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# Carbon nanotubes in the holding solution stimulate flower opening and prolong vase life in carnation

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## Abstract

**Background:** Cut flower longevity is often limited by adverse water relations or oxidative stress. The potential of single- and multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) on prolonging vase life was addressed. Dose–response curves were obtained by applying five concentrations (0–80 mg L<sup>-1</sup>) of SWCNTs or MWCNTs either once as a foliar spray or continuously in the holding solution of three carnation cultivars. Next, the optimal concentration of either SWCNTs or MWCNTs was employed to evaluate several parameters critical for vase life.

**Results:** Foliar spray application exerted minor effects on water relations, flower opening and keeping quality. By contrast, including CNTs in the holding solution sustained a positive water balance for a longer period, improved flower opening and prolonged vase life. These effects were similar between SWCNTs and MWCNTs, and were concentration-dependent. The optimal concentration for vase life was higher for MWCNTs as compared to SWCNTs, and for two cultivars as compared to the third one. At optimal concentration, SWCNTs or MWCNTs in the holding solution generally maintained turgidity, and alleviated chlorophyll degradation, electrolyte leakage and lipid peroxidation. These effects were related to increased activation of enzymatic (ascorbate peroxidase, catalase, peroxidase and superoxide dismutase) and non-enzymatic (carotenoids, polyphenols, and flavonoids) antioxidants.

**Conclusion:** CNTs in the holding solution were very effective in stimulating vase life through improved water relations and enhanced antioxidant machinery stimulation.

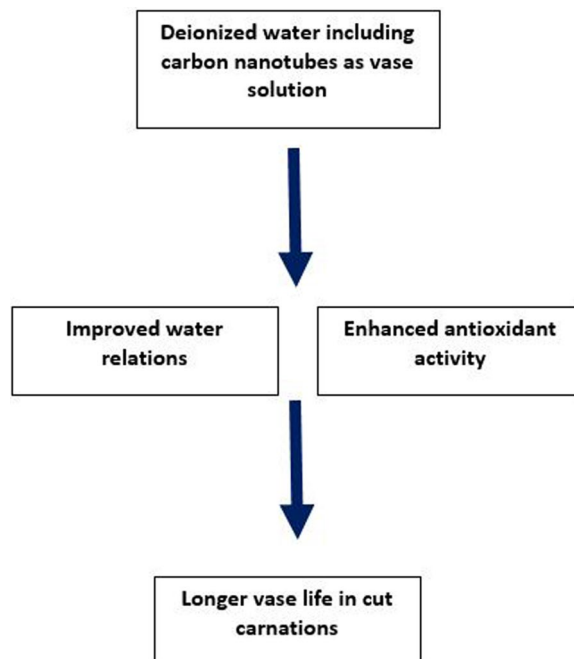
**Keywords:** Antioxidant defense, *Dianthus caryophyllus*, Keeping quality, Water status

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### Graphical Abstract



### Background

From the consumers' point of view, long vase life and adequate flower bud opening are key quality requirements [1, 2]. Short vase life or incomplete flower bud opening are associated with low perceived value (thus low consumer satisfaction), and are regarded as primary purchasing barriers [3, 4]. Overcoming these difficulties is therefore essential for the horticultural industry.

Cut flower longevity and flower bud opening are often limited by adverse water relations [5]. Water deficit develops when transpiration is not compensated by water uptake from the vase, and causes wilting symptoms ending vase life [6, 7]. Therefore, extending the period of positive water balance is a direct way of improving vase life [8]. In planta, carbon nanotubes (CNTs) have been shown to both positively affect water balance [9, 10], and stimulate tolerance against drought [11, 12]. These promotive effects, however, were dependent on the plant growth stage, as well as on the concentration and nature (single- or multi-walled) of CNTs [13]. Since previous studies were limited to developing plants, it remains unknown whether or not CNTs can also regulate the water balance during the postharvest period. If this hypothesis is validated, CNTs may provide an opportunity to alleviate the keeping quality problems associated with disturbed water relations.

Vase life terminating symptoms are also elicited by oxidative stress, which is generally triggered by unfavorable conditions [14, 15]. Oxidative stress results from excessive generation of reactive oxygen species (ROS) [16–18]. ROS accumulation stimulates lipid peroxidation via oxidation of unsaturated fatty acids, leading to membrane damage and electrolyte leakage [16, 19]. Therefore, an enhanced ability to scavenge ROS has a direct positive impact on vase life [15, 20]. To scavenge ROS, the antioxidant defense ought to be involved, encompassing both specific enzymes and metabolites. The former group includes ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), which are critical ROS detoxification enzymes [15]. Carotenoids, polyphenolics, and flavonoids represent important non-enzymatic antioxidants [16, 21]. When applied during growth, CNTs have been shown to activate the enzymatic and non-enzymatic antioxidant defense [12, 21]. However, whether or not CNTs can alleviate postharvest oxidative damage in cut flowers is still unclear.

The objectives of this study were to determine the optimal application method (spray or in the holding solution), type (single- or multi-walled) and concentration of CNTs for vase life enhancement by constructing dose–response curves in different cultivars. Our assessments encompassed key processes underlying vase life,

including water relations and critical antioxidants. Carnation was employed as model species, since it is one of the most popular cut flowers, and normally has a short vase life ( $\approx 5\text{--}10$  d) depending on the cultivar [1].

## Methods

### Plant material and growth conditions

Cut standard carnation flowers were obtained from a commercial grower (33°54'40" N 50°27'11" E; Mahallat, Markazi Province, Iran). Plants were grown in a multispan plastic greenhouse. Two harvests were conducted (1 and 21 November, 2020), supplying material for the two respective experiments. In either experiment, three cultivars were tested (White Liberty, Grand Slam, Kirs). Based on the proximity of the two harvest dates, it is safe to attribute the noted phenotypic differences to the genotype (thus limiting its interaction with the growth environment).

In either experiment, harvested shoots had a length of approximately 0.5 m, while petals have just started to elongate outside the calyx (the so-called 'paint-brush' stage). Cut flowers were further selected for uniformity based on the diameter at the bottom part of the stem ( $\approx 0.4$  cm). These were collected in the morning (08:00–10:00 h), and immediately transferred to the laboratory. Cut flowers were stored overnight in buckets filled with water at 4 °C and darkness. Following this rehydration period, stem length was shortened to 0.4 m by submerging it under water (to prevent cavitation of xylem vessels that were opened by cutting), and then all leaves were removed except for the uppermost five pairs. Following rehydration, stem length shortening and leaf number normalization practices, that fresh weight was regarded as the initial one.

Cut flower and organ (leaves or petals) level measurements were conducted (Experiments 1 and 2, respectively). For leaf-level measurements, sampled leaves had grown under direct light, were fully expanded and devoid of obvious symptoms of either pathogen infection or insect damage. For petal-level measurements, five outermost petals were detached from each flower, and further pooled. In all cases, the time between sampling and the start of the evaluation did not exceed 15 min. When this was not possible, samples (leaves or petals) were placed in vials, flash-frozen in liquid nitrogen and transferred to a freezer ( $-80$  °C) for storage. Replicate shoots were collected from separate plants, while replicate leaves or petals were sampled from separate shoots.

### Effect of CNTs applied as a foliar spray or in the holding solution on vase life, flower opening and water relations (Experiment 1)

The effect of two CNT types (single- or multi-walled, abbreviated as SWCNTs and MWCNTs, respectively) employed at different concentrations as foliar spray or

in the holding solution, on vase life was investigated. The night before the experiment, the cut flowers were kept in the dark refrigerated storage (4 °C) for 12 h, to ensure maximal turgidity [22]. Next day, vase life was determined on cut flowers that were placed in the vase (one flower per flask).

In one set of cut flowers, either SWCNTs or MWCNTs at different concentrations (0, 10, 20, 40 and 80 mg L<sup>-1</sup>) were applied via foliar spray, and then cut flowers were placed in deionized water, as holding solution. In another set of cut flowers, the holding solution contained either SWCNTs or MWCNTs at different concentrations (0, 10, 20, 40 and 80 mg L<sup>-1</sup>) by using deionized water. High-quality (> 95% purity) CNTs were purchased from a commercial supplier (Iranian Nanomaterial Pioneers Company, Mashhad, Iran). The CNTs were originally synthesized through chemical vapor deposition method by US Research Nanomaterial, Inc, USA. The average diameter of SWCNTs was 1.1 nm, while their length ranged between 5 and 30  $\mu\text{m}$ . The average inside diameter of MWCNTs varied between 5 and 10 nm, while their length ranged between 10 and 30  $\mu\text{m}$ . Both SWCNTs and MWCNTs were functionalized with a carboxylic group ( $-\text{COOH}$ ). Vase solution containing CNTs was shaken and then sonicated (VS-505 sonicator; Sonics and Materials, Inc., Newtown, CT, USA) for 30 min. This procedure was repeated four times. On a daily basis throughout the experiment, the holding solution containing CNTs was also vigorously shaken (20 min).

The vases contained 500 mL holding solution, with their top covered with Parafilm, to ensure that water loss could only occur via the flower stalks. The flasks were randomly placed in a climate-controlled room at 20 °C air temperature, 50% relative air humidity and a light intensity of 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 12 h per day, provided by fluorescent tubes (Pars Shahab Lamp Co., Tehran, Iran). The termination of vase life was determined based on the occurrence of at least one of the following criteria: (i) visible petal wilting (i.e., loss of turgor) followed by contraction and dark discoloration or shriveling; (ii) petal discoloration (edge browning, bluing or color darkening); (iii) discoloration or withering of more than 50% of the leaves, and (iv) discoloration or bending (flower angle becomes larger than 90° from the vertical position) of the stem [23]. In this study, no *Botrytis cinerea* infections were observed. The flower and flask weights were recorded separately every 2 days starting at the onset of vase life ( $\pm 0.01$  g; MXX-412; Denver Instruments, Bohemia, NY, USA). The transpiration rate was calculated per unit fresh weight and per unit time [24]. The flower diameter was also recorded every other day during vase life, by assessing the maximum diameter. Ten cut flowers

per treatment were assessed in three cultivars (White Liberty, Grand Slam, Kirsi).

