Cold plasma-ozonized water reduces petal abscission in *Polianthes tuberosa*

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Abstract

The postharvest quality and vase life of cut flowers, including tuberose (*Polianthes tuberosa*), face challenges such as incomplete floret opening and premature abscission, reducing market value. This study investigates the use of ozonized water, produced via Cold Plasma Technology (CPT), as a sustainable postharvest treatment. Tuberose flowers were treated with ozonized water for 4, 6, 8, and 10 minutes, and key parameters such as floret abscission, antioxidant enzyme activities, phenolic compounds, and lipid peroxidation were analyzed. Results showed the 8-minute treatment significantly improved vase life by enhancing antioxidant activities, increasing phenolic compound levels, and reducing lipid peroxidation, thereby delaying senescence and preserving cellular integrity. This eco-friendly method offers an effective alternative to chemical treatments, addressing industry demands for sustainable practices by extending vase life, preserving aesthetic quality, and reducing environmental impact. These findings support broader adoption of CPT-based ozonized water in postharvest management.

Keywords: *Polianthes tuberosa*, floret opening, floret abscission, vase life, oxidative stress, ozone

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Introduction

Ornamental horticulture, particularly the cut flower sector, represents a substantial global industry with a significant and growing economic impact (Gabellini and Scaramuzzi, 2022). Within this sector, tuberose (*Polianthes tuberosa* L.) stands out not only for its popularity in floral arrangements but also for its distinct fragrance, which enhances its value in the perfume industry (Bhattacharya et al., 2022). Originating from Mexico, tuberose has become an economically important crop in tropical and subtropical regions, where large-scale cultivation is viable due to favorable conditions and relatively low labor costs (Pérez-Arias et al., 2019). However, like many other cut flowers, tuberose has a notably short vase life, creating challenges in maintaining quality and freshness during transport and display, significantly affecting its marketability (Shatoori et al., 2021).

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Postharvest quality in tuberose and other cut flowers is influenced by physiological and biochemical factors, including ethylene production, microbial proliferation, and xylem blockages, which collectively accelerate senescence and petal wilting (Maiti and Mitra, 2017; Shatoori et al., 2021). These processes restrict full floret opening and lead to premature abscission, further reducing tuberose's commercial value (Bhattacharya et al., 2022; Datta, 2017). Addressing these issues, particularly through treatments that delay senescence, promote floret opening, and extend vase life is a priority for the floral industry (Kumar et al., 2024).

Traditional postharvest treatments for cut flowers, such as silver thiosulfate and synthetic preservatives have been widely used to counter these challenges by inhibiting ethylene action and microbial growth (Chen et al., 2019). However, these chemical treatments often leave harmful residues, pose health risks to consumers and handlers, and contribute to environmental toxicity (Ali et al., 2020)Additionally, long-term chemical use can lead to resistance in microbial populations, reducing their effectiveness over time (Pańka et al., 2022). Consequently, there is an urgent need for safer, more sustainable alternatives that align with industry and consumer demands for eco-friendly practices.

Cold Plasma Technology (CPT) has recently emerged as an innovative, environmentally benign approach with applications across agriculture, including seed treatment, pathogen control, and postharvest preservation (Siddique et al., 2018; Yan et al., 2022). Specifically, CPT-generated ozonated water offers unique advantages: it extends shelf life, improves quality by reducing microbial load, and delays senescence without leaving chemical residues (Attri et al., 2020). Studies on ozonized water produced with cold plasma technology treatments in horticultural crops demonstrate efficacy in freshness maintenance and shelf-life extension, positioning it as an attractive alternative for the floral industry, where preservation of quality and aesthetics are of paramount importance (Pańka et al., 2022).

The application of ozonated water, with its oxidative properties, directly influences factors like microbial load reduction and the stimulation of antioxidant activity, which are critical in mitigating early senescence and abscission in cut flowers (Siddique et al., 2018). Previous studies have shown that ozone-based treatments enhance antioxidant enzyme activity and maintain cellular integrity across a variety of horticultural products, further supporting its potential for cut flower preservation (Piechowiak et al., 2022; Zhang et al., 2020). Therefore, this study aims to investigate the efficacy of ozonized water produced with cold plasma technology as a novel postharvest treatment for tuberose cut flowers, focusing on vase life extension, petal abscission reduction, and floret opening enhancement. By comparing this eco-friendly approach with conventional chemical methods, this research seeks to provide a sustainable, residue-free alternative that aligns with the floral industry's shift towards environmentally responsible practices, supporting growing consumer demand for sustainability. Furthermore, the use of ozonized water produced with cold plasma technology may offer additional economic advantages by improving quality and reducing losses, thereby enhancing the export potential of tuberose flowers.

