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## Prolonging the Postharvest of Cut Ruscus Branches (*Ruscus hypophyllum* L.) by Different Holding Solutions.

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### ABSTRACT

The current research was conducted at the Floriculture and Vegetable Laboratory, Agriculture College, Mansoura University, Egypt during the two successive seasons of 2021 and 2022 to study the impact of different holding solutions on the vase life characteristics of cut-ruscus branches (*Ruscus hypophyllum*). These preservative solutions contained 8-hydroxyquinoline sulfate (8-HQS at 200 ppm) as a control treatment, Diphenylamine (DPA at 50, 75, and 100 ppm), and two essential oils (thyme and clove at 100 and 200 ppm for both). For a 12-hour photoperiod, all cut branches in the tested preservative solutions were maintained at  $25 \pm 2$  °C with 60%–70% relative humidity and cool-white, fluorescent lights producing 1000 lux of light. The obtained data indicated that DPA at 50 or 75 ppm had improved most of the postharvest parameters of these cut branches like the vase life, maximum rise in relative fresh weight, total water uptake, and fresh weight. In addition, these superior treatments minimized the water loss, the activity of the enzymes that break down cell walls, and the bacterial counts in the preservative solutions. Also, 8-HQS at 200 ppm (control) enhanced most of the examined postharvest characteristics of cut ruscus branches with insignificant differences between it and DPA treatments.

**Keywords:** Holding solution, 8-HQS, Diphenylamine, Clove, Thyme oils



### INTRODUCTION

Cut foliage is used in floral arrangements and bouquets as filler in the florist industry. The cut foliage of *Ruscus hypophyllum* L. has a naturally lengthy vase life, depending on the season. The time of storage and temperature after harvest have a significant impact on how long cut foliage keeping its quality (Pacifci et al., 2013).

Since weight is the primary method of sale for most cut foliage, weight reductions seen within the distribution chain result in financial losses. Usually, metabolism (respiration) and water loss (water potential imbalance) are the primary causes of weight fluctuations. Weight loss may be impacted by the systems and storage conditions (Awuchi et al., 2021). Many ornamentals have longer vase lives and storage periods when the respiration rate is reduced, allowing for the retention of energy reserves such as carbohydrates (Cavalcante et al., 2021). Commercialization cooperatives are therefore motivated to ensure a satisfactory vase life following storage and to prolong the storage time as long as feasible without reducing the ornamental value (Drechsler and Holzapfel, 2023).

The floral industry uses 8-hydroxyquinoline sulfate (8-HQS) as a crucial preservative germicide. It enhances water absorption and functions as an anti-microbial agent. It has been shown that the postharvest quality of cut gerbera flowers can be improved by applying chemical preservative solutions containing 8-HQS, calcium chloride, and either 4% sucrose or none. It stated that sucrose can enhance the effect of 8-HQS while extending the vase life of cut rose flowers. Also, 8-HQS treatment increased the vase life and postharvest quality of various cut flowers by increasing water uptake,

fresh weight, and carbohydrate content. Despite the useful studies on the effective use of several phytochemicals for extending the life of freshly cut flowers, floriculture must screen for and develop an accurate, affordable, and simple-to-use preservative (Mirjalili et al., 2018).

In addition, according to (Mostafa, 2020), diphenylamine (DPA) has the structural formula  $[(C_6H_5)_2NH]$ , making it an aromatic antioxidant amine. It serves as a fungicide and a regulator of plant growth. Additionally, it is frequently used to protect apple and pear crops from post-harvest degradation (storage scald) as it has numerous effects on the plants that have been treated. Additionally, the postharvest treatment of "Granny Smith" apples with DPA decreased the activity of oxidative enzymes while increasing the total antioxidant content and activity in apples. Additionally, by preventing the passage of mitochondrial electrons, the DPA decreases plant cell respiration. Additionally, the DPA decreased the formation of ethylene, which in turn decreased the synthesis and oxidation of  $\alpha$ -farnesene and decreased surface scald in apples from the "Cortland" variety. Additionally, Gonge et al. (2021) found that the metabolomic analysis revealed accumulations of  $\alpha$ -farnesene oxidation products, methyl esters, phytosterols, and some chemicals linked to loss of chloroplast integrity and oxidative stress response.

On the other side, (Wang et al., 2022), reported that clove essential oil (CEO) is mostly composed of phenolic compounds such as eugenol and its derivatives and has a variety of biological activities including analgesic, insecticide, and antibacterial. However, the usage of essential oil is restricted because of its readily oxidized and low

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solubility features. In the past, scientists used high-energy emulsification to create a highly stable clove/cinnamon oil nanoemulsion. Additionally, the chitosan nanoparticles made from clove essential oil using the ion gel technique displayed effective antibacterial and antioxidant capabilities. El-Sayed and El-Ziat (2021) reported that the thyme (*Thymus vulgaris*) which belongs to the Lamiaceae family contains thymol, borneol, and carvacrol as the major constituents of its oil. It contains antibacterial, antifungal, and antioxidant qualities. The vase solution, which was fortified with thyme oils had extended the shelf life of cut gerbera (*Gerbera jamesonii* cv. "Dune") flowers.

Clove, or *Syzygium aromaticum*, is a plant that belongs to the Myrtaceae family and offers a variety of therapeutic benefits, including antibacterial and antiviral characteristics. Hydroxybenzoic acids, flavonoids, hydroxy phenylpropenes, and hydroxycinnamic acids are all primarily found in the clove plant. Additionally, the EOs extracted from the plant's flowers, buds, stems, and leaves showed a range in chemical composition, color, and flavor. Clove essential oils have been demonstrated to have antibacterial, anti-inflammatory, antioxidant, analgesic, anti-parasitic, anti-convulsant, and anticarcinogenic properties (Chen et al., 2017; Radünz et al., 2019; Shen et al., 2021).

