



Research Paper

Synergistic effects of oxygen nanobubble, nano-silicon and seaweed extract on promoting quality and postharvest performance of two cut rose flowers

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ABSTRACT

The application of novel and cost-effective methodologies to prolong the vase life and improve the quality of harvested flowers, while reducing post-harvest loss, has become increasingly important. Therefore, the purpose of this study was to investigate the efficacy of seaweed extract (SWE), oxygen nanobubble (NB), and nano-silicon (NSi) in a hydroponic system to improve the pre-harvest and post-harvest qualities of cut rose flowers. The experimental design comprised four variables: two cut rose cultivars, namely Samurai and Jumilia; the utilization of NB at two different concentrations (0 and 20 mg L⁻¹); the foliar application of NSi at two different concentrations (0 and 2 ml L⁻¹); and foliar application of SWE at two different concentrations (0 and 1 ml L⁻¹). The quality and longevity of the flowers post-harvest were significantly enhanced by the treatments that were implemented. Comparing Jumilia cv to the control (31 days), the application of NB increased post-harvest life by 47.6 %. In addition, the petals exhibited the lowest Malondialdehyde concentrations. When NSi was applied to the flower, its quality improved by 43.7 % in comparison to the control. The combined treatment of NSi and SWE or individual application of NB resulted in the Samurai cv flower reaching its maximum length of 111.3 cm and 110.7 cm, respectively. The combined application of NB, NSi, and SWE resulted in a significant increase in some nutrient concentrations (N, P, Ca, and Mg) of two cut rose flowers. The utilization of NB, and NSi treatments increased N by 16.5 % and 11.5 %, respectively, compared to the absence of these treatments. The utilization of SWE resulted in a 3.82 % rise in N and a 46.4 % increase in Zn, as compared to the control plants. Based on our findings, the combined application of NB, NSi, and SWE in hydroponic cultivation for cut flowers may improve the flowers' quality and postharvest life. In protected cultivation, NB are an additional promising treatment.

1. Introduction

Roses are considered one of the top three cut flowers and are highly desired in the perfume and cosmetic industries (Kono et al., 2023). Reducing post-harvest losses and enhancing the quality of cut flowers are essential and unavoidable due to the rising consumption and demand (Gupta and Dubey, 2018). Sometimes the symptoms of lack of nutrients are due to lack of oxygen in the rhizosphere (Mercurio, 2007). The study by Bhattarai et al. (2005) found that increasing the oxygen availability in the rhizosphere enhances a plant's capacity to collect water from the soil, this, in turn, leads to the development of a larger and more efficient root system. The roots of most plant species require

sufficient oxygen levels to enhance yield and fulfill the water and nutritional needs of the aboveground parts (Meek et al., 1983). Studies indicate that an essential level of oxygen (2 mg L⁻¹) is required for the roots to operate well (Bhattarai and Midmore, 2009). Rectifying oxygen insufficiency via rhizosphere oxygenation enhances root oxygenation, leading to increased root access to oxygen (Bhattarai and Midmore, 2009). This directly impacts plant root growth and nutrient absorption, as well as supplies the necessary oxygen for root respiration, thereby enhancing metabolic activity and overall plant performance (Wu et al., 2019).

Aerated irrigation is recognized as an innovative irrigation technique that saves water. Bhattarai et al. (2005) were the first to document that

Abbreviation: SWE, seaweed extract; NB, oxygen nanobubbles; NSi, nano-silicon; Si, silicon; MDA, malondialdehyde; H₂O₂, hydrogen peroxide; ROS, reactive oxygen species.

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introducing aeration into irrigation systems can enhance both agricultural productivity and irrigation efficiency. By integrating pure oxygen with nanobubbles, the concentration of dissolved oxygen can be increased, hence promoting the growth of plants in aerobic circumstances. Nanoparticles are transferred to different organs faster than bulk materials and easily penetrate into plant tissues because their diameter is smaller than leaf opening and cell wall pores (El-Serafy, 2019). Studies have demonstrated the efficacy of nanomaterials in promoting plant growth, controlling diseases, and managing abiotic stress (Baram et al., 2022). Nanobubbles are particles with a diameter smaller than 200 nm (Baram et al., 2022). Nanobubbles enhance soil oxygen levels by delivering an ample supply of oxygen to the rhizosphere (Zhou et al., 2019). The presence of a substantial number of nanoscale bubbles in the irrigation water during nanobubble oxygenation has been found to result in significantly increased nutritional levels, as reported by Wu et al. (2019). According to reports, employing sub-surface drip irrigation with water containing nanobubbles enhances the productivity, water utilization efficiency, and fruit quality of cucumber, tomato, and maize (Zhou et al., 2019; Baram et al., 2022). There has been a growing interest in the use of biostimulants, such as nano-silicon and seaweed extract (SWE), for greenhouse products in recent years (Battacharyya et al., 2015; Antón-Herrero et al., 2023). Among the nanomaterials that have dimensions <100 nm and can be incorporated into the cut flowers and thus increase the absorption of this material is silicon (Si) (Seddon et al., 2012; Mathur and Roy, 2020). Plants can exclusively absorb silicon in the form of mono-silicic acid (H_4SiO_4). The physiology and development of plants are positively impacted by silicon nanoparticles, which also offer defense against several diseases and pests (Mathur and Roy, 2020). They effectively improve the antioxidant defense system by considerably enhancing reactions such as chlorophyll content, nutrient absorption, Rubisco activity, photosynthetic rate, and leaf gas exchange, as well as by stimulating metabolic processes. An increase in photosynthesis caused by the presence of Si results in greater carbohydrate production, which enhances the stem and postpones senescence (Seddon et al., 2012; Asgari and Diyanat, 2021). Simultaneously, research has shown that improving the antioxidant system prolongs the vase life of cut flowers (Seddon et al., 2012). This process enhances the durability and longevity of cell walls, resulting in reduced degradation and a longer lifespan of the cells (Seddon et al., 2012). Consequently, the combination of these factors helps prolong the post-harvest life of flowers.