Materials and Methods

Cut flowers were obtained from a commercial greenhouse located in Pakdasht, Tehran. Immediately after harvesting, the flowers were placed upright in buckets filled with water and transported to the laboratory. Once in the laboratory, the stem ends were pre-cut underwater to prevent air embolism, and the stems were cut to a uniform length of 30ccm. Only the flowers that had no mechanical damage, infections, and exact sizes were selected for the subsequent treatments. All experiments were conducted in a growth room with a 16/8h day/night light/dark photoperiod and a temperature of 25 ˚C. The relative humidity was 50%, and photon flux density was 200 µmol m^{-2} s^{-1}. Flowers were placed in a solution containing ozonized water (OW) of 4, 6, 8, and 10 min in a laboratory with a temperature of 25 ˚C. To conduct an experiment on the longevity of cut

flowers, six flowers were placed in a 250ml flask filled with 200ml of solution as one replicate. Distilled water was used as a control, and each treatment had three replicates. After four days of vase life, when all the flowers were fresh, the petals of flowers under each treatment were chosen to measure physiological parameters. Three samples from each replicate were used for biochemical analysis, while another three flowers were left to evaluate their vase life. The vase life of cut flowers under each treatment was determined after they lost their ornamental value.

Ozonized water preparation

The reactor was made up of two coaxial cylinder electrodes, each 15 cm long. The inner electrode was made of stainless steel with a diameter of 10mm. The outer electrode was a copper foil installed on the Pyrex tube's outer surface. The electrodes were powered by a pulsed DC high voltage with a frequency of 200 Hz and adjustable pulse height. The Pyrex tube was 2 mm thick and acted as a dielectric, preventing a sharp increase in electric current. This created a dielectric barrier discharge (DBD) in the gap between the electrodes, where oxygen gas flew with a 99% purity. A digital flow controller, regulated the gas flow rate (Breezens GP Series). The ozone concentration in the gas that flew out of the reactor was controlled by the flow rate and pulse height of the applied voltage. To dissolve this gas in water, a diffuser was installed at the bottom of a cylindrical water container, which was then fed by the gas (Fig. I). The samples of ozonized water were prepared by treatment of 1.0 liter of distilled water in the container for six different treatment times of 4, 6, 8, and 10 minutes. To determine the concentration of ozone in water samples, a setup was used that included a spectrometer (AVANTES AvaSpec), a homemade absorption cell, a cuvette with its holder, and a UV light source (BloorAzma Deuterium Light Source). The absorbance spectra obtained were analyzed using the reference spectra of O_3 which (Noori et al., 2021), yielding ozone concentrations of approximately 0.43 ppm for 4 minutes, 0.71 ppm for 6 minutes, 0.96 ppm for 8 minutes, and 1.45 ppm for 10 minutes.

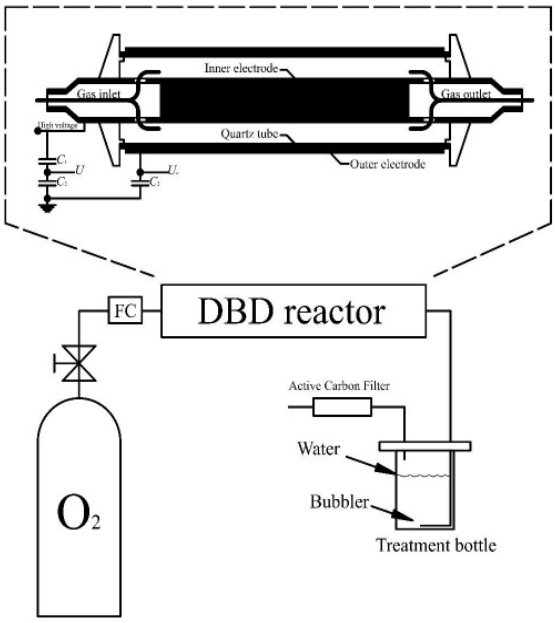

Fig. I. Schematic image of a plasma reactor set up which was used to produce ozone gas

Determination of vase life and quality index

The vase life of each flower was measured as the number of days from the initial placement in the solution until observable senescence compromised their ornamental appearance. The end of vase life was determined by observing specific indicators of senescence, primarily focusing on petal wilting and stem bending. To assess flower quality daily, we monitored several parameters, including the rate of petal wilting, degree of flower opening, neck bending, and overall freshness. A quality index was used to evaluate these characteristics, with values ranging from 1 to 4: 1 indicating excellent, 2 good, 3 average, and 4 poor. A flower was considered to have reached the end of its vase life when it received a quality index score of 4, indicating that the petals had significantly wilted, or the stem had bent to a degree affecting its ornamental appearance. The vase life duration for each treatment is presented in the relevant chart within the Results section.