Based on the studies, the utility of natural-origin products as antimicrobials was clear. The investigation of the biological activity of chemical compounds found in cut foliage ruscus (*Ruscus hypophyllum* L.). Thus, the purpose of this study was to investigate the impact of some holding solutions containing 8-HQS, DPA, thyme, and clove oils under different concentrations on the postharvest characteristics of cut ruscus branches.

## MATERIALS AND METHODS

This research was conducted throughout two consecutive seasons, 2021 and 2022, at the Laboratory of Floriculture and Vegetable Plants, Faculty of Agriculture, Mansoura University, Egypt. The purpose of this study was to examine the effects of various holding solution concentrations of 8-HQS, DPA, clove oil, and thyme oil on the postharvest properties of cut ruscus (*Ruscus hypophyllum*, L.) branches to maintain quality and increase the vase life. To achieve this goal, these materials and methods were used.

### Plant material

The cut-ruscus branches were obtained from Mansoura University's Faculty of Agriculture's Floriculture Unit. The branches were brought to the control vase life room in the early morning after being cut and potted in an icebox collar. Also, the stems were cut to a length of 35 cm. Upon arrival at the laboratory, branches were recut from the base (5 cm) under the surface of distilled water, to prevent air embolism. The leaves at the base of the branches were removed.

### Holding preservative solutions treatments

Ruscus branches were processed into eight holding preservative solutions simultaneously. Stock solutions from each preservative treatment were prepared before the dilution process to reach the final concentration in each holding treatment. Furthermore, the concentrations of both studied essential oils (thyme and clove oils) were prepared according to their bulk density. Moreover, treatments of both essential oils were dissolved in Tween 20 (1 ml L<sup>-1</sup>) before adding to

the holding solutions (El-Sayed and El-Ziat, 2021). The holding solutions treatments were arranged as follows: T<sub>1</sub>: 8-HQS control at 200 ppm; T<sub>2</sub>: DPA at 50 ppm; T<sub>3</sub>: DPA at 75 ppm; T<sub>4</sub>: DPA at 100 ppm; T<sub>5</sub>: clove oil at 100 ppm; T<sub>6</sub>: clove oil at 200 ppm; T<sub>7</sub>: thyme oil at 100 ppm; and T<sub>8</sub>: thyme oil at 200 ppm. Cut ruscus branches were held in a glass cylinder (100 ml), and the preservative solutions were refreshed and prepared with distilled water every 2 days during the vase life. All preservative solutions were fortified with 20 g/l sucrose as a carbon source and left in laboratory conditions for a 24-hour photoperiod at 25 ± 2 °C with 60%–70% relative humidity and cool-white, fluorescent lights producing 1000 lux of light. All parameters were read every 10 days during the vase life duration.

### Experimental design

Eight treatments, each with four replicates, and each duplicate consisting of three cut branches were applied. The trial design was straightforward complete randomized design (CRD).

### Data measurements

#### Postharvest parameters

**Vase life (days).** The duration of the cut branches in the vase solution from the start of the experiment until 25% of the ruscus stem leaves became yellowish was used to calculate the vase life.

**Change in fresh weight (CFW %).** Every ten days, it was calculated as the difference between the initial weight (g) and the weights of the same cut branches (g) on the first day of the vase's life. The result was then divided by the fresh weight of the cut branches on the first day of the vase's life. The result was then divided by the fresh weight of the cut branches on the first day and converted to 100g fresh weight (He et al., 2006).

**Maximum increase in fresh weight (%).** The fresh weight of the cut branches on the first day was divided by the fresh weight of the ruscus branches, and the result was then subtracted from the greatest fresh weight of branches to get the percentage.

**Total water uptake (g/cut).** It was calculated using the total amount of water that the ruscus branches absorbed throughout their shelf life, as per Rahman et al. (2018).

**Change of water uptake (g/cut).** According to Hatamzadeh et al. (2012), it was recorded every ten days during the vase life of ruscus branches, after correction with the mean evaporation value with a small amount of adjustment by applying the formula:

$$\frac{\text{Change in water uptake (g)} = \text{solution uptake on the day(n) of the cut stick}}{\text{fresh weight (g) on the day(n) of the same cut stick}}$$

**Change of water loss (g/cut):** Computed with the below formula:  $(WU_d - (\pm CFW_d) / FW_{d1}) \times 100$  is the water loss. WU= Water uptake (absorption) for ruscus branches was tested every 10, 20, and 30 days. The formula below was used to determine the absorption of water:  $((S_{t-1}) - S_t / FW_{d1}) = WU$ . S<sub>t</sub> is the weight of the solution absorbed each day of the vase life; S<sub>t-1</sub> is the weight of the solution from the day before; FW<sub>d1</sub> is the fresh weight of the cut flower on the first day (Ghale-Shahi et al., 2015) (Ghale-Shahi et al., 2015).

**Change of water balance (g/cut):** It was computed with the accompanying formula: Water balance is equal to the difference between water loss and intake.

#### Cell wall degradation enzyme activities (CWDE)

The CWDE samples were collected four times during the vase life. The first sample was on the initial day (day 0), the

second after 10 days, 20 days, and the last one was after 10 days. One gram of ruscus stem ground with 20 mM of Tris-HCL and homogenized at pH 7. The resulting mixture was cooled to 4 °C and centrifuged for 20 minutes at 15000 rpm. To identify the enzymes of cellulose (CEL), xylanase (XLN), and polygalacturonase (PG), the clear supernatant was stored at -20 °C. The diminishing end of galacturonic acid, xylose, and carboxymethyl cellulose as sources of sucrose were measured to track the enzyme activity (Miller, 1959). A fair proportion of the combination in an aggregate volume of 1 ml was included in the reaction blend (1000 UI), which also included 200 UI of 200 mM sodium acetic acid derivation buffer pH 5.0, 100 UI of NaCl (200 mM), and 300 UI of polygalacturonic acid. The substrate's expansion displays the response. The reaction mixture was placed in a water bath and incubated for an hour at 37 °C. Subsequently, 500 µl of dinitro-salicylic acid reagent was added and allowed to rise in a water bath for ten minutes. A spectrophotometer was used to measure the mixes' CEL activity at 540 nm and PG and XLY at 560 nm. One unit of compound development is defined as the percentage of the impulse that releases one uM of decreasing sugar every minute at 37 °C. Pectinase (PT), activity was determined (Collmer et al., 1988). 500 µl of 0.36% (w/v) polygalacturonic corrosive, 0.05 M of Tris-HCL support pH 8.5, 300 µl of 4 mM CaCl<sub>2</sub>, 600 µl protein, and 600 µl water were combined to assess the activity of PT. For three hours, the reaction mixture was kept at 37 °C. By measuring the concomitant absorbance at 232 nm, the PT activity was determined.