The addition of biostimulants, such as SWE, is a contemporary approach that has emerged in recent years to improve the sustainability of agricultural production systems (Schiattoni et al., 2023). Biostimulants are substances, either natural or synthetic, that induce modifications in the essential and structural functions of plants (Parinitha et al., 2022). Their primary objective is to enhance the plant's ability to absorb and utilize nutrients, as well as increase its resistance to biotic or abiotic stresses (Antón-Herrero et al., 2023). The use of SWE enhances plant development, promotes root growth, and retards senescence, as demonstrated by Tavakoli and Asadi-Gharnehin (2020) on gerbera flowers.

According to Battacharyya et al. (2015) and Parinitha et al. (2022), SWE contains a variety of substances that promote plant growth, including minerals, abscisic acid, cytokinin, gibberellins, betaines, polyamines, and auxins, the presence of these substances, especially auxin and cytokinin, delay senescence by increasing antioxidant levels. Prolonging the postharvest life of these flowers directly impacts consumer demand and the economic worth of cut flowers.

The scientific literature is devoid of dependable information regarding the impact of oxygen nanobubble technology on the nutrition of cut roses, as well as the improvements in the quality and quantity of cut rose flowers grown in hydroponic media induced by nano-silicon and SWE. Hence, this study aimed to assess the impact of injecting oxygen nanobubble into the nutrient solution, as well as foliar spraying of NSi and SWE, on the growth, performance, quality, and post-harvest

longevity of 'Jumilia' and 'Samurai' cut rose flowers.

2. Materials and methods

2.1. Experimental site

From March to November 2022, this experiment was conducted in the research greenhouses of Isfahan University of Technology, Isfahan, Iran (39°43'N, 51°32'E; 1683 m) throughout 2022 and 2023. Normal Hoagland's solution was used while standardizing the nutrients schedule for cut roses. The cultivars used in this study consist of two rose varieties, including 'Jumilia' and 'Samurai', which were grown hydroponically from grafted-cutting plants within a controlled-climate greenhouse compartment. The seedlings were transplanted in media at mixing ratios of 3:2 (v/v) composed of cocopeat and perlite. The temperature regulation was set at 24–32 °C for the day and 18–20 °C at night.

2.2. Experimental design and treatments

The experimental conditions comprised two cultivars of rose flowers (*Rosa hybrida* cv. 'Jumilia' and 'Samurai'), foliar application of nano-silicon (NSi) at concentrations of 0 and 2 ml L^{-1} , spaced 10 days apart, and SWE ("Stimplex®" liquid commercial concentrate of *Ascophyllum nodosum* from Acadian SeaPlants, Canada, at two concentrations of 0 and 1 ml L^{-1} , spaced 10 days between each application). 20 mg L^{-1} of the oxygen nanobubbles (NB) was incorporated into the nutrient solution and the control plants did not receive any NB treatment (0 mg L^{-1} NB). At a pH of 5.5–5.8, the nutrient solution comprised calcium nano-chelate, magnesium nano-chelate, iron nano-chelate, and nano-chelate super plus ZFM (12–12–36) (Table 1).

As a daily dosing for 4 min, once every hour, this nutrient solution containing dissolved oxygen in water (0 or 20 mg L^{-1}) was administered to the plants. NB was produced in a 500 L plastic water-filled reservoir utilizing a two-phase vortex pump (SVP40 S 22-B) manufactured by UYDantek Company (Isfahan, Iran) with an oxygen container connected to the pump. The oxygen nanobubble practice injection into the vessel had a size of <100 nm, as determined by the dynamic light string method. The Sodour Ahrar Shargh Company (Tehran, Iran) supplied the NSi. The particle dimension of nano-silicon is 40 nm (Fig 1).