#### Effect of CNTs in the holding solution on parameters affecting cut flower longevity (Experiment 2)

Based on the results of Experiment 1, the optimum CNTs' concentration in the holding solution for vase life was selected. For SWCNTs, that concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup>. For MWCNTs, the respective concentration was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup>. The holding solution for controls was deionized water. Cut flowers were submitted to the same conditioning methods and test room conditions as described for Experiment 1. Measurements were performed during vase life at 4-day intervals by starting at time 0. The parameters under study are described below. In all cases, 4 replicates were considered per treatment in three cultivars (White Liberty, Grand Slam, Kirsi).

#### Leaf and petal water status

Leaf and petal water status during vase life was assessed by measuring relative water content (RWC; also referred as relative turgidity). Samples were collected 3 h following the onset of the photoperiod [25]. Following excision, fresh weight was gravimetrically obtained ( $\pm 0.0001$  g; Mettler AE 200, Giessen, Germany). Immediately after, samples were floated on distilled water inside a Petri dish, covered with a lid. Following 24 h of incubation, the weight was recorded, and was regarded as turgid (saturated) weight. Then, dry weight (48 h at 80 °C) was determined. RWC was calculated according to Taheri-Garavand et al. [26].

#### Leaf chlorophyll and carotenoid contents

Decreases in chlorophyll content are associated with leaf discoloration, which is a vase life terminating symptom [23]. Carotenoids are important non-enzymatic

China). Chlorophyll *a*, chlorophyll *b*, total chlorophyll (i.e., chlorophyll *a* + chlorophyll *b*), and carotenoid contents were calculated according to Lichtenthaler and Wellburn [28].

#### Leaf total phenolic and total flavonoid contents

Phenols and flavonoids represent important non-enzymatic antioxidants [16]. For extraction, finely ground leaves (0.5 g) were incubated in methanol (80%). After 15 min at room temperature, it was centrifuged (14,000g) for 15 min. The contents of total phenolic and flavonoid were determined by using the Folin–Ciocalteu assay and aluminum chloride colorimetric assay, respectively, following Chen et al. [16]. The absorbance against prepared reagent blank was determined using a spectrophotometer (Mapada UV-1800; Shanghai Mapada Instruments Co., Ltd., Shanghai, China). For total phenolic content, gallic acid was used as the standard reference and gallic acid equivalent (GAE) was expressed as mg per g fresh mass. For total flavonoid content, quercetin was used as the standard reference and quercetin equivalent (QUE) was expressed as mg per g fresh mass.

#### Free radicals' neutralization ability in the leaves

The extraction for antioxidant activity determination was similar as that of the total phenolic and flavonoid content determination. The ability of the extracts to neutralize the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined by the method described by [29]. Briefly, a volume of 20  $\mu$ L of sample extract with methanol was made up to 100  $\mu$ L. Then, 350  $\mu$ L freshly prepared DPPH was mixed to it. The mixture was then incubated for 20 min in the dark at room temperature. Afterwards, the absorbance measurements were conducted at 517 nm using a spectrophotometer (Mapada UV-1800; Shanghai Mapada Instruments Co., Ltd., Shanghai, China). A positive control (ascorbic acid) was prepared in the same way as samples. The DPPH radical scavenging of each plant extract was calculated using the following equation:

$$\text{Scavenging activity(\%)} = [A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}] \times 100,$$

antioxidants [16]. The leaf chlorophyll and carotenoid contents under different holding solution compositions were therefore assessed. Samples were processed immediately after collection. Following fine chopping, portions weighing 0.1 g were homogenized with the addition of 10 mL of 100% acetone. The extract was then centrifuged (4000g for 15 min), and the supernatant was collected. Since chlorophyll is light sensitive, extraction took place in a dark room [27]. The obtained extract was subjected to reading on a spectrophotometer (Mapada UV-1800; Shanghai Mapada Instruments Co., Ltd., Shanghai,

where *A* control is the absorbance of the control, and *A* sample is the absorbance in the presence of extracts [29]. The decrease on absorption at 517 nm was used for calculating the IC 50 (mg/mL). The antioxidant concentration (mg extract mL<sup>-1</sup>), at which 50% inhibition of free radical activity was observed, was estimated (the so-called IC 50). The IC 50 value is inversely related to the overall effectiveness of the antioxidant.

### Leaf and petal electrolyte leakage

The holding solution effect on the relative ion content in the apoplastic space, taken as an indication of membrane stability, was evaluated by measuring electrolyte leakage [19]. Freshly cut leaf and petal discs (0.79 cm<sup>2</sup> each) were rinsed 3 times (3 min) with deionized water (to remove surface-adhered electrolytes), and subsequently floated on 10 mL of deionized water. The electrolyte leakage in the solution was measured after 24 h of floating at room temperature (25 °C) using a conductimeter (Crison 522, Crison Instruments, S.A., Spain). Samples were then autoclaved for 20 min at 120 °C, and total conductivity was obtained after equilibration at 25 °C. Results were expressed as percentage of total conductivity. Four discs were assessed per replicate sample.

### Leaf and petal lipid peroxidation

The role of holding solution on the malondialdehyde (MDA) content, taken as an indication of lipid peroxidation level, was evaluated during vase life by employing the thiobarbituric acid reactive substance assay [27]. Freshly cut leaf and petal discs (0.1 g) were homogenized, and then added in 5 mL of 20% (w/v) trichloroacetic acid and 0.5% (w/v) thiobarbituric acid. The suspension was subsequently centrifuged (6000g for 15 min). The obtained solution was heated (100 °C for 25 min). After equilibration at 25 °C, the precipitate was removed by centrifugation (6000g for 5 min). The amount of MDA was calculated from the absorbance at 532 nm after subtracting the non-specific absorption at 450 and 600 nm (Mapada UV-1800; Shanghai Mapada Instruments Co., Ltd., Shanghai, China). The extinction coefficient of 156 mmol MDA L<sup>-1</sup> cm<sup>-1</sup> was used. Four discs were assessed per replicate sample.

### Leaf and petal enzymatic activity

APX activity was assessed using the method described by Nakano and Asada [30] with some modifications. Fresh frozen leaf and petal segments (0.1 g) were ground in liquid N<sub>2</sub>, homogenized with 1 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM EDTA and 1% polyvinylpyrrolidone (PVP), and centrifuged (14,000g) for 20 min at 4 °C. APX activity in the supernatant was assessed by following the decrease in absorbance at 290 nm for 2 min (10 s intervals) in a reaction mixture containing sodium phosphate buffer, ascorbic acid, and H<sub>2</sub>O<sub>2</sub>. The extinction coefficient of 2.8 mM<sup>-1</sup> cm<sup>-1</sup> was used. APX activity was expressed as μmol of ascorbate oxidized min<sup>-1</sup> g<sup>-1</sup> tissue.

POD activity was assayed as described by MacAdam et al. [31] with some modifications. Fresh frozen leaf and petal segments (0.3 g) were ground in liquid N<sub>2</sub>, homogenized with 1.5 mL of 50 mM potassium phosphate buffer

(pH 7.0), and centrifuged at 14,000g and 4 °C for 20 min. POD activity in the supernatant was assessed by following the decrease in absorbance at 470 nm for 2 min (10 s intervals) in a reaction mixture containing potassium phosphate buffer, guaiacol, and H<sub>2</sub>O<sub>2</sub>. The extinction coefficient of 26.6 mM<sup>-1</sup> cm<sup>-1</sup> was used. POD activity was expressed as μmol of H<sub>2</sub>O<sub>2</sub> reduced min<sup>-1</sup> g<sup>-1</sup> tissue.

CAT activity was measured as described by Chance and Maehly [32] with some modifications. Fresh frozen leaf and petal segments (0.3 g) were ground in liquid N<sub>2</sub>, homogenized with 1.5 mL of potassium phosphate buffer (containing 1 mM EDTA and 2% PVP), and centrifuged (14,000g for 20 min) at 4 °C. CAT activity in the supernatant was assessed by following the decrease in absorbance at 240 nm for 2 min (10 s intervals) in a reaction mixture containing potassium phosphate buffer and H<sub>2</sub>O<sub>2</sub>. The extinction coefficient of 39.4 M<sup>-1</sup> cm<sup>-1</sup> was used. CAT activity was expressed as μmol of H<sub>2</sub>O<sub>2</sub> reduced min<sup>-1</sup> g<sup>-1</sup> tissue.

SOD activity was determined by the method of Wu et al. [33] with adaptations and was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT). Fresh frozen leaf and petal segments (0.5 g) were ground in liquid N<sub>2</sub>, homogenized with 1 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM EDTA and 1% polyvinylpyrrolidone (PVP), and centrifuged at 14,000g for 20 min at 4 °C. A reaction mixture of sodium phosphate buffer, methionine, NBT, EDTA, and riboflavin was used. The mixture was placed for 20 min at 25 °C under a fluorescent light (30 Watt). Absorbance at 560 nm was monitored using a spectrophotometer (Mapada UV-1800; Shanghai Mapada Instruments Co., Ltd., Shanghai, China). A SOD enzyme activity unit was considered as 50% of the NBT photoreduction and expressed as unit min<sup>-1</sup> g<sup>-1</sup> tissue.