**Bacterial counts**

Total bacterial count averages (CFU/ml): After the tenth day of the vase life, was noted in the preservation

solution. From the first dilution to the sixth dilution, one milliliter of each sample was diluted using ten milliliters of distilled water that had been sterilized. Additionally, 1 milliliter of every dilution was kept on nutrient agar substrate on Petri plates. On the tenth day, all dishes were maintained at 28 °C, and the colonies were counted (Morisaki et al., 1995). This study was conducted twice at the Microbiology Department Laboratory, Faculty of Agriculture, Mansoura University, using three replicates for each treatment.

**Statistical Analysis**

Using the Costat (1986) V. 3.30 software, data were analyzed using ANOVA, and Snedecor and Cochran (1990) recommended applying the Duncan multiple Range test with 5% probability to compare means.

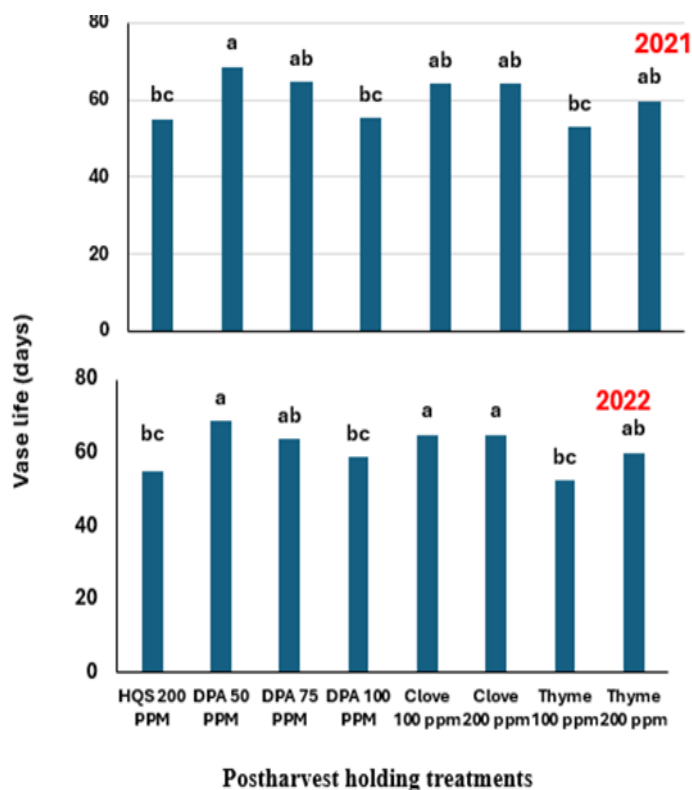
**RESULTS AND DISCUSSION**

**Results**

**Postharvest characteristics**

**Vase life (days).**

The results received from this analysis showed a significant exchange of vase life plotted as a function of treatments for ruscus branches (Figure 1). The DPA at 50 or 75 ppm treatments significantly recorded the highest vase life periods during both seasons. Followed by the treatments of thyme at 200 ppm, and clove at 100 or 200 ppm, and insignificant differences appeared between them and the previous superior treatment. However, the 8-HQS at 200 ppm treatment came in the second order after the previous treatments in that respect during both seasons with insignificant differences between it and most of the other holding solutions except for the treatment of DPA at 50 ppm.

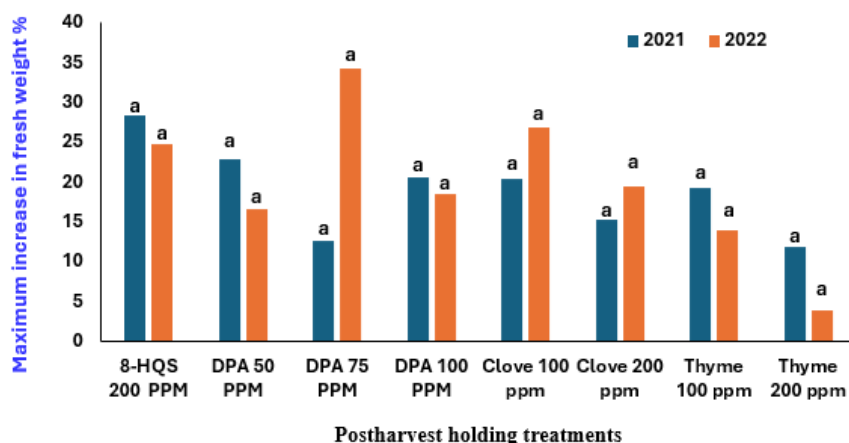


**Figure 1.** The vase life of Ruscus was exposed to different holding solutions during both seasons of 2021 and 2022. According to Duncan's Test at 5%, the uppercase letters signify differences. Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

**Maximum increase in fresh weight (%)**

The MIFW% of ruscus experiment-based change in fresh weight of ruscus branches has been attended (Figure 2). In general, the overview of results, both seasons are approximately the same trends since there are insignificant differences among all the examined holding treatments with this parameter. However, both 8-HQS at 200 ppm, clove at

100 ppm, and DPA at 100 treatments presented a higher maximum increase in fresh weight percentage in both seasons compared with other treatments. Also, the DPA at 75 ppm presents a higher value of MIFW% during the second season. The lowest MIFW% observed with the rest of the essential oil treatments was about below 15% in both seasons.