Measurement of lipid peroxidation

The lipid peroxidation index of petal tissue, known as malondialdehyde (MDA) content, was estimated using the methods described by Heath

and Packer (1968). To calculate the MDA content, the non-specific absorption value at 600 nm was subtracted from the 532 nm reading. The MDA content was then calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as µmol MDA per gram fresh weight.

Enzyme extraction and activity determination

To prepare the petal extract, 300 mg of fresh petals were homogenized with 3 ml of 50 mM potassium phosphate buffer. The mixture was then centrifuged at 10000 × g for 20 minutes, and the supernatant was collected to measure enzyme activity and protein content. The protein content was determined using bovine serum albumin as a standard, according to Bradford's method (Bradford, 1976).

Catalase activity (CAT) (EC 1.11.1.6)

The activity of CAT (EC 1.11.1.6) was measured using the method described by Dhindsa et al. (1981). To perform the measurement, a reaction mixture was prepared that consisted of 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 100 microliters of the enzyme extract. The decrease in absorbance of the mixture was then determined at 240 nm (Ɛ=40 mM^{-1} cm^{-1}). The enzyme activity was expressed in U per milligram protein (one unit= 1 micromole of H_2O_2 reduction per min per mg protein).

Guaiacol peroxidase (GPX) (EC1.11.1.7)

Based on the method described by Plewa et al. (1991), the GPX (EC 1.11.1.7) activity was measured. A reaction mixture consisting of 50 mM potassium phosphate (pH 7.0), 0.3 % (v/v) H_2O_2, 1 % (v/v) guaiacol, and the enzyme extract was used. The enzyme activity was recorded as unit per milligram protein, where one unit (U) of enzyme activity was considered as the amount of enzyme that produced 1 micromole of tetra guaiacol per minute.

Ascorbate peroxidase (APX) (EC 1.11.1.11)

The activity of APX (EC 1.11.1.11) was measured using the method described by Nakano and Asada (1981). A mixture of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, and 0.1 mM H_2O_2 was prepared in a test tube, following which 150 µl of enzyme extract was added to it. The absorbance was measured at 290 nm (Ɛ=2.8 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in unit per milligram of protein.

Polyphenol oxidase (PPO) activity assay (EC 1. 14. 18. 1)

The activity of polyphenol oxidase was determined using the method described by Nicoli et al. (1991). To carry out the reaction, a solution was prepared containing 50 mM potassium phosphate buffer (pH=7.0), 20 mM pyrogallol, and 100 µl enzyme extract. After 3 minutes, the absorbance of the solution was recorded at 420 nm and the activity was determined using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Phenylalanine ammonia-lyase (PAL) activity assay (EC 4.3.1.5)

The activity of PAL was measured using the method described by D'Cunha et al. (1996). To prepare the reaction mixture, 100 mM Tris-HCl buffer with a pH of 8.5, 1 mM 2-mercaptoethanol, 50 mM L-Phenylalanine, and 100 µl of enzyme extract were combined. The mixture was then incubated at 30 °C for 15 minutes. To stop the reaction, 0.5 mL of 6 M HCl was added, and the absorbance of the supernatant was measured at 290 nm. The conversion of one unit of enzyme activity was represented by the reaction of 1 µmol of substrate to cinnamic acid per minute**.**

Determination of polyphenol contents

The polyphenol compounds were quantified using the method described by Gao et al. (2000) and Folin-Ciocalteu reagent. To extract polyphenols, 100 mg of petal tissue was homogenized in 1 ml of 80% ethanol, and the extract was kept at room temperature for 24 hours (preferably in darkness). After that, the samples were centrifuged for 10 minutes at 2000 × g, and the supernatants were used to measure the polyphenols at a wavelength of 765 nm. The amounts of polyphenols were expressed as mg/g DW.

Determination of total soluble sugars

Total soluble sugar was measured using anthrone reagent, with glucose as the standard Fales (1951). To the petal tissue extract, 6 ml of anthrone reagent (containing 150 mg of anthrone in 72% H_2SO_4) was added, and the mixture was then heated in a water bath at 100 ˚C for 10 minutes. After this, the test tubes were cooled in ice for 10 minutes and further incubated for 20 minutes at 25 ˚C. The resulting absorbance was read at 625 nm. To construct the standard curve, different concentrations of glucose were used.

Statistical Analysis

The study employed a complete randomized design, with three replicates for each treatment, and six flowers in each replicate. The data were analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as the mean of three replications. The means were compared using one-way analysis of variance and Duncan's multiple range test, with a 5% significance level.