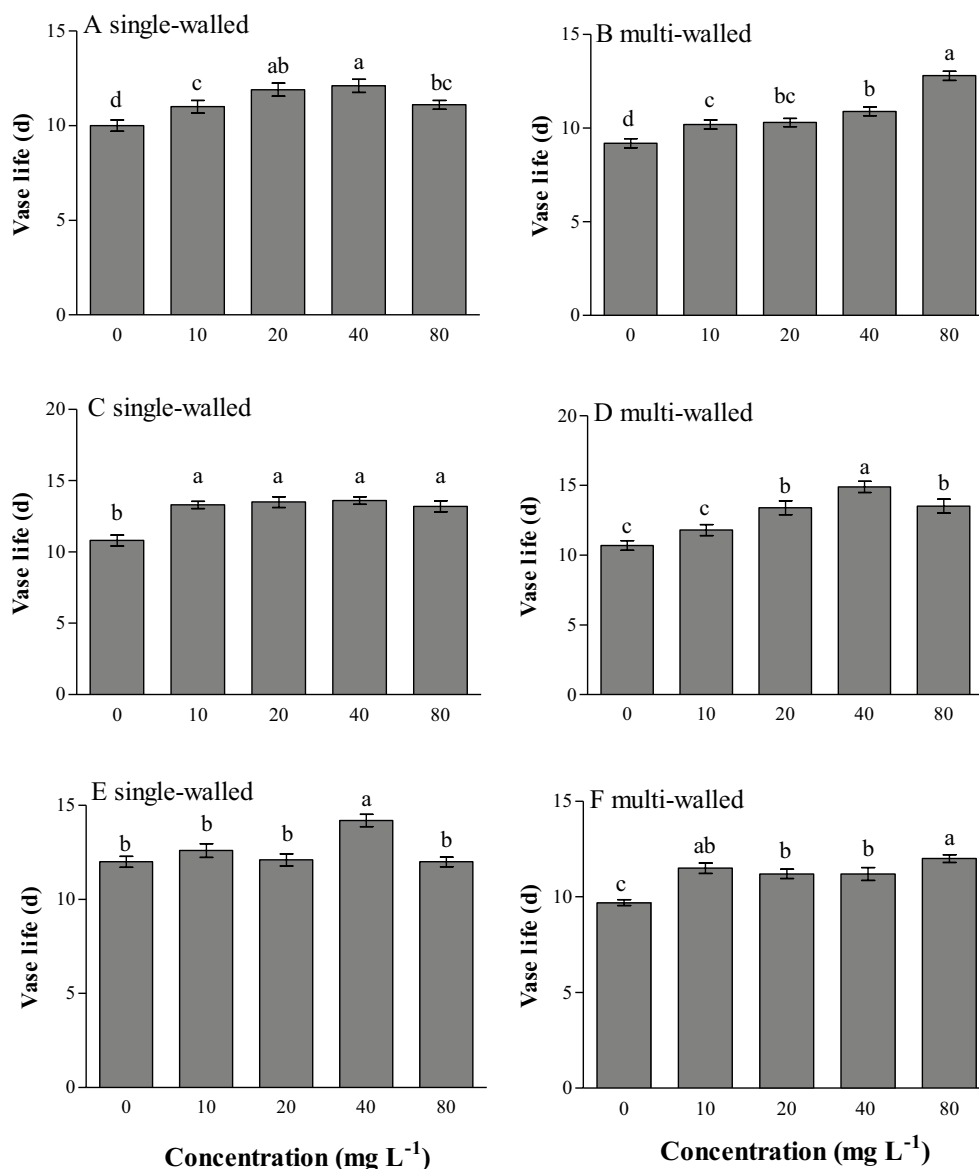
### Statistical analyses

Data were subjected to analysis of variance by using SPSS 23 (SPSS Inc., Chicago, IL, USA). Data were firstly tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). Subsequently, estimated least significant differences (LSD) of treatment effects were determined ( $P=0.05$ ).

## Results

### Effect of CNTs applied as a foliar spray or in the holding solution on vase life, flower opening and water relations (Experiment 1)

CNTs in the holding solution generally extended vase life (Fig. 1). The maximum effect on vase life depended on both the CNTs' type and the cultivar. For SWCNTs, the optimal concentration was 10 mg L<sup>-1</sup> for cultivar White liberty and 40 mg L<sup>-1</sup> for cultivars Grand slam and Kirsi



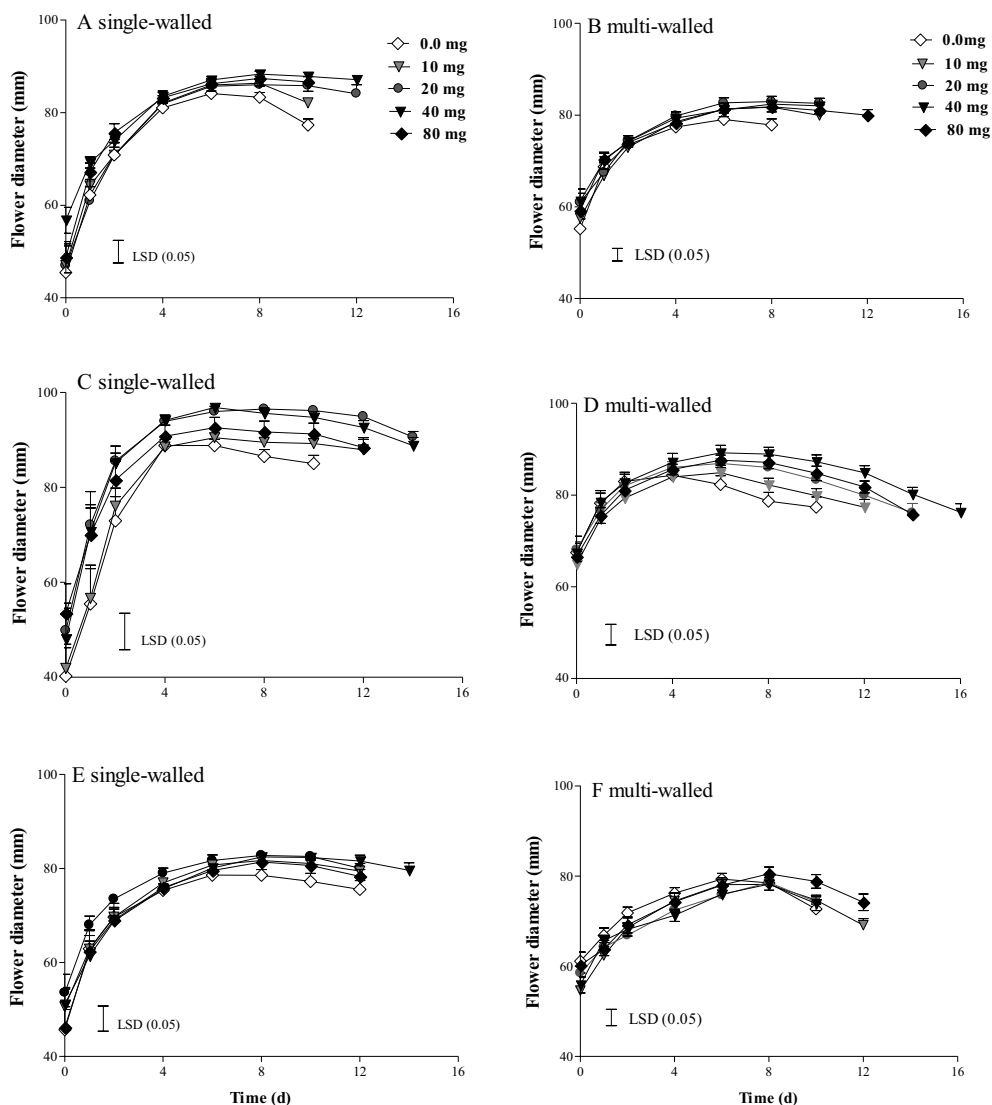
**Fig. 1** Vase life as a function of different concentrations of single- or multi-walled carbon nanotubes (left and right panels, respectively) in the holding solution of three cut carnation cultivars (**A, B**: Grand Slam; **C, D**: White liberty; **E, F**: Kirsi; Experiment 1). Values are the mean of 10 cut flowers  $\pm$  sem. Within each insert, different letters indicate significant differences. Differences in the y-axis scale among panels ought to be noted

(Fig. 1A, C, E). For MWCNTs, the optimal concentration was 40 mg L<sup>-1</sup> for cultivar White liberty and 80 mg L<sup>-1</sup> for cultivars Grand slam and Kirsi (Fig. 1B, D, F).

CNTs in the holding solution generally promoted flower diameter, and extended the period of maximum flower opening (Fig. 2). The greatest effect on flower diameter and the period of maximum flower opening varied among CNTs' types and cultivars. For SWCNTs, the optimal concentration was 20 mg L<sup>-1</sup> for cultivar White liberty and 40 mg L<sup>-1</sup> for cultivars Grand slam and Kirsi (Fig. 2A, C, E). For MWCNTs, the optimal concentration

was 20, 40 and 80 mg L<sup>-1</sup> for cultivars Grand slam, White liberty and Kirsi, respectively (Fig. 2B, D, F).

In all cases, CNTs in the holding solution promoted both cut flower water uptake (Additional file 1: Fig. S1) and loss (Additional file 1: Fig. S2). This effect was different between CNTs' types and cultivars. Cut flower fresh weight changes depending on the difference between water loss and uptake. CNTs in the holding solution generally promoted the cut flower fresh weight increase, and extended the period of increased fresh weight (Fig. 3). The largest effect depended on



**Fig. 2** Flower diameter during vase life as a function of different concentrations of single- or multi-walled carbon nanotubes (left and right panels, respectively) in the holding solution of three cut carnation cultivars (**A, B**: Grand Slam; **C, D**: White liberty; **E, F**: Kirsi; Experiment 1). Values are the mean of 10 flowers  $\pm$  sem. Statistics are provided in Additional file 2: Tables S1–S6