**Figure 2.** The maximum increase in fresh weight of ruscus branches exposed to different holding solutions during both seasons of 2021 and 2022. According to Duncan's Test at 5%, the uppercase letters signify differences. Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

**Change in fresh weight (%).**

Generally, data in Table (1) showed that all preservative solutions gained an increase in relative fresh weight values during the vase life until the 30<sup>th</sup> day. Holding solutions fortified with 8-HQS at 200 ppm or DPA at 100 produced the highest CFW% during the 10<sup>th</sup> and 20<sup>th</sup> days in both seasons. Moreover, the DPA at different concentrations (50, 75, and 100 ppm) increases gradually until the end of the experiment. Secondly, the 8-HQS at 200 ppm also be stable through the vase life period even lower than the DPA treatment at 100 ppm. However, both essential oil treatments at two concentrations (100, and 200 ppm), presented the lowest values of CFW% during both seasons. However, the thyme at 100 ppm presents slight increases in RFW% especially at the 30<sup>th</sup> days of the vase life.

**Total water uptake (g/cut branch)**

The results obtained from this investigation indicate differences in the total water uptake (Figure 3). The DPA at 50 ppm significantly increased the total water uptake over 40 ml stick<sup>-1</sup> during both seasons (2021 and 2022), compared with other different DPA concentrations and treatments. Moreover, clove oil at 200 ppm and thyme oil at 100 or 200 ppm came in the second order after the previous superior

treatments of DPA at 50 or 75 ppm during both seasons without any significant differences among them. On the other side, holding solutions supplemented with 8-HQS at 200 ppm or clove oil show the lowest effect than other treatments during both seasons.

**Change in water uptake (g/cut branch).**

The variation of water uptake of ruscus branches as a function of the vase life duration at different holding treatments was observed in Table (2). Initially, it recorded a higher decrease from the 10<sup>th</sup> day of vase life with all preservative solutions up to the end of the experiment time. Moreover, all treatments significantly recorded a decrease in the water uptake by ruscus branches at the beginning of the vase life until the 10<sup>th</sup> day of vase life with insignificant differences among all the examined holding treatments, especially in the first season. Moreover, it was very noticeable that 8-HQS and DPA treatments increased the change in water uptake during the vase life duration than most of the other treatments starting from the beginning of the vase life until the end. Also, both essential oil treatments under their concentrations recorded higher changes in water uptake starting from the 20<sup>th</sup> day to the 30<sup>th</sup> day of the vase's life.

**Table 1.** Change in fresh weight (CFW%) of ruscus branches (10<sup>th</sup>: 30<sup>th</sup> days of vase life) exposed to different holding solutions during both seasons of 2021 and 2022. According to Duncan's Test at 5%, the uppercase letters signify differences within the same column.

Holding treatments (ppm)	Change in fresh weight (%) during vase life days					
	2021			2022		
	10	20	30	10	20	30
8-HQS 200	-9.72 <sup>a</sup>	-7.36 <sup>a</sup>	-39.72 <sup>ab</sup>	-8.62 <sup>a</sup>	-11.12 <sup>a</sup>	-18.44 <sup>a</sup>
DPA 50	-13.06 <sup>a</sup>	-15.34 <sup>a</sup>	-25.32 <sup>ab</sup>	-5.39 <sup>a</sup>	-9.75 <sup>a</sup>	-28.21 <sup>a</sup>
DPA 75	-14.17 <sup>a</sup>	-19.73 <sup>a</sup>	-50.32 <sup>b</sup>	-8.80 <sup>a</sup>	-17.65 <sup>a</sup>	-29.45 <sup>a</sup>
DPA 100	-3.84 <sup>a</sup>	-5.77 <sup>a</sup>	-44.80 <sup>b</sup>	-9.19 <sup>a</sup>	-14.59 <sup>a</sup>	-40.15 <sup>a</sup>
Clove 100	-16.11 <sup>a</sup>	-5.97 <sup>a</sup>	-28.47 <sup>ab</sup>	-9.37 <sup>a</sup>	-15.97 <sup>a</sup>	-48.95 <sup>a</sup>
Clove 200	-20.71 <sup>a</sup>	-19.28 <sup>a</sup>	-0.71 <sup>a</sup>	-15.62 <sup>a</sup>	-25.89 <sup>a</sup>	-32.14 <sup>a</sup>
Thyme 100	-10.53 <sup>a</sup>	-13.03 <sup>a</sup>	-19.37 <sup>ab</sup>	-19.82 <sup>a</sup>	-16.69 <sup>a</sup>	-27.85 <sup>a</sup>
Thyme 200	-10.77 <sup>a</sup>	-11.81 <sup>a</sup>	-32.21 <sup>ab</sup>	-23.80 <sup>a</sup>	-7.14 <sup>a</sup>	-17.85 <sup>a</sup>

Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

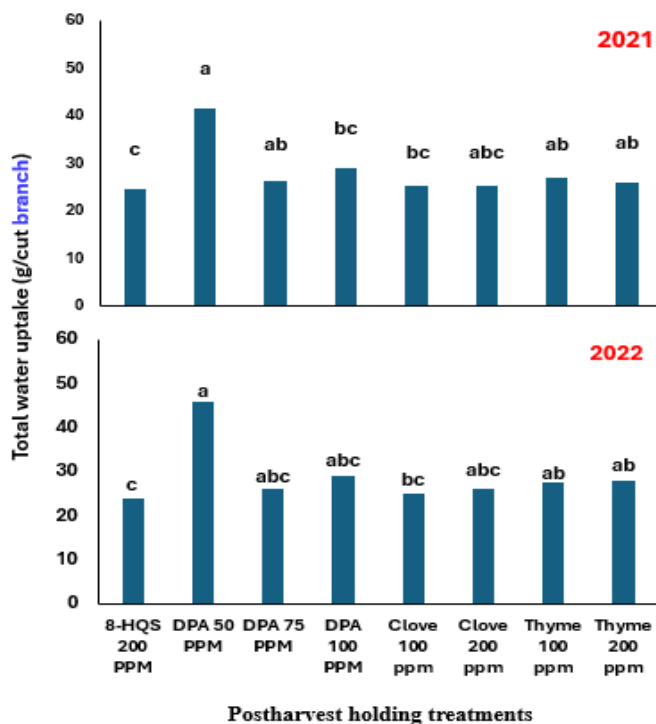


Figure 3. Total water uptake (g/cut/vase life) of ruscus branches exposed to different holding solutions during two seasons (2021 and 2022). According to Duncan's Test at 5%, the uppercase letters signify differences. Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

Table 2. Change in water uptake (g/branch) of ruscus (10<sup>th</sup>: 30<sup>th</sup> days of vase life) exposed to different holding solutions during two seasons (2021 and 2022). According to Duncan's Test at 5%, the uppercase letters signify differences within the same column.

Holding treatments (ppm)	Change in water uptake (g/branch) during vase life days					
	2021			2022		
	10	20	30	10	20	30
8-HQS 200	1.50 <sup>a</sup>	0.50 <sup>c</sup>	1.75 <sup>ab</sup>	2.50 <sup>a</sup>	2.00 <sup>bc</sup>	2.25 <sup>a</sup>
DPA 50	0.25 <sup>a</sup>	0.25 <sup>c</sup>	0.50 <sup>bc</sup>	2.25 <sup>a</sup>	1.50 <sup>bc</sup>	2.50 <sup>a</sup>
DPA 75	0.50 <sup>a</sup>	0.75 <sup>c</sup>	2.25 <sup>a</sup>	1.25 <sup>ab</sup>	1.00 <sup>bc</sup>	0.25 <sup>c</sup>
DPA 100	0.50 <sup>a</sup>	0.25 <sup>c</sup>	0.25 <sup>bc</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	0.50 <sup>c</sup>
Clove 100	2.50 <sup>a</sup>	3.25 <sup>ab</sup>	1.00 <sup>ab</sup>	2.00 <sup>a</sup>	4.50 <sup>ab</sup>	1.00 <sup>ab</sup>
Clove 200	1.00 <sup>a</sup>	0.50 <sup>c</sup>	0.50 <sup>bc</sup>	1.00 <sup>ab</sup>	8.50 <sup>a</sup>	0.25 <sup>c</sup>
Thyme 100	1.25 <sup>a</sup>	5.25 <sup>a</sup>	1.00 <sup>ab</sup>	1.00 <sup>ab</sup>	7.00 <sup>a</sup>	0.75 <sup>ab</sup>
Thyme 200	1.00 <sup>a</sup>	7.25 <sup>a</sup>	2.25 <sup>a</sup>	0.25 <sup>c</sup>	6.25 <sup>ab</sup>	1.50 <sup>ab</sup>

Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

**Change in water loss (g/cut branch)**

In general, there are insignificant differences among most of the holding solutions until the 10<sup>th</sup> day of the vase's life (Table 3). However, it was quite clear that the treatments of both essential oils (clove and thyme) under both concentrations recorded the lowest water loss values during the 10<sup>th</sup> day of the vase's life. On the 20<sup>th</sup> day, holding solutions fortified with 8-HQS at 200 ppm or DPA at all concentrations recorded the lowest change in water loss values compared with most of the other treatments. In contrast, the highest water loss values were recorded for preservative solutions supplemented with thyme oil at 100 or 200 ppm during the 30<sup>th</sup> day of the vase life.

**Change in water balance (g/cut branch)**

The water balance of all treatments started higher and decreased steadily based on treatment throughout the vase life duration (Table 4). Moreover, there were insignificant differences among all preservative solutions at the 10<sup>th</sup> of the

vase life in that parameter. But on the 20<sup>th</sup> day of the vase life holding solutions supplemented with DPA at 100 ppm, produced the highest water balance values in comparison with most of the other treatments, although there were insignificant differences among all holding solutions. Starting from the 10<sup>th</sup> day until the 30<sup>th</sup> day of the vase's life, holding solutions fortified with DPA at 50, 75, and 100 ppm, or clove oil at 100 ppm produced the highest water balance values. However, it was quite clear that the control treatment (200 ppm 8-HQS) produced the maximum water balance value on the 30<sup>th</sup> day from the start of the vase's life.

**Cell wall degradation enzyme activities (CWDEs).**

Data presented in Figures 4A, and 4B show the variation of the cell wall degradation enzyme activities (unit g<sup>-1</sup> FW) as a function of holding treatments. Initially, the influence of preservative solutions on cell wall degradation enzyme activities showed that the CWDEs decreased gradually during the vase life duration with all enzymes

monitored compared to the initial day. The highest increases in activities were observed with both essential oil treatments at both concentrations (100 and 200 ppm) during both seasons. However, the HQS presents the lowest activities

during the vase life duration. Along with DPA concentrations (50, 75, 100 ppm) decreased enzyme activities based on increasing DPA concentrations during the vase life period.