2.3. Morphological traits

The plant's vegetative growth parameters, such as the height of the flowering stem, the diameter of the flower stem, the size of the flower, the length of the peduncle, the number of leaves with 3, 5, and 7 leaflets, the fresh weight of the flowering stem, and the quality of the flower, were all measured. The sampling was conducted over a period of 60 days with three replications.

Table 1

Formulation of nutrition solution for cut rose flowers of 'Samurai' and 'Jumilia' under control and injection of oxygen nanobubble treatment. The mentioned amounts are in nano-chelate form application for 1000 L of water.

Element	Concentration of elements (g)	Source
N	50	NPK 12–12–36, Ca nano chelate 7 %, Mg nano chelate 6 % and nitric acid
P	20.4	NPK 12–12–36
K	59.7	NPK 12–12–36
Ca	17.5	Ca nano chelate 7 %
Mg	6	Mg nano chelate 7 %
Fe	3.8	Fe nano chelate 9 %, Complete micro nano chelate
Zn, Mn	0.45	Complete micro nano chelate
Cu, Mo, and B	0.15	Complete micro nano chelate

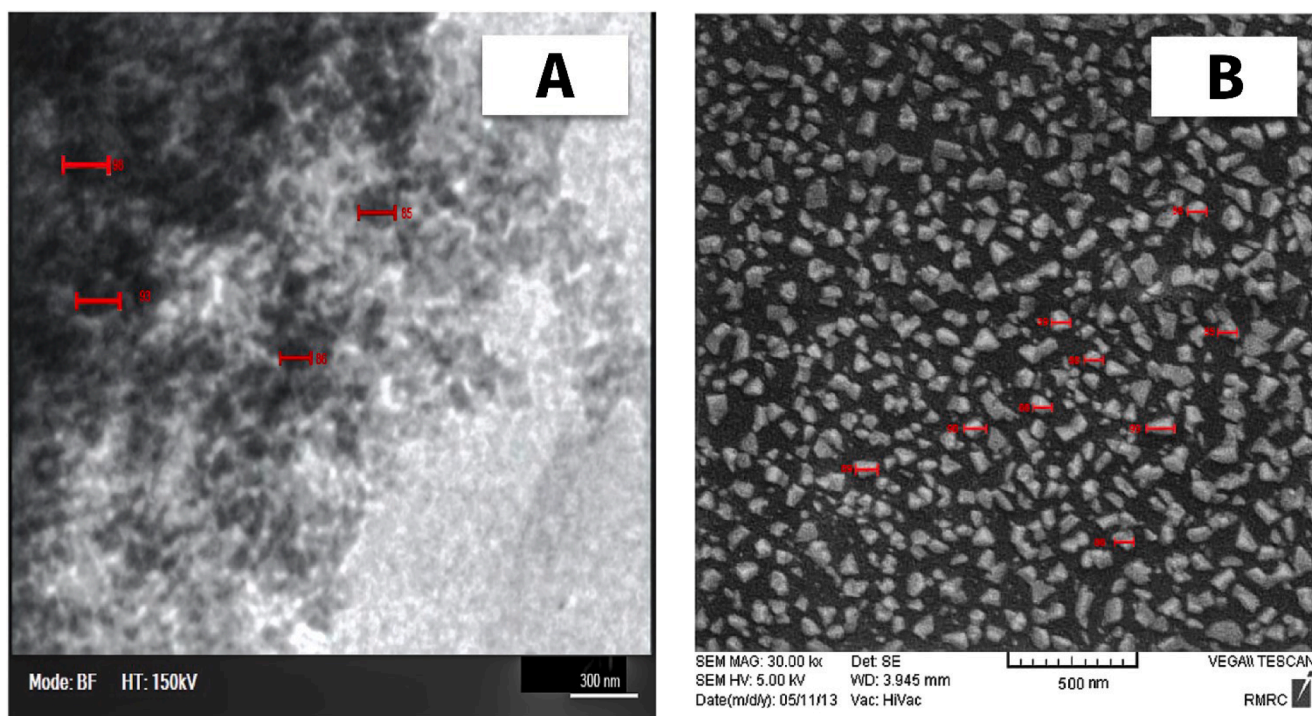


Fig. 1. Transmission Electron Microscopy (TEM) image of Khazra nano-silicon fertilizer (a). As can be seen in the marked image, the particles are approximately between 80 and 90 nm. This image was taken by EM900 device (150KV) at University of Tehran. SEM (Scanning Electron Microscope) image of Khazra nano-silicon fertilizer (b). As can be seen that particle are approximately 40–50 nm. This image was taken by HITACHI SU-5000 device (500 KV) at Research Institute of Petroleum Industry.