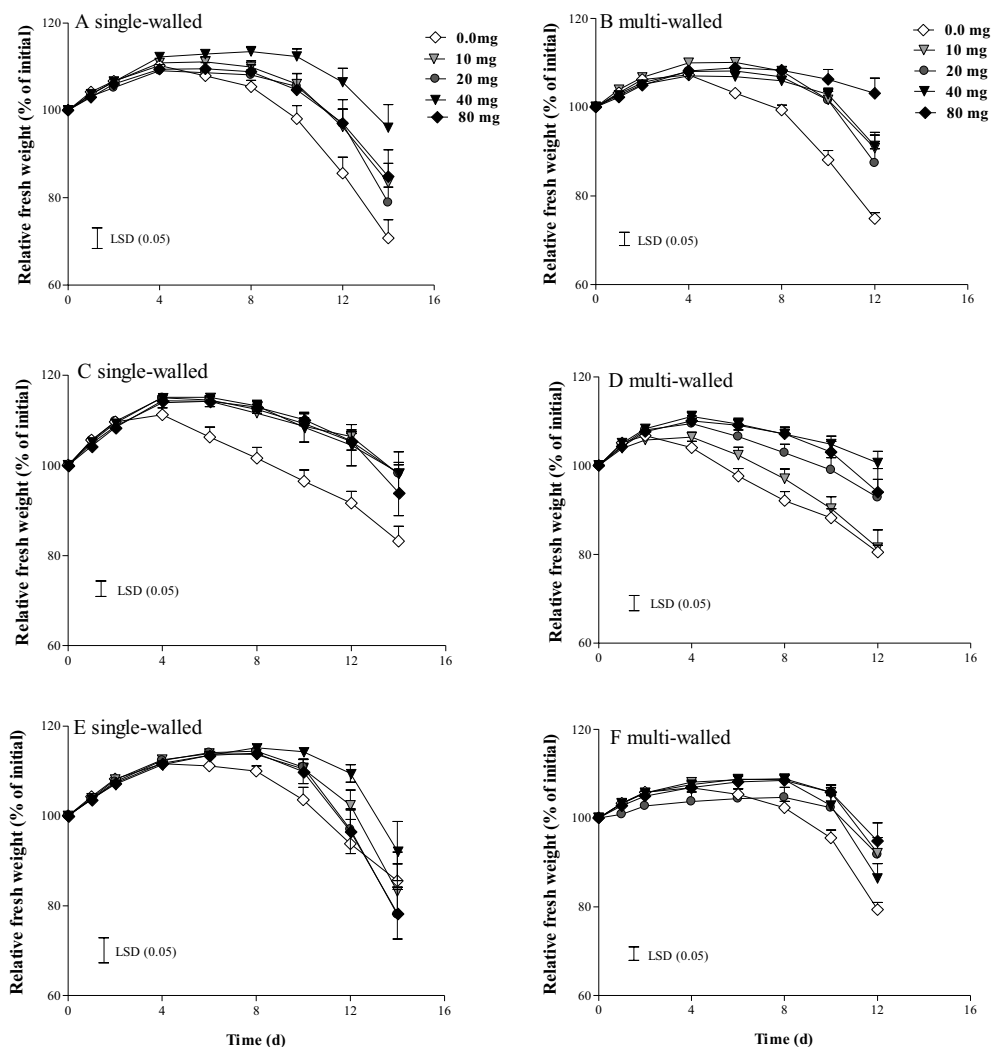
both the CNTs' type and the cultivar. For both SWCNTs and MWCNTs, the optimum concentration for cut flower fresh weight was the same as the one for vase life (Figs. 1, 3). An exception to this trend was cultivar White liberty treated with SWCNTs in the holding solution, where the promotive effect was similar among different concentrations (Fig. 3C).

CNTs applied once (before evaluation) as a foliar spray generally exerted minor effects on vase life (Additional file 1: Fig. S3), flower opening, and cut flower fresh weight dynamics (data not shown).

#### Effect of CNTs in the holding solution on parameters affecting cut flower longevity (Experiment 2)

Based on the results of Experiment 1, the optimal concentration of SWCNTs and MWCNTs in the holding solution for vase life (Fig. 1) was selected for Experiment 2. Several parameters critical to vase life were assessed at 4-day intervals for a period of 12 days.

RWC was assessed in situ, as an indication of hydration status. Leaves were more hydrated than petals (Fig. 4). In both organs, CNTs in the holding solution generally improved RWC throughout the evaluation period. An exception to this trend was cultivar White liberty, where the positive effect of SWCNTs and MWCNTs on RWC



**Fig. 3** Fresh weight (relative to initial one) during vase life as a function of different concentrations of single- or multi-walled carbon nanotubes (left and right panels, respectively) in the holding solution of three cut carnation cultivars (**A, B**: Grand Slam; **C, D**: White liberty; **E, F**: Kirsi; Experiment 1). Cut flowers were well-hydrated at the onset of the experiment, while starting intact cut flower weight was similar among cultivars. Values are the mean of 10 cut flowers  $\pm$  sem. Statistics are provided in Additional file 2: Tables S7–S12

was minor (Fig. 4C). The promotive effect of CNTs was more prominent in petals as compared to leaves.

Leaf discoloration, owing to chlorophyll content decrease, is a common vase life terminating symptom. CNTs in the holding solution generally stimulated leaf chlorophyll content throughout the evaluation period (Fig. 5A, C, E). This pattern was not apparent in cultivar Kirsi, where SWCNTs negatively affected leaf chlorophyll content at 4 and 8 days (Fig. 5E).

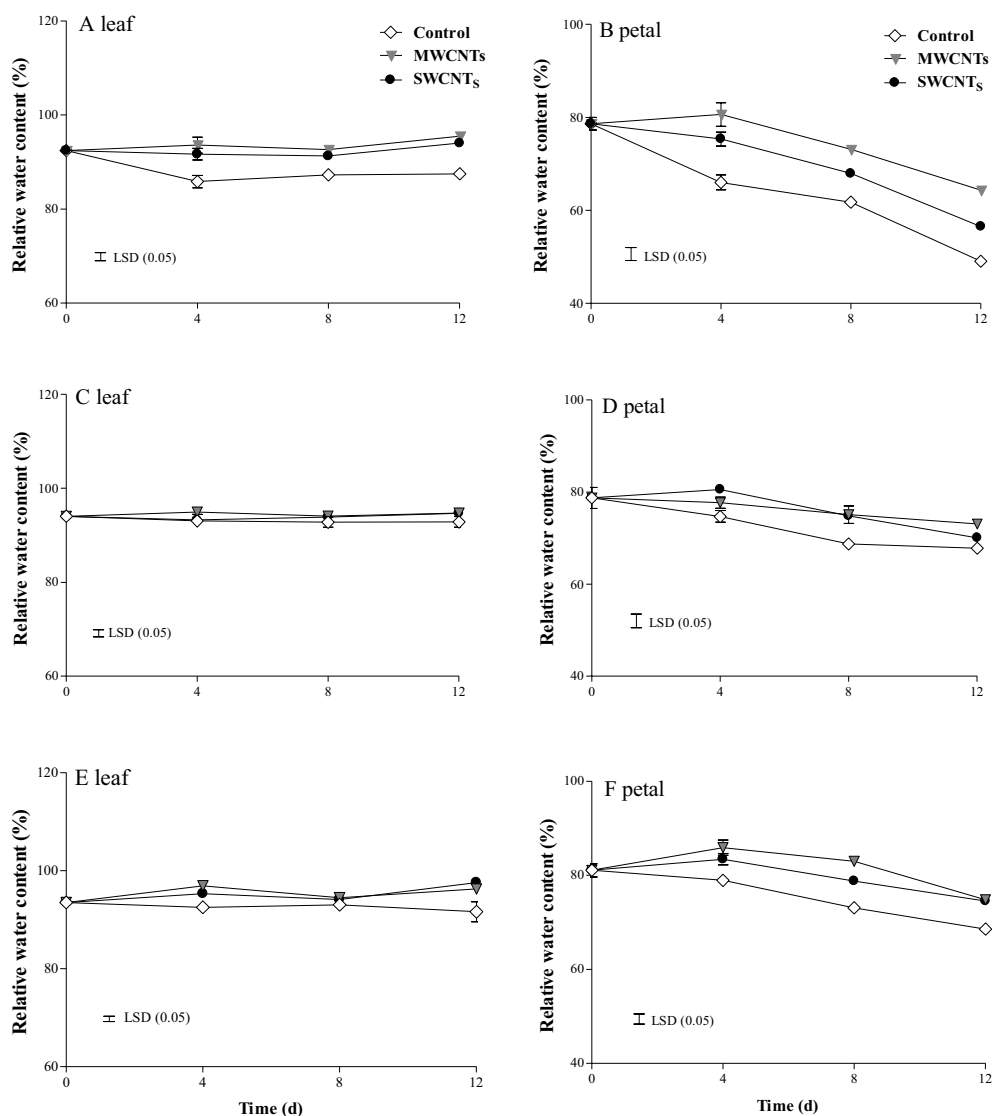
Carotenoids, polyphenolics, and flavonoids represent important non-enzymatic antioxidants. These compounds were generally enhanced when CNTs were included in the holding solution (Figs. 5B, D, F, 6).

Exceptions were noted for carotenoid content in cultivar Kirsi when using SWCNTs (4 and 8 days; Fig. 5F), and for total phenolic content in cultivar Grand Slam (8 days; Fig. 6A).

Similarly, lower IC 50 value (indicative of greater overall antioxidant effectiveness) was noted in leaves of cut flowers placed in a holding solution containing CNTs (Fig. 6C, E, K). This trend was not observed in cultivar Grand Slam at 8 days (Fig. 6C).

The relative ion content in the apoplasmic space, taken as an indication of membrane stability, was evaluated by measuring electrolyte leakage. CNTs in the holding





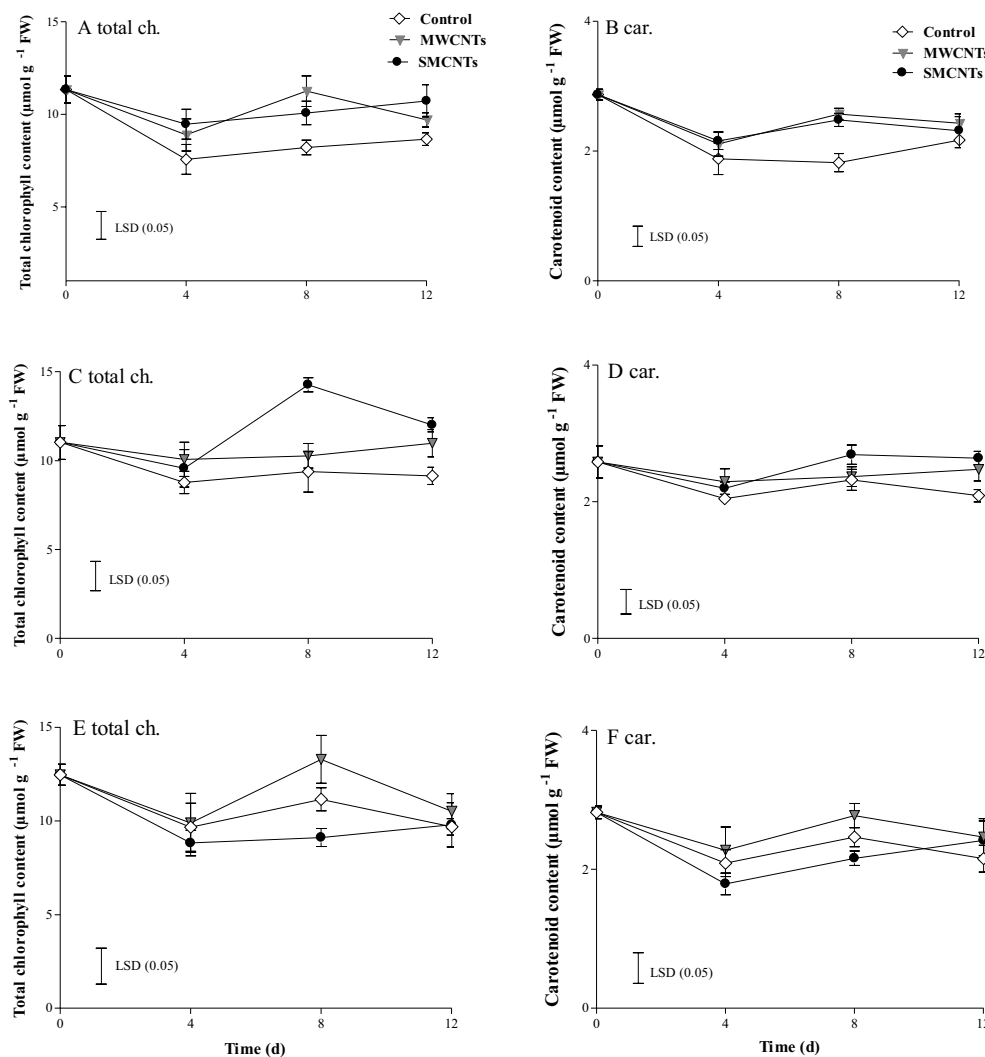
**Fig. 4** Leaf and petal relative water content during vase life of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi)  $\text{mg L}^{-1}$  for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi)  $\text{mg L}^{-1}$  for MWCNTs. Cut flowers were well-hydrated at the onset of the experiment. Values are the mean of 4 replicates  $\pm$  sem. Statistics are provided in Additional file 2: Tables S13–S18

solution generally led to lower electrolyte leakage in both leaves and petals (Fig. 7).