Results

Characterization of ozonized water

The physicochemical characteristics of ozonized water were determined by measuring O_3. Table 1 displays O_3 concentrations in ozonized water samples for different treatment times.

Vase life, quality index, floret opening, and floret abscission

Based on the findings presented in Fig. (II), the use of ozonized water has a significant impact on the vase life of tuberose cut flowers. The most effective treatment time was found to be 8 minutes, resulting in a 2-fold increase in vase life. However, when the plasma time was increased beyond 8 minutes, the effect decreased, and the vase life of the flowers also decreased significantly, becoming similar to the control flowers. The use of ozonized water also improved the quality of the cut flowers. This was particularly noticeable from the fourth day of the experiment, as shown in Table 2. Overall, the experiment results indicated that using ozonized water as a preservation solution had a positive effect on the

Table 1
Concentrations of O_3 species in the ozonized water samples for different treatment times

Time (min)	4	6	8	10
O_3 (ppm)	0.43	0.71	0.96	1.45

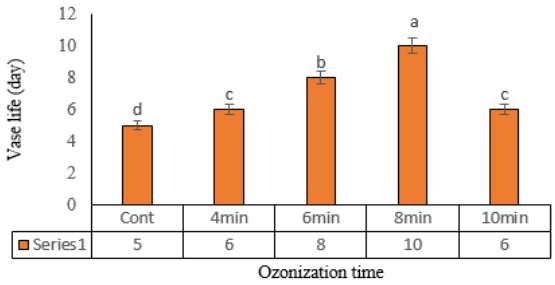

Fig. II. Effect of 4-, 6-, 8-, and 10-minute ozonized water as preservation solutions on the vase life of Tuberose cut flowers; distilled water was used as a control preservation solution. Data are means of tree replicates. The mean comparisons among treatments were compared using DMRT with a significance level of $P<0.05$. Different letters indicate significant differences among treatments.

Table 2
Effect of 4-, 6-, 8-, and 10-minute ozonized water treatment as preservation solutions on quality of Tuberose cut flowers; distilled water was used as a control preservation solution (1 excellent, 2 good, 3 = average, 4 bad).

	Control	4 min	6 min	8 min	10 min
1th day	1	1	1	1	1
2th day	1	1	1	1	1
3th day	2	2	1	1	2
4th day	3	2	2	1	2
5th day	4	3	2	1	3
6th day	4	4	3	1	3
7th day	4	4	3	2	4
8th day	4	4	4	2	4
9th day	4	4	4	3	4
10th	4	4	4	4	4

Table 3
Effect of 4-, 6-, 8-, and 10-minute ozonized water treatment as preservation solutions on Tuberose cut flowers' floret opening; distilled water was used as a control preservation solution. Data are means of three replicates. The mean comparisons among treatments were determined by DMRT, taking P<0.05 as significant. Different letters indicate significant differences among treatments.

	Control (%)	4 min (%)	6min (%)	8min (%)	10min (%)
1th day	38(a)	40(a)	41(a)	40(a)	40(a)
2th day	40(a)	42.9(a)	52.7(b)	56.7(b)	41(a)
3th day	47(c)	48.7(c)	58.1(d)	61(d)	47(c)
4th day	49.8(c)	51(b)	61(d)	62.9(d)	50(b)
5th day	51.4(b)	57(b)	64.6(d)	63.7(d)	55(b)
6th day	Destroyed	59.9(d)	75(e)	69(d)	Destroyed
7th day	Destroyed	Destroyed	82(f)	75.8(e)	Destroyed
8th day	Destroyed	Destroyed	82.5(f)	77.9(e)	Destroyed
9th day	Destroyed	Destroyed	Destroyed	79(e)	Destroyed
10th	Destroyed	Destroyed	Destroyed	83(f)	Destroyed
Standard Deviation	5.95(a)	7.75(c)	14.57(e)	12.91(d)	6.27(b)

Table 4
Effect of 4-, 6-, 8-, and 10-minute ozonized water treatment as preservation solutions on floret abscission of Tuberose cut flowers. Distilled water was used as a control preservation solution. Data are means of three replicates. The mean comparisons among treatments were determined by DMRT, taking *P < 0.05* as significant. Different letters indicate significant differences among treatments.