**Table 3. Change in water loss (g/branch) of ruscus (10<sup>th</sup>: 30<sup>th</sup> days of vase life) exposed to different holding solutions during two seasons (2021 and 2022). According to Duncan's Test at 5%, the uppercase letters signify differences within the same column.**

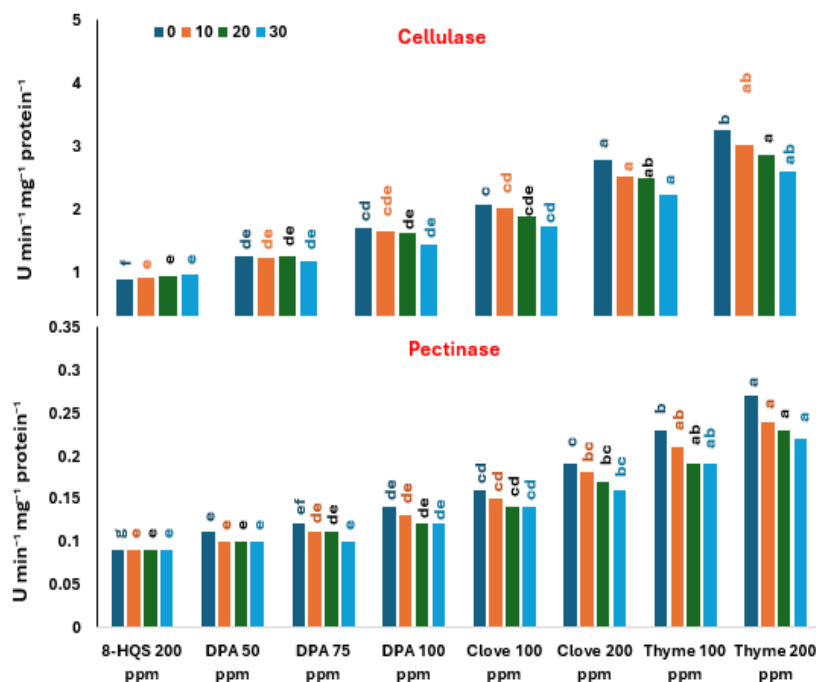
Holding Treatments (ppm)	Change in water loss (g/ branch) during vase life days					
	2021			2022		
	10	20	30	10	20	30
8-HQS 200	4.25 <sup>a</sup>	3.75 <sup>bc</sup>	4.50 <sup>ab</sup>	4.50 <sup>a</sup>	4.00 <sup>c</sup>	2.75 <sup>b</sup>
DPA 50	4.25 <sup>a</sup>	4.50 <sup>bc</sup>	6.25 <sup>ab</sup>	5.00 <sup>a</sup>	3.00 <sup>c</sup>	6.25 <sup>a</sup>
DPA 75	3.75 <sup>a</sup>	3.00 <sup>c</sup>	6.25 <sup>ab</sup>	2.50 <sup>b</sup>	3.00 <sup>c</sup>	4.00 <sup>a</sup>
DPA 100	1.75 <sup>a</sup>	2.25 <sup>c</sup>	8.25 <sup>a</sup>	2.50 <sup>b</sup>	3.50 <sup>c</sup>	5.00 <sup>a</sup>
Clove 100	3.25 <sup>a</sup>	7.50 <sup>ab</sup>	4.50 <sup>bc</sup>	2.25 <sup>b</sup>	8.00 <sup>ab</sup>	5.50 <sup>a</sup>
Clove 200	3.00 <sup>a</sup>	9.50 <sup>ab</sup>	6.75 <sup>ab</sup>	1.00 <sup>bc</sup>	8.50 <sup>ab</sup>	4.50 <sup>a</sup>
Thyme 100	3.50 <sup>a</sup>	10.25 <sup>a</sup>	4.00 <sup>bc</sup>	4.25 <sup>a</sup>	10.50 <sup>a</sup>	5.75 <sup>a</sup>
Thyme 200	3.00 <sup>a</sup>	10.25 <sup>a</sup>	2.50 <sup>c</sup>	4.00 <sup>a</sup>	9.50 <sup>a</sup>	5.00 <sup>a</sup>

Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

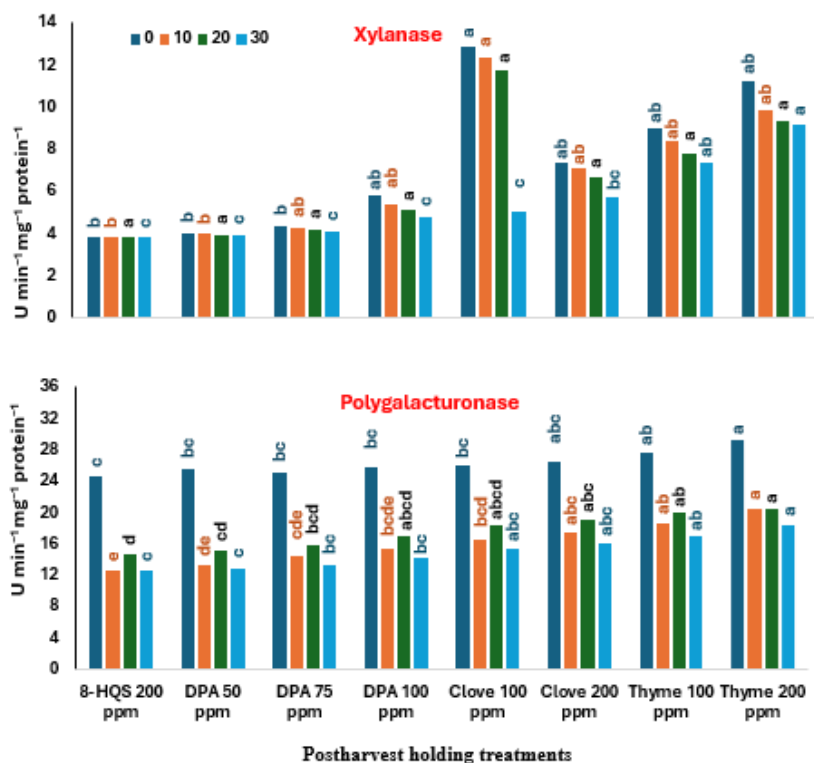
**Table 4. Change in water balance (g/branch) of ruscus (10<sup>th</sup>: 30<sup>th</sup> days of vase life) exposed to different holding solutions during two seasons (2021 – 2022). According to Duncan's Test at 5%, the uppercase letters signify differences within the same column.**