MDA content, indicative of lipid peroxidation, was also quantified. A higher MDA content was generally noted in controls, as compared to cut flowers placed in a holding solution containing CNTs (Fig. 8). An exception was noted in cultivar Kirsi, where the MDA content of SWCNT-treated cut flowers exceeded that of

controls at 8 (leaves; Fig. 8E) and 12 (leaves and petals; Fig. 8F) days.

APX, POD, CAT, and SOD are main ROS detoxification enzymes. The activity of these enzymes was generally enhanced in both leaves and petals when the holding solution contained CNTs, as compared to controls (Figs. 9, 10, 11, 12).



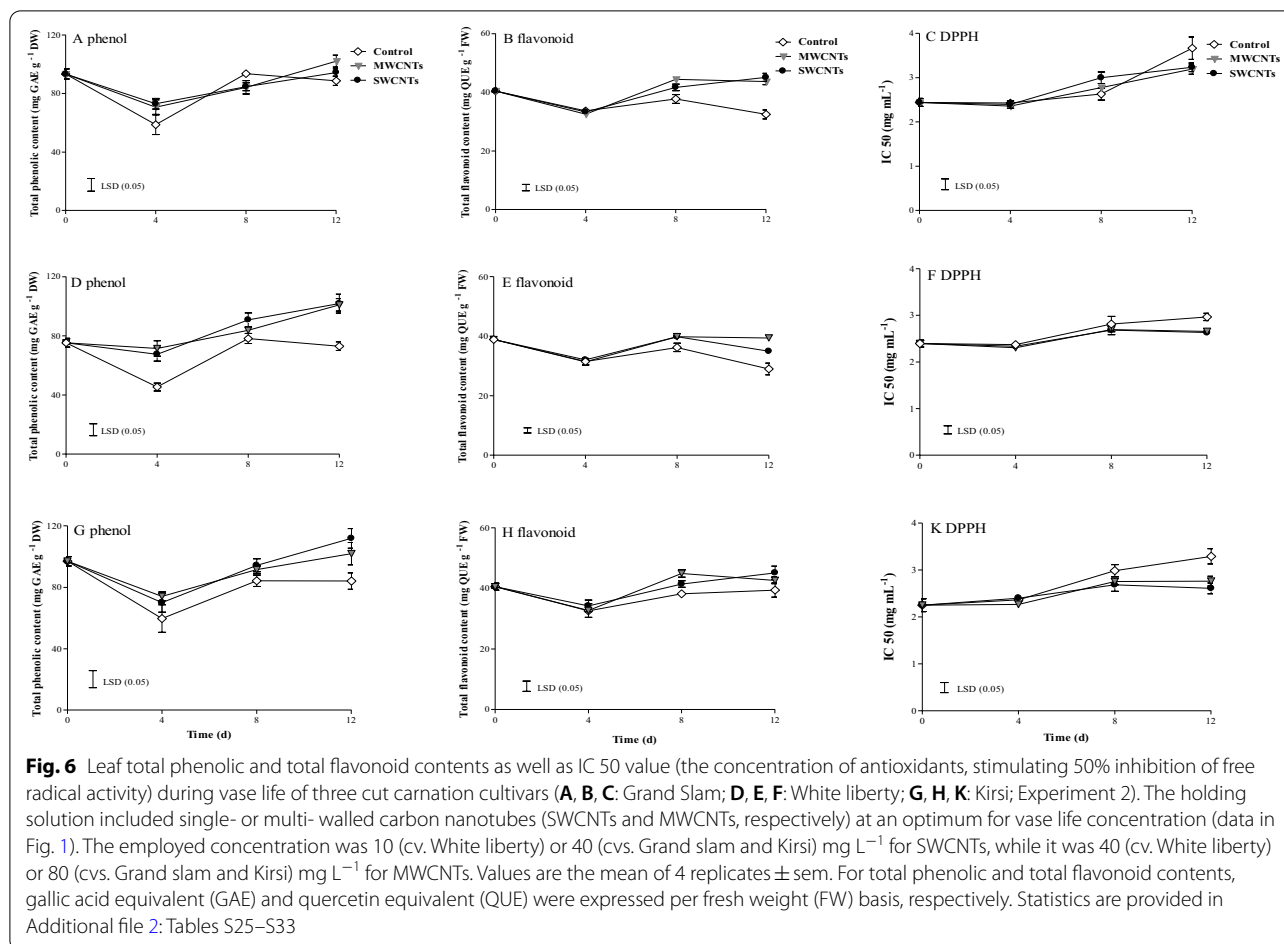
**Fig. 5** Total chlorophyll and carotenoid contents during vase life of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Contents were expressed per fresh weight (FW) basis. Statistics are provided in Additional file 2: Tables S19–S24

## Discussion

Several studies have shown that CNTs are beneficial when applied during cultivation (i.e., pre-harvest period). For instance, they can improve vegetative growth and yield, as well as drought tolerance [12, 13, 21]. In this paper, we focus on the possibility of using CNTs during the postharvest period in order to maintain the quality and extend the vase life of cut flowers.

Application of CNTs not only through the root, but also via foliar spray has been shown to be feasible in developing plants [21, 34, 35]. Foliar spray-applied CNTs effectively penetrate into the leaves and readily

translocate to systemic sites [36]. Successful foliar uptake, however, depends on additional factors, including species and environmental conditions [36, 37]. In this study, CNTs triggered beneficial effects when applied via the holding solution (Fig. 1), whereas minor effects were noted when applied via foliar spray (Additional file 1: Fig. S3). The small size of the leaves may have impeded the foliar intake of CNTs. However, a conclusive picture of the factors limiting the foliar intake of CNTs cannot be elucidated by the results of this study and deserve further investigation.

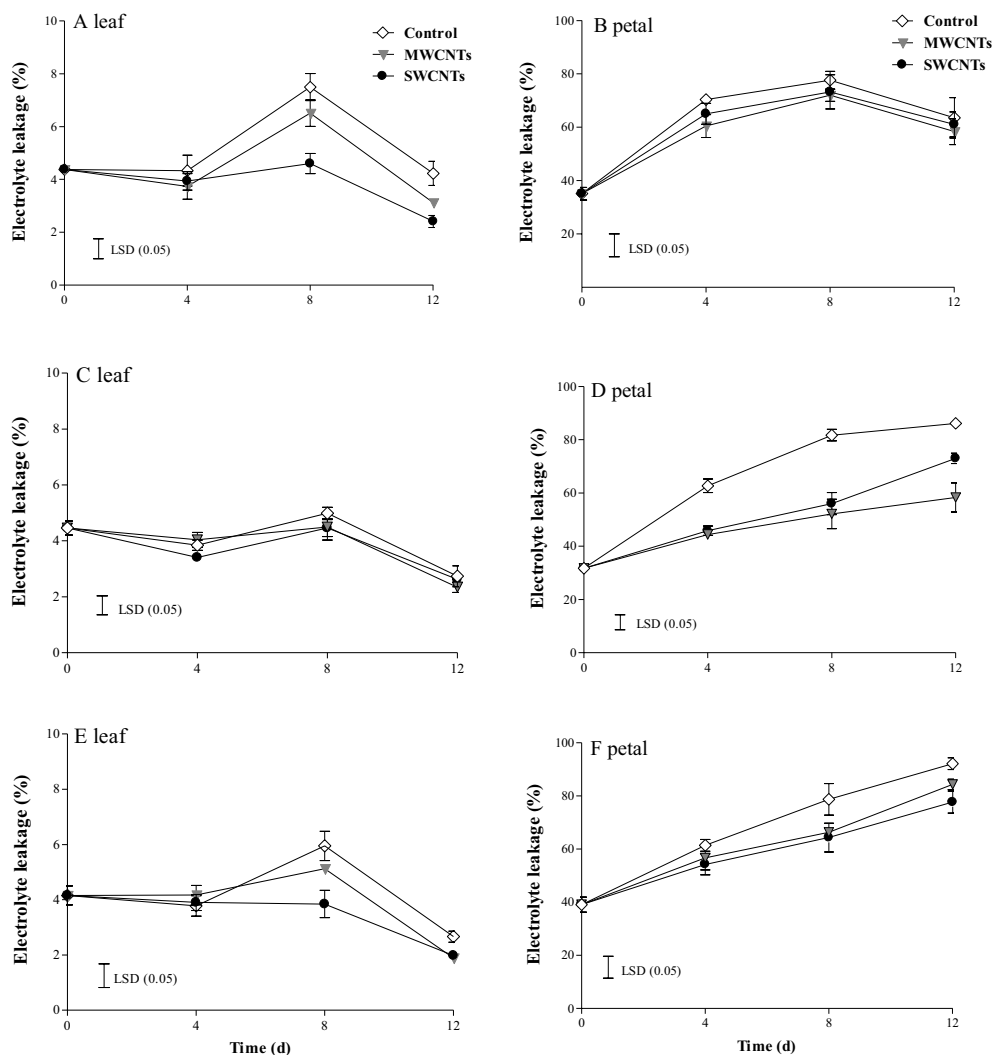


Including CNTs in the holding solution promoted cut carnation vase life (Fig. 1). Although this promotive effect was similar between the two CNTs' types, the optimal concentration was higher in MWCNTs as compared to SWCNTs (10–40 and 40–80 mg L<sup>-1</sup>, respectively). A direct influence of the CNTs' structure on their properties has been suggested [13]. For instance, SWCNTs have smaller diameter and shorter length as compared to MWCNTs. The particle size, in turn, has been negatively related to the translocation capacity [36, 37]. Our findings indicate that for postharvest applications the required concentration is negatively associated with the CNTs' particle size.