	Control (%)	4 min (%)	6 min (%)	8 min (%)	10 min (%)
1th day	0(a)	0(a)	0(a)	0(a)	0(a)
2th day	0(a)	0(a)	0(a)	0(a)	0(a)
3th day	21(b)	2(a)	0(a)	0(a)	25(b)
4th day	36(c)	8.9(a)	3(a)	0(a)	30(c)
5th day	38(c)	13(b)	11.2(b)	6.2(a)	45(d)
6th day	Destroyed	14.7(b)	12.5(b)	8.25(b)	Destroyed
7th day	Destroyed	Destroyed	12.5(b)	11(b)	Destroyed
8th day	Destroyed	Destroyed	16.3(c)	14.2(c)	Destroyed
9th day	Destroyed	Destroyed	Destroyed	29(d)	Destroyed
10th	Destroyed	Destroyed	Destroyed	35(e)	Destroyed
Standard Deviation	17.14(d)	6.63(a)	6.84(b)	12.56(c)	18.03(e)

quality and vase life of the cut flowers. The treatment with ozonized water also increased the number of open florets on the cut flower. On the tenth day, the cut flowers treated with ozonated water for 8 minutes had up to 83% of the florets opened while the control flowers, which perished on the fifth day, had only 50% of the open florets (Table 3). The amount of floret abscission in the control cut flowers on the fifth day of the vase life was about 38% while the measures in the cut flowers treated with ozonated water for 4, 6, and 8 minutes were 13, 11.2, and 6.2, respectively. However, it is worth noting that despite the positive effect of ozonated water treatments for 4, 6, and 8 minutes, ozonated water for 10 minutes increased the rate of floret abscission compared to control flowers (Table 4).

Lipid peroxidation

The data indicated that treatment with ozonated water effectively reduced lipid peroxidation in petal tissues of cut flowers. Specifically, treatments with 6 and 8 minutes of ozonated water showed the most pronounced reduction in lipid peroxidation, decreasing levels by approximately 50%. In contrast, the 10-minute ozonated water treatment did not produce a significant effect on lipid peroxidation (Fig. III).

Antioxidant enzyme activity

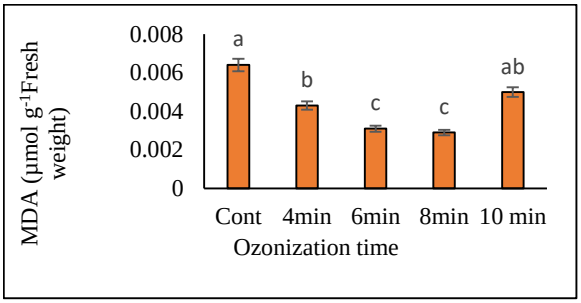

Fig. III. Effect of 4-, 6-, 8-, and 10-min ozonized water as preservation solutions on lipid peroxidation of Tuberose cut flowers; distilled water was used as a control preservation solution. Data are means of three replicates. The mean comparisons among treatments were performed using DMRT with a significance level of P<0.05. Different letters indicate significant differences among treatments.

The results of enzyme activity measurement are displayed in Fig. (IV). According to this figure, GPX enzyme activity increased in flowers that received 6, 8, and 10 min-ozonized water treatments while it had no significant effect on flowers treated with 4 min-ozonized waters (a). APX enzyme activity increased in flowers that received 4-min ozonized water treatment but decreased in those receiving 6- and 8-min ozonized water treatment (b). CAT enzyme activity showed a significant increase in all treatments (c). On the other hand, PPO enzyme activity decreased in flowers treated with 6- and 8-min ozonized water while there was no significant change in flowers treated with 4 and 10-min ozonized water (d).

PAL activity and total phenol content

PAL enzyme activity assay and measurement of total phenol content showed that treatment of tuberose cut flowers with ozone-activated water increased PAL activity and total phenol content of petal tissue in all treatments (Fig. V)

Total soluble sugars

Figure (VI) illustrates the variation in soluble sugar content across different ozonized water treatments. Flowers exposed to an 8-minute ozonized water treatment displayed the highest level of soluble sugar content. Conversely, a 10-minute exposure led to a noticeable reduction in soluble sugar content. The 6-minute treatment showed relatively high sugar levels while the 4-minute treatment, though had an increasing effect