Holding Treatments (ppm)	Change in water balance (g/ branch) during vase life days					
	2021			2022		
	10	20	30	10	20	30
8-HQS 200	-2.75 <sup>a</sup>	-3.25 <sup>ab</sup>	-2.75 <sup>a</sup>	-2.00 <sup>ab</sup>	-2.00 <sup>a</sup>	0.50 <sup>a</sup>
DPA 50	-4.00 <sup>b</sup>	-4.25 <sup>ab</sup>	-5.75 <sup>b</sup>	-2.75 <sup>ab</sup>	-1.50 <sup>a</sup>	-3.75 <sup>b</sup>
DPA 75	-3.25 <sup>ab</sup>	-2.25 <sup>a</sup>	-4.00 <sup>ab</sup>	-1.25 <sup>ab</sup>	-2.00 <sup>a</sup>	-3.75 <sup>b</sup>
DPA 100	-1.25 <sup>a</sup>	-2.00 <sup>a</sup>	-8.00 <sup>b</sup>	-2.25 <sup>ab</sup>	-3.25 <sup>ab</sup>	-4.50 <sup>bc</sup>
Clove 100	-0.75 <sup>a</sup>	-4.25 <sup>ab</sup>	-3.50 <sup>ab</sup>	-0.25 <sup>a</sup>	-3.50 <sup>ab</sup>	-4.50 <sup>bc</sup>
Clove 200	-2.00 <sup>ab</sup>	-9.50 <sup>bc</sup>	-6.25 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	-4.25 <sup>bc</sup>
Thyme 100	-2.25 <sup>ab</sup>	-5.00 <sup>ab</sup>	-3.00 <sup>ab</sup>	-3.50 <sup>bc</sup>	-3.50 <sup>ab</sup>	-5.00 <sup>bc</sup>
Thyme 200	-2.00 <sup>ab</sup>	-3.50 <sup>ab</sup>	-0.25 <sup>a</sup>	-3.25 <sup>bc</sup>	-3.25 <sup>ab</sup>	-3.25 <sup>b</sup>

Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.



**Figure 4A. Cell wall degradation enzyme activities (cellulase and pectinase) of ruscus branches exposed to different holding solutions measured every 10-day intervals (0, 10, 20, and 30 days). According to Duncan's Test at 5%, the uppercase letters signify differences. Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.**



**Figure 4B.** Cell wall degradation enzyme activities (xylanase and polygalacturonase) of ruscus branches exposed to different holding solutions measured every 10 days intervals (0, 10, 20, and 30 days). According to Duncan's Test at 5%, the uppercase letters signify differences. Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

**Bacterial counts**

The average bacterial account was recorded in the preservative solutions of all holding treatments after the 10<sup>th</sup> day of the vase life for ruscus branches during both seasons of 2021 and 2022. The results tabulated in Table (5) revealed that the least average bacterial counts ( $2.70 \times 10^5$  and  $2.30 \times 10^7$ ), ( $1.18 \times 10^7$  and  $1.85 \times 10^7$  CFU ml<sup>-1</sup>) were accounted for using 8-HQS at 200 ppm and DPA at 50 ppm in both seasons, respectively compared with other treatments of ruscus. Moreover, the clove oil treatment at 200 ppm recorded  $1.03 \times 10^7$  CFU ml<sup>-1</sup> and  $1.00 \times 10^3$  during both seasons, respectively.

**Table 5** Average of total bacterial count CFU ml<sup>-1</sup> of different postharvest treatments under study during two seasons (2021 - 2022)

Holding treatments (ppm)	Bacterial numbers	
	2021	2022
8-HQS 200	$2.70 \times 10^5$	$2.30 \times 10^7$
DPA 50	$1.18 \times 10^7$	$1.85 \times 10^7$
DPA 75	$3.20 \times 10^7$	$5.20 \times 10^8$
DPA 100	$6.10 \times 10^7$	$1.20 \times 10^5$
Clove 100	$2.30 \times 10^7$	$1.75 \times 10^4$
Clove 200	$1.03 \times 10^6$	$1.00 \times 10^3$
Thyme 100	$4.30 \times 10^6$	$5.70 \times 10^6$
Thyme 200	$2.50 \times 10^7$	$3.00 \times 10^3$

Since, 8-HQS; 8-hydroxyquinoline sulfate, DPA; Diphenylamine.

**Discussion**

The results presented in this investigation indicate that ruscus branches are indeed different according to the difference of these holding solutions. Both 8-HQS and DPA were shown to be the most effective for enhancing the postharvest characteristics of this important cut foliage plant. The greater effect that often occurred with DPA compared to most of the examined preservative treatments was also

unexpected. Since holding solutions contained DPA at 50 and 75 ppm, 8-HQS at 200, and clove oil at 200 improved the longevity of the cut branches, enhanced the water relations (water uptake and water balance), reduced the water loss, reduced the cell wall degradation enzymes activities, and minimized the bacterial counts in holding solutions. The primary cause of the varying vase life of ruscus branches is occlusion, which is mostly found at the stem end of the plant and is most likely brought on by microbial development, vascular obstruction, and increased water loss through transpiration of the leaves (Manzoor et al., 2024). Our results were in line with the finding of Mostafa (2020) who explained that diphenylamine (DPA) has the structural formula  $[C_6H_5)_2 NH]$ , making it an aromatic antioxidant amine. It serves as a fungicide and a regulator of plant growth. Also, the DPA decreased the formation of ethylene, which in turn decreased the synthesis and oxidation of  $\alpha$ -farnesene which in turn could prolong the shelf life of cut foliage.