In addition, the optimal concentration of either SWCNTs or MWCNTs was consistently lower for cultivar White liberty as compared to cultivars Grand slam and Kirsi. Genetic variation in the CNT-induced effects on plant growth has been recently reported [38]. Therefore, the required concentration can be adjusted depending on the cultivar. In cultivars requiring smaller concentrations, the economic gain will be significant in combination with reduced environmental impact.

Water balance has been shown to be very critical for vase life [8, 22]. Cut flower weight decreases (i.e., water balance becomes negative), when transpiration exceeds water uptake. CNTs stimulated water uptake (Additional file 1: Fig. S1) more than transpiration (Additional file 1: Fig. S2), since cut flower fresh weight increased (Fig. 3). In intact plants, the CNT-induced simultaneous increase in both transpiration and water uptake is an indication of enhanced symplastic water movement, through an amplified abundance or enhanced functionality of aquaporins [39]. Aquaporins are the primary water transport channels across membranes, and are abundant in tonoplast (vacuolar) and plasma (cell) membranes [40]. The promotive effect of CNTs in the holding solution on turgidity was also evident when examining leaf and petal RWC (Fig. 4). Therefore, the improved cut flower water relations most likely underlie the positive effect of CNTs on vase life.

Upon placement in the vase, cut flower weight initially increased (Fig. 3) owing to flowering opening (Fig. 2). CNTs in the holding solution stimulated flower opening and extended the period of maximum flower diameter

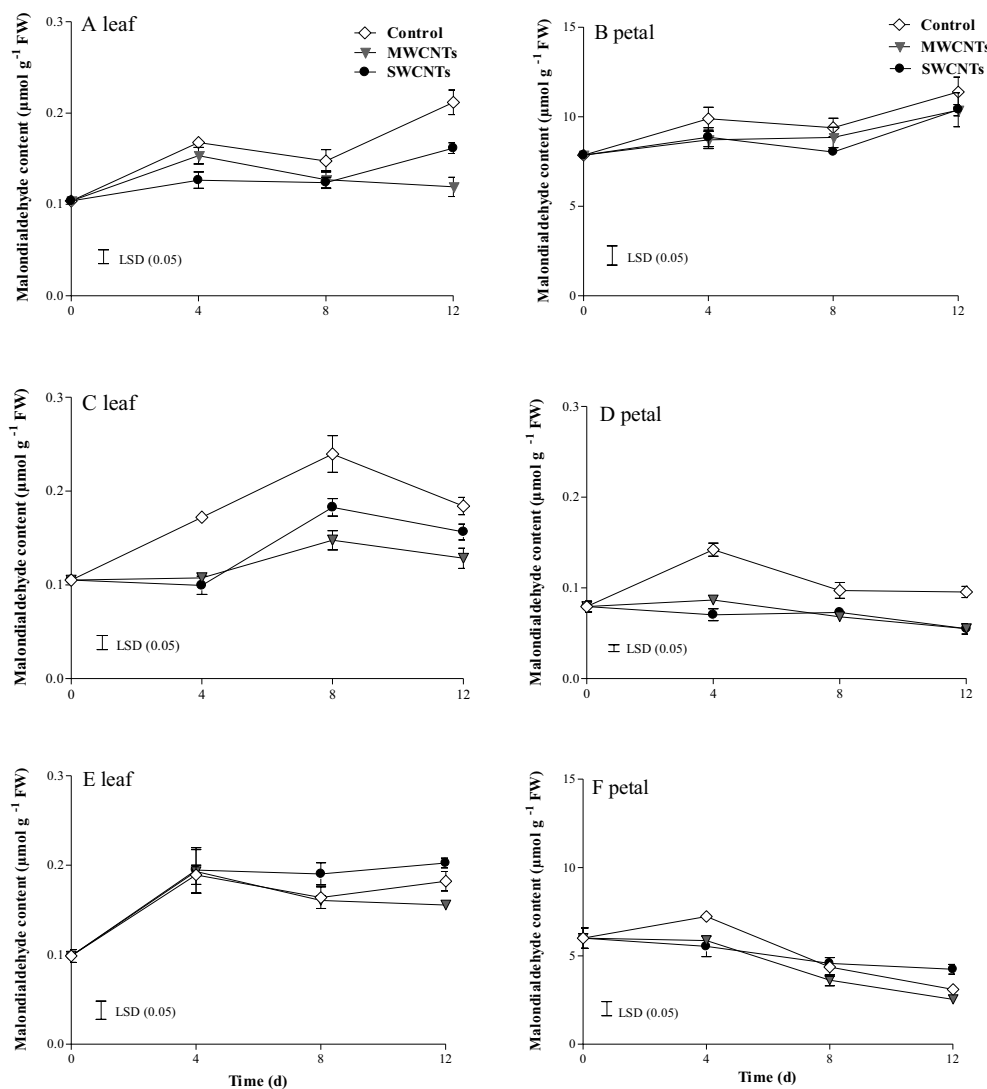


**Fig. 7** Leaf and petal electrolyte leakage during vase life of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Statistics are provided in Additional file 2: Tables S34–S39

(Fig. 2), which were associated with a more pronounced fresh weight increase, persisting for longer (Fig. 3). Therefore, flowers placed in deionized water failed to open properly during vase life, and this was partly counteracted by employing CNTs. Flower bud opening has been related to both petal water and carbohydrate status [41]. Petal RWC was indeed improved by CNTs in the holding solution (Fig. 4B, D, F). By contrast, no effect on carbohydrate status is to be expected, given that postharvest light conditions were well below the light compensation point, as commonly applied both in retail facilities and consumer's home [1, 2, 7]. Under this background, the positive

effect of CNTs on flower bud opening is possibly related to enhanced flower bud hydration (Fig. 4B, D, F).

The development of senescence symptoms was assessed by employing the assays for the estimation of chlorophyll (leaves; Fig. 5) and membrane (leaves and petals; Fig. 7) degradation. These methods indicated that senescence symptoms were attenuated as well as in many cases delayed when CNTs were included in the holding solution. Both electrolyte leakage and chlorophyll loss have been related to cell membrane disruption [16]. This might be taken to indicate that improved membrane



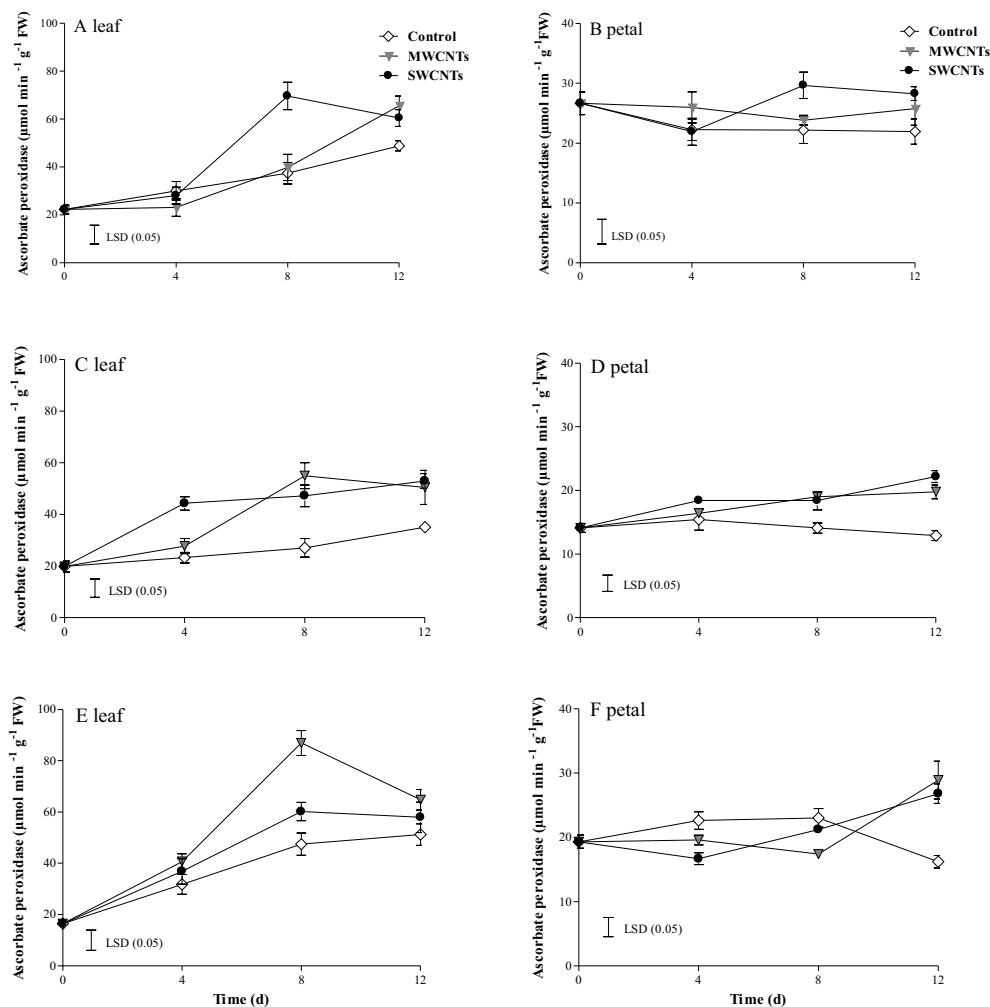
**Fig. 8** Leaf and petal malondialdehyde content during vase life of three cut carnation cultivars (**A, B**: Grand Slam; **C, D**: White liberty; **E, F**: Kirsi; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Content was expressed per fresh weight (FW) basis. Statistics are provided in Additional file 2: Tables S40–S45

stability also contributed to the increased vase life of CNT-treated cut flowers.