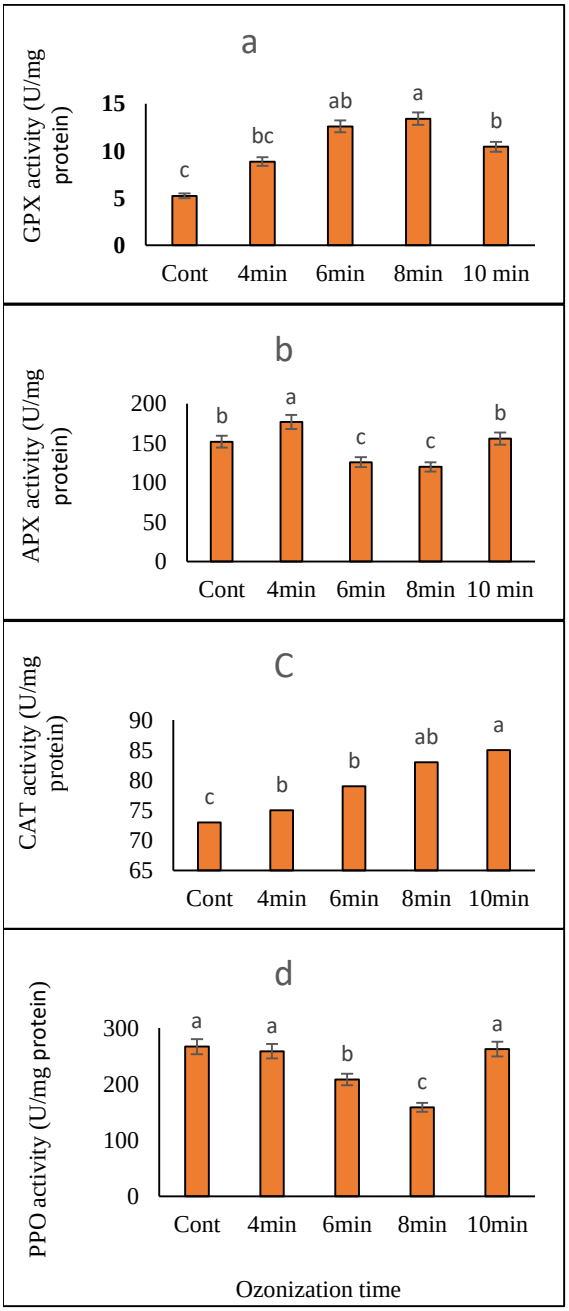

Fig. IV. Effect of 4-, 6-, 8-, and 10-min ozonized water as preservation solutions on the antioxidant activity of Tuberose cut flowers; distilled water was used as a control preservation solution. Data are means of three replicates. The mean comparisons among treatments were compared using DMRT with a significance level of P < 0.05. Different letters indicate significant differences among treatments.

compared to the control, was less effective than the 6- and 8-minute treatments.

These results suggest that soluble sugar content peaks with the 8-minute treatment, with lower levels observed at both shorter and longer exposure times.

Discussion

Postharvest quality is a critical factor in the cut flower industry, and extending vase life is essential for the commercial success of flowers (García-González et al., 2022; In and Lim, 2018; Li et al., 2021). The aging process in cut flowers involves a series of tightly regulated physiological and biochemical changes, including increased production of reactive oxygen species (ROS), degradation of proteins and nucleic acids, lipid peroxidation, and increased membrane leakage (Horibe, 2020; Sharifi and Naderi, 2019; Veluru et al., 2018). Additionally, factors such as the regulation of oxidative enzymes, changes in plant hormones, petal wilting, color shifts, leaf yellowing, and weight loss also contribute to senescence (Jing et al., 2021; Sao and Verma, 2020; Wang et al., 2023). For successful marketing, extended postharvest life and high aesthetic quality are essential attributes in cut flowers

Vase life is influenced by both genetic and environmental factors, and a variety of treatments, including growth regulators, essential oils, antioxidants, signaling molecules, and nanomaterials, have been applied to prolong it (Li et al., 2021). Among common chemical preservatives, silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) are notable for their ability to inhibit ethylene action, thereby delaying senescence. However, these preservatives pose significant environmental and health risks; for example, STS leaves toxic silver residues that can contaminate soil and water sources while prolonged use of 1-MCP may lead to resistance in treated flowers, potentially diminishing its efficacy (García-González et al., 2022; Muraleedhran et al., 2022). Consequently, there is growing interest in eco-friendly alternatives such as natural antioxidants and treatments like cold plasma, which hold potential for enhancing postharvest quality sustainably and without harmful residues (Jia et al., 2022; Šerá et al., 2021; Stryczewska et al., 2022).