Also, our results presented that the 8-HQS treatment has a positive influence on the vase life and postharvest quality of ruscus branches in different measuring times. The application of 8-HQS at 200 ppm increased the vase life of ruscus branches. These results were in line with the results of (Comby et al., 2012). This is because 8-HQS functions as an anti-microbial agent, which may inhibit xylem occlusion and reduce the buildup of microorganisms in the xylem vessel (Manzoor et al., 2021). Furthermore, when the 8-HQS plays a part in excessively fast cellular water loss, it may also lessen the plasmolysis of cells. These findings agree with the reports of (Nazemi Rafi and Ramezani, 2013; El-Shawa et al., 2022;). Also, it preserves membrane stability during vase life (Kazemi et al., 2018). In addition, 8-HQS maintains the water uptake and controls the microbial population in vase solutions

which extends the longevity of cut foliage. The present study showed that 8-HQS and DPA had the strongest effect on vase life solution uptake microbial population suppression, and fresh weight loss inhabitation. During the vase life, the cell wall degradation enzymes exhibit a degree of growth followed by a fall in activity. Remarkably, the addition of essential oils also causes increases in enzyme activity, in certain instances, the increases are highest when the concentrations of both essential oils are at 100 and 200 ppm.

Regarding DPA, selecting the appropriate treatment and storage period to preserve cut foliage should be based in large part on the material's intended purpose. The DPA has been applied worldwide to minimize oxidative stress (Dias et al., 2020). Also, it used in the horticultural product industry for more than 50 years (Dehnen-Schmutz et al., 2010). It is considered an aromatic antioxidant amine (Osman, 2014), and it increases the total antioxidant too. Thus, it delays blooming senescence and prevents cell and tissue damage by acting as a scavenger (Mostafa, 2020). In addition, compared to previous treatments, applying any amount of DPA shortened the time required for blooming. These findings could be explained by the fact that DPA can influence the composition of proteins, auxins, and enzymes (Karagiannis et al., 2018). Overall, cell wall degradation enzyme activities are less with holding the cut branches of ruscus in preservative solutions containing 8-HQS at 200 ppm or DPA at 50 ppm than the essential oil treatments. This strength might originate from the idea that oxidative damage is happening, and that the redox homeostasis model suggests that the rate of damage is inversely reliant upon antioxidant capability (Skopelitis et al., 2006; El-Batrawy, 2022).

In addition, holding solutions fortified with 8-HQS at 200 ppm, DPA at 75 ppm, and clove at 100 ppm recognized the highest increase in fresh weight, relative fresh weight, and the total amount of water absorbed, and delayed cell wall degradation enzyme activities. Essential oils (Eos) were found or extruded from plants from blossoms, seeds, fruits, fruit peels, leaves, bark, wood, and roots. The main effect of EOs is considered as an antimicrobial preparative against some pathogens (Al-Maqtari et al., 2022). Nonetheless, they all exhibit some degree of growth during the vase life, followed by a decline in the activity of CWDEs (Figures 4A and 4B). Where the activity is dependent on the type of essential oil (clove and thyme), it increases with both tested concentrations (100 and 200 ppm). Furthermore, because of the induction that occurs between the cationic surface and microorganisms, which results in a modification of the cell membrane and programmed cell death, the EOs reduced the antibacterial mechanisms. Additionally, other investigations revealed that by stifling the essential nutrients for microbial development, EOs can inhibit the transcription of RNA from DNA or function as chelating agents like metal (Valdivieso-Ugarte et al., 2019).

## CONCLUSION

However, the information provided in this study shows that holding solutions supplemented with diphenylamine (DPA) at 50 or 75 ppm could take the place of 8-HQS at 200 ppm (control) in improving the postharvest characteristics of cut ruscus branches. In addition to delaying the activities of the enzymes that break down cell walls and the number of bacteria in the preservative solutions, it also created the longest vase life period, the greatest rise in fresh weight, total water absorption, and relative fresh weight.

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## إطالة فترة ما بعد الحصاد لأفرع السفندر المقطوعة باستخدام بعض محاليل الحفظ المختلفة

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### الملخص

أجريت الدراسة الحالية بمعامل قسم الخضار و الزينة بكلية الزراعة جامعة المنصورة بمصر خلال الموسمين المتتاليين 2021 و 2022 لدراسة تأثير بعض محاليل التثبيت على بعض صفات ما بعد الحصاد لفريعات نبات السفندر (*Ruscus Hypophyllum*). تحتوي هذه المحاليل الحافظة على المركبات التالية، 8-هيدروكسي كينولين سلفات (8-HQS) بتركيز 200 جزء في المليون كعامل الكنترول، داي فينيل أمين (DPA) بتركيزات 50، 75، 100 جزء بالمليون، وإيثان من الزيوت العطرية (الزعر و القرفل بتركيز 100 و 200 جزء في المليون لكليهما). تم الاحتفاظ بجميع الفريعات المقطوعة الموجودة في محاليل التثبيت بدرجة حرارة  $25 \pm 2$  درجة مئوية تحت رطوبة نسبية 60% - 70% ومصباح الفلورسنت ذات اللون الأبيض البارد المقامة بواسطة ضوء 1000 لوكن لفترة ضوئية مدتها 12 ساعة. أشارت البيانات التي تم الحصول عليها إلى أن DPA عند تركيز 50 أو 75 جزء في المليون قد أدى إلى تحسين معظم صفات ما بعد الحصاد لهذه الفريعات المقطوعة مثل فترة البقاء بعد القطف، والحد الأقصى للزيادة في الوزن الطازج، والوزن الطازج النسبي، وإجمالي امتصاص الماء. بالإضافة إلى ذلك، أدت هذه المعاملات المتوقعة إلى تقليل فقدان الماء، وأنشطة إنزيمات تحلل جدار الخلايا، وأعداد البكتيريا في المحاليل الحافظة. كما أن 8-HQS بتركيز 200 جزء في المليون (الكنترول) عزز معظم خصائص ما بعد الحصاد المدروسة لفريعات السفندر المقطوعة مع وجود اختلافات غير معنوية بينها وبين معاملات DPA.