MDA is a by-product of the membrane lipids' oxidation, and accumulates when plants are exposed to oxidative stress [19]. Therefore, the increase in lipid peroxidation level was alleviated by the presence of CNTs (Fig. 8). Thus, reduced levels of membrane lipid peroxidation, owing to alleviation of oxidative stress, is another process contributing to the increased vase life of CNT-treated cut flowers.

Antioxidants accumulate to detoxify ROS [17, 21]. Enhanced antioxidants' levels have been related to

increased vase life [1, 14, 15]. Including CNTs in the holding solution stimulated both enzymatic and non-enzymatic ROS defense mechanisms, enhancing antioxidant effectiveness (Fig. 6C, F, K). The former mechanism was evaluated by measuring the activity of four main detoxification enzymes (APX, CAT, POD and SOD; Figs. 9, 10, 11, 12). The latter was examined by assessing carotenoids' (Fig. 5B, D, F), polyphenols' (Fig. 6A, D, G), and flavonoids' (Fig. 6B, E, H) contents. Activation of the antioxidant defense has also been shown upon application of CNTs in growing plants [12, 21]. Therefore, the reduced oxidative damage (as indicated by chlorophyll



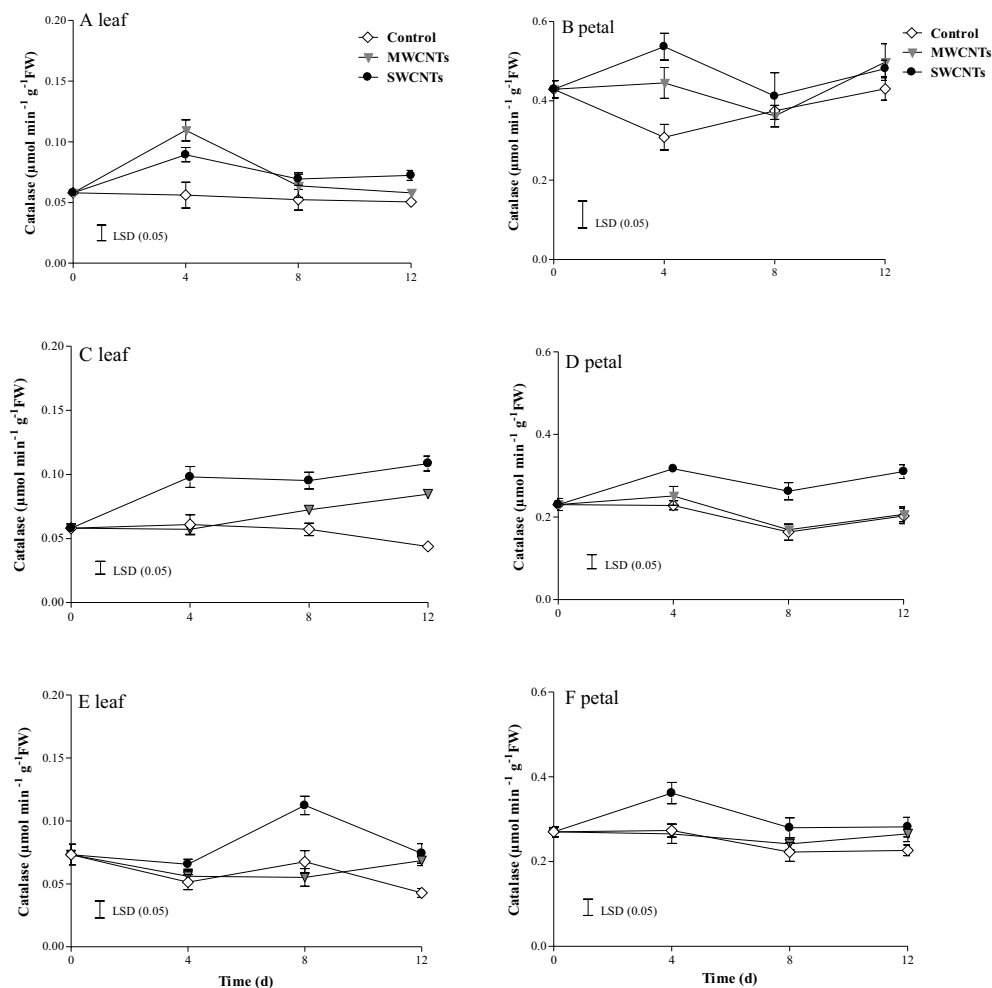
**Fig. 9** Leaf and petal ascorbate peroxidase activity during vase life of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi)  $\text{mg L}^{-1}$  for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi)  $\text{mg L}^{-1}$  for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Enzyme activity was expressed per fresh weight (FW) basis. Statistics are provided in Additional file 2: Tables S46–S51

and membrane degradation, as well as MDA content; Figs. 5, 7, and 8) in cut flowers treated with CNTs was associated with the stimulation of the antioxidant defense mechanism.

Ethylene is a critical factor in determining the vase life of climacteric cut flowers, including carnation [1, 4]. A lower ethylene production may have contributed to the vase life enhancement owing to CNTs' application via the holding solution, though not assessed in the current study. The effect of CNTs' application on ethylene production of climacteric cut flowers has not been currently addressed, and deserves further investigation.

Nanomaterials (including CNTs) are currently explored as a viable means of improving plant growth

and productivity [13], and this work presents promising results for employment in the postharvest sector too. In edible crops, adverse effects of nanomaterials on human health and environment have been suggested [13]. In this regard, no laxity in application ought to be tolerated, and disposal issues ought to be deliberated before commercial use. However, it deserves to be noted that environmental and public health concerns have also been raised for many chemicals currently used in floral preservative solutions, especially in ethylene-sensitive species as the one under study [42].

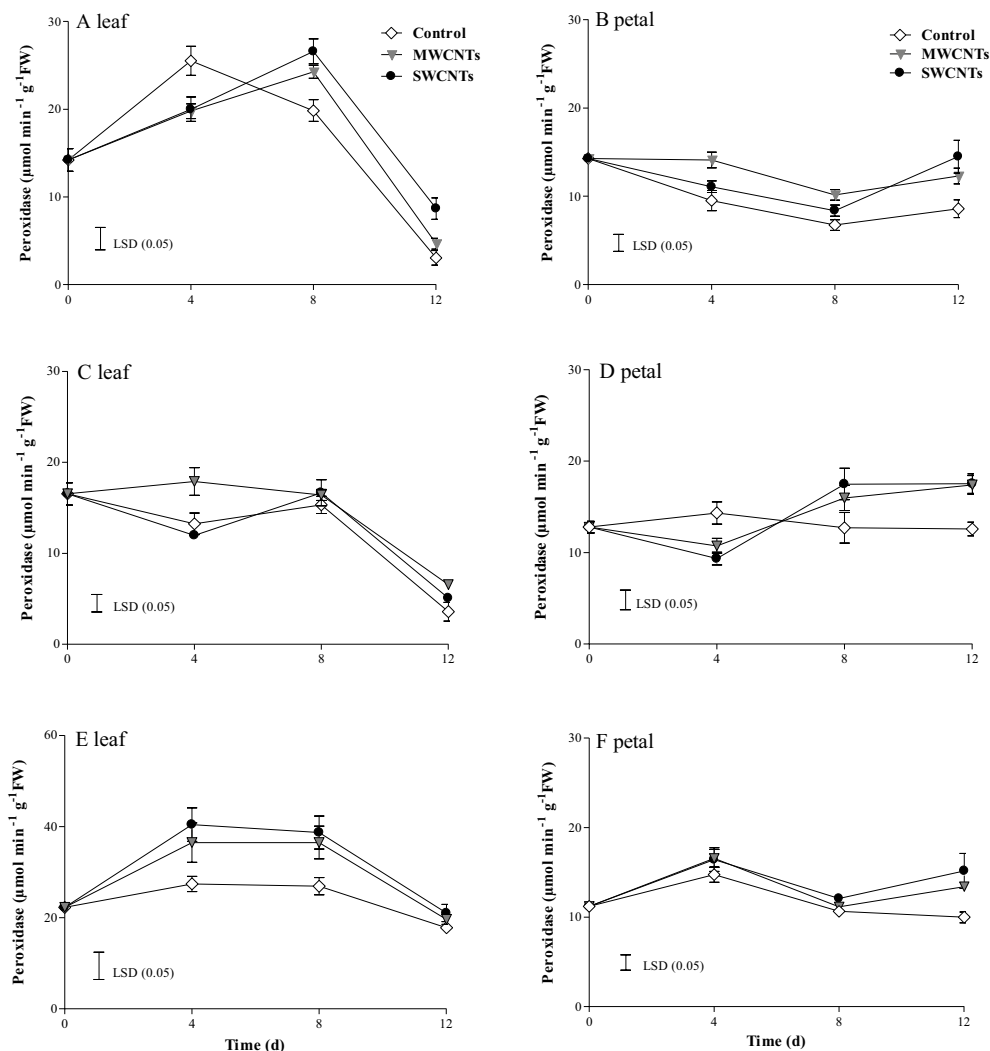


**Fig. 10** Leaf and petal catalase activity during vase life of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Enzyme activity was expressed per fresh weight (FW) basis. Statistics are provided in Additional file 2: Tables S52–S57