Tuberose (*Polianthes tuberosa*), a fragrant flower known for its spike-shaped inflorescence, is highly valued in the floral industry; however, its postharvest quality largely depends on the

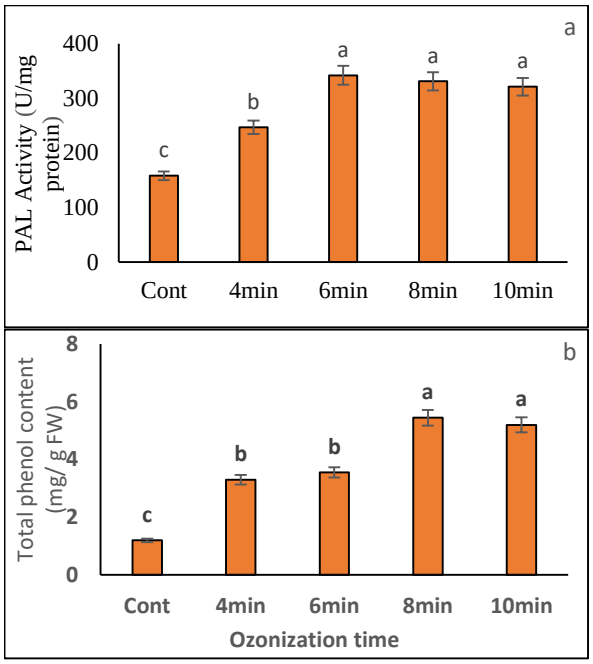

Fig. V. Effect of 4-, 6-, 8-, and 10-min ozonized water as preservation solutions on phenyl alanine ammonia lyase (PAL) activity and total phenol content of Tuberose cut flowers; distilled water was used as a control preservation solution. Data are means of three replicates. The mean comparisons among treatments were compared using DMRT with a significance level of P<0.05. Different letters indicate significant differences among treatments

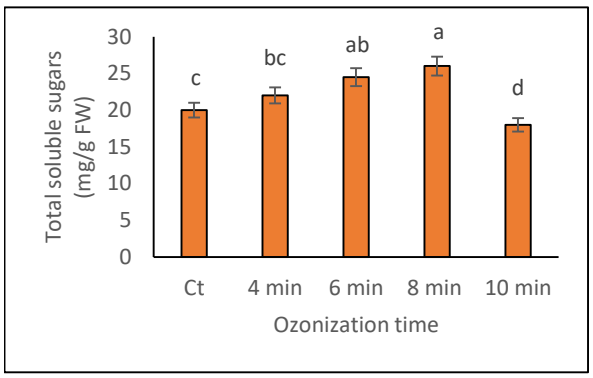

Fig. VI. Effect of 4-, 6-, 8-, and 10-min ozonized water as preservation solutions on the total soluble sugar of Tuberose cut flowers; distilled water was used as a control preservation solution. Data are means of three replicates. The mean comparisons among treatments were compared using DMRT with a significance level of P<0.05. Different letters indicate significant differences among treatments.

freshness and full opening of its florets (Paul et al., 2021). Tuberose faces specific challenges, including incomplete floret opening along the spike, premature petal shedding, limited terminal floret development, and short longevity of opened florets. These limitations hinder the commercial viability of tuberose, especially in export markets

where high quality and longevity are of paramount importance (Karimian et al., 2021). Given these challenges in maintaining the postharvest quality of cut flowers, particularly tuberose, there is a critical need for innovative and sustainable treatments that enhance longevity and quality. Future research should focus on developing environmentally compatible methods, such as natural antioxidants and cold plasma, to offer viable alternatives to conventional chemical preservatives like STS and 1-MCP. Such advancements could support the efforts in cut flower industry toward sustainability and improved commercial outcomes (Abedi et al., 2020; Filipić et al., 2020; Šimek and Homola, 2021).

The application of ozonized water in this experiment significantly improved the vase life and overall quality of tuberose cut flowers. Treated flowers displayed a higher number of open florets and a substantial reduction in floret abscission, with the exception of the 10-minute treatment group, where prolonged exposure appeared to diminish these benefits. These observations suggest that ozonized water can delay the senescence process in cut flowers, probably by mitigating common senescence-related physiological and biochemical changes. In untreated flowers, senescence is usually characterized by an increase in reactive oxygen species (ROS), leading to petal wilting, floret abscission, lipid peroxidation, and membrane degradation. Here, lower malondialdehyde (MDA) levels, a marker of oxidative stress, in ozonized flowers indicated enhanced membrane stability and cellular integrity, thus extending vase life through these protective mechanisms.

Ozonized water also activated key antioxidant enzymes, a mechanism essential for reducing oxidative damage by scavenging ROS. Elevated antioxidant enzyme activity aligns with previous findings in ozone-treated fruits, such as red pitaya, kiwi, chili, *Actinidia arguta*, raspberries, and strawberries, where ozone exposure enhanced the antioxidant system, extended shelf life, and delayed senescence (Janhom and Whangchai, 2023; Piechowiak and Balawejder, 2019; Piechowiak et al., 2022; Starič et al., 2020; Wang et al., 2023).

