulsing in H₂O treatment which gave 3.07 and 3.04% in the first and second seasons, respectively. These effects agreed with the findings of Ahmad *et al.* (2013) who concluded that cold storage treatment before transfer to holding solutions improved iris species membrane integrity, sugar content and soluble proteins.

Table 7: Effect of pulsing and pinching treatments on reducing sugars (%) and Ca% of cut *Moluccella laevis* during 2017 and 2018 seasons.

Treatments	Reducing sugars							
	First season				Second season			
	STS pulsing		H ₂ O pulsing		STS pulsing		H ₂ O pulsing	
	Pinching	Non pinching	Pinching	Non pinching	Pinching	Non pinching	Pinching	Non pinching
No storage	4.38	4.63	4.27	4.31	4.30	4.61	4.24	4.28
Storage for 3 days	4.46	4.72	4.32	4.36	4.43	4.65	4.27	4.34
Storage for 6 days	3.83	3.96	3.50	3.64	3.74	3.88	3.46	3.59
Storage for 9 days	3.17	3.44	3.07	3.29	2.95	3.40	3.04	3.23
Treatments	Ca%							
No storage	0.31	0.33	0.28	0.30	0.28	0.31	0.26	0.27
Storage for 3 days	0.30	0.35	0.27	0.29	0.29	0.34	0.25	0.26
Storage for 6 days	0.28	0.30	0.26	0.26	0.28	0.29	0.25	0.24
Storage for 9 days	0.26	0.29	0.23	0.24	0.24	0.27	0.22	0.22

As shown in Table (7) the stored cut flowers for 3 days and pulsed in STS gave a maximum percentage of Ca in non-pinched ones 0.35% compared to 0.23% pinched and pulsed cut flowers in H₂O which recorded a minimum percentage. The increase in Ca% is an important factor that decreased the susceptibility of the cut flower to a sprayed conidial suspension, which exposed the flowers to extreme attack by the disease. Although the correlation of petal Ca concentration with the susceptibility to *Botrytis cinerea* was not very high, there is enough evidence in the literature that Ca is involved in the resistance mechanism of the plant to infection by this disease (Volpin and Elad, 1991, Elad and Volpin 1993, Elad and Evensen 1995) as well as in the post-harvest flower longevity and bud opening and senescence process of rose flower is controlled by membrane protein and phospholipids, ethylene and ATPase activity (Michalczuk *et al.*, 1989; Torre *et al.*, 1999). The petal Ca concentration is therefore a secondary factor in controlling the resistance to the disease.

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