## Conclusions

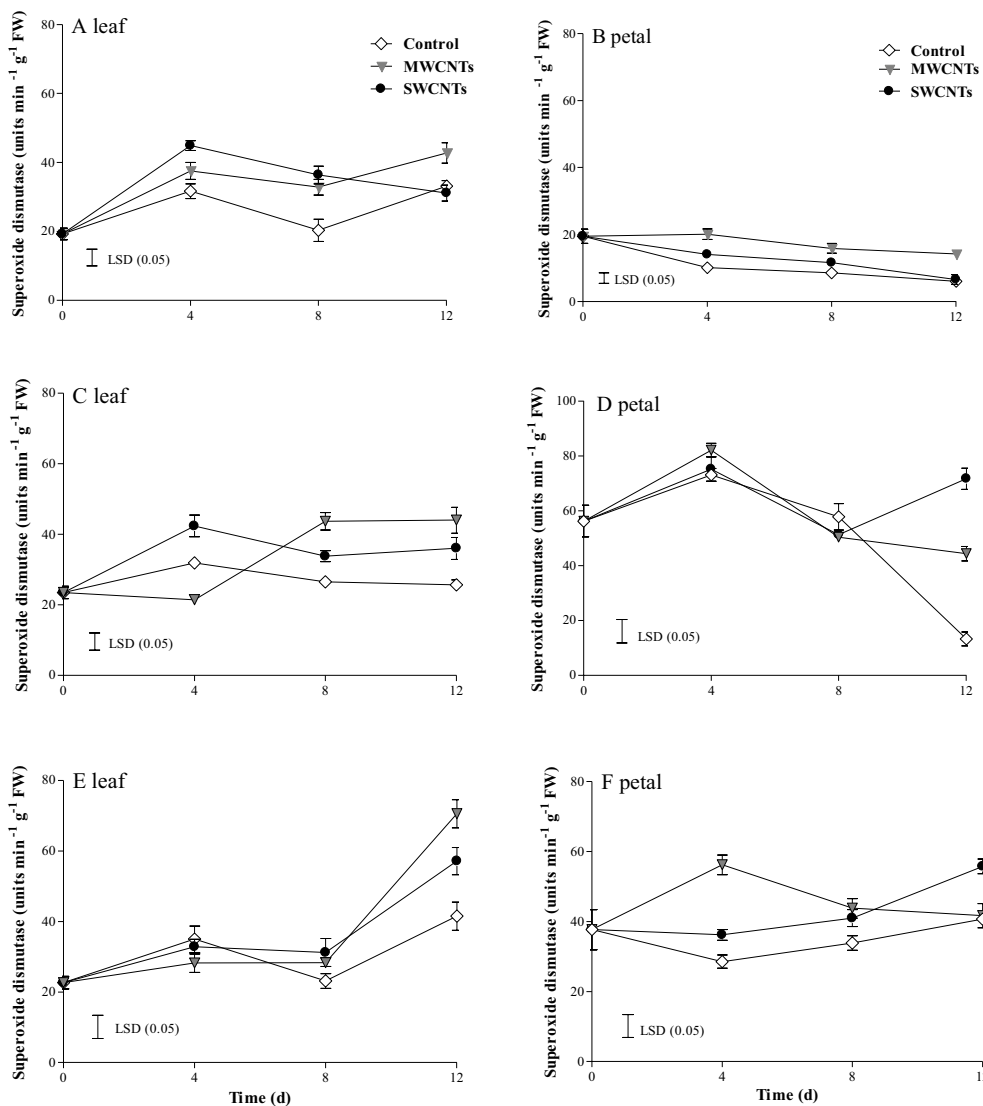
The possibility of using single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) during the postharvest period to enhance cut carnation vase life was deciphered. No effect on vase life was elicited by foliar spray of CNTs. The small size of the leaves may have impeded CNTs' foliar intake. When applied in the holding solution, SWCNTs or MWCNTs were effective in promoting turgidity, flower opening and keeping quality. The CNT-induced effect was clearly concentration-dependent, while the required concentration to induce the maximal response was dependent

on both the CNTs' type and the cultivar. By employing the optimal CNTs' concentration in the holding solution, several leaf and petal traits were improved. CNTs alleviated chlorophyll degradation, electrolyte leakage and lipid peroxidation. These effects were associated with enhancement in enzymatic (APX, CAT, POD and SOD) and non-enzymatic (carotenoids, polyphenols, and flavonoids) antioxidants. Overall, our results indicate that CNTs in the holding solution enhanced vase life by both improving water relations and stimulating antioxidant defense.



**Fig. 11** Leaf and petal peroxidase activity during vase life of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 2). The holding solution included single- or multi- walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi)  $\text{mg L}^{-1}$  for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi)  $\text{mg L}^{-1}$  for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Enzyme activity was expressed per fresh weight (FW) basis. Statistics are provided in Additional file 2: Tables S58–S63





**Fig. 12** Leaf and petal superoxide dismutase activity during vase life of three cut carnation cultivars (**A, B**: Grand Slam; **C, D**: White liberty; **E, F**: Kirsi; Experiment 2). The holding solution included single- or multi- walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). The employed concentration was 10 (cv. White liberty) or 40 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for SWCNTs, while it was 40 (cv. White liberty) or 80 (cvs. Grand slam and Kirsi) mg L<sup>-1</sup> for MWCNTs. Values are the mean of 4 replicates  $\pm$  sem. Enzyme activity was expressed per fresh weight (FW) basis. Statistics are provided in Additional file 2: Tables S64–S69

## Abbreviations

APX: Ascorbate peroxidase; CAT: Catalase; CNTs: Carbon nanotubes; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; GAE: Gallic acid equivalent; IC 50: The concentration of antioxidants, stimulating 50% inhibition of free radical activity; MDA: Malondialdehyde; MWCNTs: Multi-walled CNTs; NBT: Nitro-blue tetrazolium chloride; POD: Peroxidase; QUE: Quercetin equivalent; ROS: Reactive oxygen species; RWC: Relative water content; SOD: Superoxide dismutase; SWCNTs: Single-walled CNTs.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-021-00264-1>.

**Additional file 1 : Figure S1.** Water uptake rate during vase life as a function of different concentrations of single- or multi-walled carbon nanotubes (left and right panels, respectively) in the holding solution of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 1). The water uptake was expressed per unit of time and cut flower fresh weight (FW). Cut flowers were well-hydrated at the onset of the experiment, while starting intact cut flower weight was similar among cultivars. Values are the mean of 10 cut flowers  $\pm$  sem. Statistics are provided in Supplementary Tables S70-75. **Figure S2.** Water loss rate during vase life as a function of different concentrations of single- or multi-walled carbon nanotubes (left and right panels, respectively) in the holding solution of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 1). The water loss was expressed per unit of time and cut flower fresh weight (FW). Cut flowers were well-hydrated at the onset of the experiment, while starting intact cut flower weight was similar among cultivars. Values are the mean of 10 cut flowers  $\pm$  sem. Statistics are provided in Supplementary Tables S76-81. **Figure S3.** Vase life as a function of different concentrations of single- or multi-walled carbon nanotubes applied by spray on leaves of three cut carnation cultivars (A, B: Grand Slam; C, D: White liberty; E, F: Kirsi; Experiment 1). Values are the mean of 10 cut flowers  $\pm$  sem. Within each insert, different letters indicate significant differences.

**Additional file 2: Table S1.** Flower diameter during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Fig. 2A; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S2.** Flower diameter during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Fig. 2C; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S3.** Flower diameter during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsi (data in Fig. 2E; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S4.** Flower diameter during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Fig. 2B; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S5.** Flower diameter during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Fig. 2D; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S6.** Flower diameter during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsi (data in Fig. 2F; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S7.** Fresh weight (relative to initial one) during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Fig. 3A; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S8.** Fresh weight (relative to initial one) during vase life as a function of different concentrations of single-walled carbon nanotubes in the

holding solution of cut carnation cultivar White Liberty (data in Fig. 3C; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S9.** Fresh weight (relative to initial one) during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsi (data in Fig. 3E; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S10.** Fresh weight (relative to initial one) during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Fig. 3B; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S11.** Fresh weight (relative to initial one) during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Fig. 3D; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S12.** Fresh weight (relative to initial one) during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsi (data in Fig. 3F; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S13.** Leaf relative water content during vase life of cut carnation cultivar Grand Slam (data in Fig. 4A; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S14.** Leaf relative water content during vase life of cut carnation cultivar White Liberty (data in Fig. 4C; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S15.** Leaf relative water content during vase life of cut carnation cultivar Kirsi (data in Fig. 4E; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S16.** Petal relative water content during vase life of cut carnation cultivar Grand Slam (data in Fig. 4B; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S17.** Petal relative water content during vase life of cut carnation cultivar White Liberty (data in Fig. 4D; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S18.** Petal relative water content during vase life of cut carnation cultivar Kirsi (data in Fig. 4F; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S19.** Leaf total chlorophyll content during vase life of cut carnation cultivar Grand Slam (data in Fig. 5A; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S20.** Leaf total chlorophyll content during vase life of cut carnation cultivar White Liberty (data in Fig. 5C; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and MWCNTs, respectively) at an optimum for vase life concentration (data in Fig. 1). Values are the mean of 4 replicates. Within each column, different letters indicate significant differences. **Table S21.** Leaf total chlorophyll content during vase life of cut carnation cultivar Kirsi (data in Fig. 5E; Experiment 2). The holding solution included single- or multi-walled carbon nanotubes (SWCNTs and





Water uptake rate during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Supplementary Fig. S1A; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S71.** Water uptake rate during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Supplementary Fig. S1C; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S72.** Water uptake rate during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsí (data in Supplementary Fig. S1E; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences.

**Table S73.** Water uptake rate during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Supplementary Fig. S1B; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S74.** Water uptake rate during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Supplementary Fig. S1D; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S75.** Water uptake rate during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsí (data in Supplementary Fig. S1F; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences.

**Table S76.** Water loss rate during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Supplementary Fig. S2A; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S77.** Water loss rate during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Supplementary Fig. S2C; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S78.** Water loss rate during vase life as a function of different concentrations of single-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsí (data in Supplementary Fig. S2E; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S79.** Water loss rate during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Grand Slam (data in Supplementary Fig. S2B; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences. **Table S80.** Water loss rate during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar White Liberty (data in Supplementary Fig. S2D; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences.

**Table S81.** Water loss rate during vase life as a function of different concentrations of multi-walled carbon nanotubes in the holding solution of cut carnation cultivar Kirsí (data in Supplementary Fig. S2F; Experiment 1). Values are the mean of 10 flowers. Within each column, different letters indicate significant differences.

#### Acknowledgements

The authors would like to thank Lorestan University (Iran) for providing the facilities. We are grateful to the laboratory staff for their contributions, continued diligence, and dedication to their craft. The valuable comments of the editor and two anonymous reviewers are greatly acknowledged.

#### Authors' contributions

MAM performed the experimental work. MAM and SMF carried out the data analysis and interpretation. ARN, SMF, and DF designed and supervised the study. All authors have read and agreed to the final version of the manuscript.

#### Funding

This research was financed by Lorestan University (Iran).

#### Availability of data and materials

Additional data are available on request from the corresponding author.

#### Declarations

#### Ethics approval and consent to participate

Ethical approval and consent to participate were not required for this research, as it did not involve human or non-human animals.

#### Consent for publication

Not applicable.

#### Competing interests

The authors have no conflict of interest to declare.

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Received: 18 July 2021 Accepted: 12 October 2021

Published online: 19 January 2022

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