A key finding in this experiment was the increase in guaiacol peroxidase (GPX) and catalase (CAT) activities, two enzymes crucial for ROS scavenging. The 6- and 8-minute ozonized treatments significantly enhanced GPX and CAT activities, which are essential for breaking down hydrogen peroxide (H_2O_2), a ROS that accelerates oxidative stress and tissue senescence. Elevated GPX and CAT activities indicate a robust ROS-scavenging mechanism that protects cellular structures and delays visible signs of senescence (Mildaziene and Sera, 2022). The reduction in ascorbate peroxidase (APX) activity under optimal ozonized treatments may suggest a redistribution of antioxidant roles, with GPX and CAT serving as the primary ROS neutralizers (Song et al., 2020; Wang et al., 2023). Such adaptive antioxidant responses are essential for maintaining redox homeostasis under moderate oxidative stress from ozonized water.

Lipid peroxidation, indicated by MDA levels, was notably lower in the 6- and 8-minute ozonized water treatments, which supported enhanced membrane stability and reduced oxidative stress. These results align with prior research showing that controlled ozone exposure can reduce lipid peroxidation, thereby extending the vase life of flowers (Jing et al., 2021; Priatama et al., 2022). However, the 10-minute treatment led to increased MDA levels, suggesting that excessive exposure may compromise antioxidant defenses, accelerate membrane degradation, and promote senescence. This emphasizes the importance of optimized ozone dosage in postharvest applications (Li et al., 2021).

Furthermore, ozonized water at an optimal exposure also elevated soluble sugar content, a key factor in maintaining cellular turgor and metabolic activity. Soluble sugars are essential for sustaining cellular functions in cut flowers, providing energy and osmotic balance that are crucial for petal firmness and color retention (Li et al., 2021; Shelar et al., 2022). The 8-minute treatment, in particular, preserved higher sugar levels, contributing to longer vase life. Conversely, the 10-minute treatment showed a decline in

sugar content, probably due to rapid sugar depletion under high oxidative stress, leading to premature senescence (Wang et al., 2023).

The treatment also notably increased phenolic compounds, potent non-enzymatic antioxidants in plant cells. Phenylalanine ammonia lyase (PAL), an enzyme vital for synthesizing phenolic compounds, showed increased activity in treated flowers, with phenolic compounds positively correlating with antioxidant capacity. While PAL is not traditionally categorized as an antioxidant enzyme, its role in oxidative stress tolerance has been documented in numerous studies (Naing et al., 2022). Similarly, in ozone-treated fruits like *Actinidia arguta* and raspberries, increased polyphenolic content and PAL activity were reported (Piechowiak et al., 2022). This enhancement in phenolic compounds probably contributed to extended vase life of tuberose flowers by providing non-enzymatic antioxidant protection.

Interestingly, ozonized water treatment also reduced polyphenol oxidase (PPO) activity, an enzyme that typically degrades phenolic compounds and accelerates browning in cut flowers. Reducing PPO activity is crucial for maintaining the visual quality of flowers, as browning negatively affects their aesthetic appeal. By limiting PPO activity, ozonized water not only preserved phenolic compounds but also helped sustain aesthetic quality over time (Hajizadeh Namin et al., 2021).

Reduced floret abscission observed in 6- and 8-minute ozonized treatments suggests a potential modulation of ethylene sensitivity. Ethylene, a plant hormone involved in senescence and abscission, can be mitigated by antioxidant treatments that reduce ROS levels (Seyed Hajizadeh et al., 2024) . By enhancing antioxidant enzyme activity, ozonized water may reduce ethylene-induced abscission, thereby preserving postharvest quality (Asrey et al., 2024). However, the 10-minute treatment, which showed increased abscission, underscores the need for controlled ozone exposure, as excessive ROS may activate stress pathways that accelerate abscission(Hasan et al., 2021).

While traditional preservatives like silver thiosulfate (STS) are effective for extending vase life, they raise environmental and health concerns due to residual toxicity (Bagheri and Abbaszadeh, 2020; García-González et al., 2022). Ozonized water offers an eco-friendly alternative, supporting industry trends toward sustainable postharvest methods. Its ability to boost antioxidant defenses, reduce microbial load, and extend vase life makes it a promising, residue-free solution for the floral industry (Abbaszadeh et al., 2018; Jing et al., 2021).

Conclusion

This study shows that treating *Polianthes tuberosa* cut flowers with ozonized water is an effective way to boost antioxidant enzyme levels and phenolic compounds while also reducing PPO activity. These changes help delay aging and protect the cells in the flowers, leading to longer vase life and better postharvest quality. The results suggest that ozonized water could serve as a sustainable, residue-free method for preserving cut flowers, meeting the floral industry's growing interest in eco-friendly treatments.

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