2.4. Leaf area measurement

Ten leaves were selected from each plant to measure the area of the leaves. The leaves were then scanned using a scanner (CanoScan LIDE 700F) and the Digimizer image analysis software [(V 4.1.1.0) (Schoonjans, 2019)] to determine the leaf surface area.

2.5. Chlorophyll and carotenoid content

The chlorophylls and carotenoid contents were assessed using the methodology outlined by Lichtenthaler (1987). In summary, a sample of fresh leaf tissue weighing 0.5 g was combined with 80 % acetone. The mixture was then filtered and adjusted to a volume of 10 ml. The absorption of chlorophyll a, b, and carotenoid was measured at wavelengths of 663 nm, 645 nm, and 470 nm, respectively, using a spectrophotometer (UV-160A UV-Visible Recording Spectrophotometer; Shimadzu, Tokyo, Japan).

2.6. Petal total anthocyanin content

To determine the overall amount of anthocyanin, 0.2 g of fresh petal tissues were crushed in 3 ml of methanol that was mixed with hydrochloric acid in a ratio of 99:1. The mixture was then placed in a dark place at a temperature of 25 °C for a period of 24 h. The sample was subjected to centrifugation at a force of 4000 g for 10 min. The absorbance of the sample was then measured using a spectrophotometer (Shimadzu, Japan) at a wavelength of 550 nm, as described by Wagner (1979). Ultimately, the content of anthocyanins was determined using the subsequent equation:

$$(1) A = \epsilon bc$$

where A represents the absorbance at a wavelength of 545 nm. The extinction coefficient (ϵ) is $33,000 \text{ cm}^2 \text{ mol}^{-1}$, b is the area of a 1 cm square cell, and c is the amount of anthocyanin.

2.7. Malondialdehyde and hydrogen peroxide assays

The malondialdehyde (MDA) content was quantified by analyzing fresh petal samples. Initially, a quantity of 0.1 g of petal tissue was subjected to centrifugation for a duration of 10 min at 12,000 g, using 2 ml of a solution containing 0.1 % (w/v) trichloroacetic acid (TCA). The thiobarbituric acid (TBA) was mixed with 4 ml of a standard solution containing 0.5 % TBA in a 20 % solution. After being subjected to a 30 min heating process in a hot bath at a temperature of 95 °C, the reaction mixture was then centrifuged at 2000 g for a duration of 15 min. The quantity of MDA was subsequently quantified using a spectrophotometer (UV-160A UV-Visible Recording Spectrophotometer; Shimadzu, Tokyo, Japan) at wavelengths of 532 and 600 nm (Velikova et al., 2000).

The assay for hydrogen peroxide (H_2O_2) was conducted using the procedure outlined by Velikova et al. (2000). The spectrophotometer was used to detect the optical absorption of the supernatant at a wavelength of 390 nm to quantify the concentration of H_2O_2 ($\epsilon=0.28 \mu\text{M}^{-1} \text{ cm}^{-1}$). The results were represented as micromoles per gram of fresh weight.

2.8. Vase life evaluation

After harvest, the cut flowers were placed immediately in distilled water at 4 °C and in the dark for 24 h. Next, cut roses were kept at 25 °C with a 16:8 h light:dark cycle, 3500 lux of daylight provided by white fluorescent lamps, and 50–60 % relative humidity. Flowers were transferred to vases containing 300 ml of distilled water, which was replaced every two days, and 2 cm was removed from the base of each stem. A total of 9 flowers per treatment were assayed. In this research, the term "end of vase life" refers to the period at the start of the transport to the laboratory until flowers exhibit at least one of the following symptoms: petal curling or wilting, stem bending ($\geq 90^\circ$), neck bending, or petal abscission. Specifically, it is defined as the time when approximately 50 % of the flower exhibit wilted petals or petal curling as

described by Rezaei et al. (2023).

2.9. Nutrient concentrations

In order to determine the elements, samples were collected from the first leaf with five leaflets and subjected to a drying process in an oven at a temperature of 65 °C for a duration of 48 h. Subsequently, the samples were ground. The Kjeldahl method, as described by Eaton et al. (1995), was used to measure the total N. Other nutrients extracted from samples with 2 N HCl after dry ashing at 550 °C for 5.5 h (Aalipour et al., 2019). Next, nutrient concentrations determined using inductively coupled plasma atomic emission spectrometry (Varian vista pro, ICP-OES axial-mode, Varian, Australia).

2.10. Statistical analysis

Split plot design was applied using Proc linear model of SAS 9.4. Main plot was a combination of 2 cultivars of rose flowers. In sub-plots, NSi, NB, and SWE with 2 concentrations of each one were taken into consideration, making 8 combinations in total. The experiment was conducted in triplicate and each replication consisted of four plants, leading to a total of 192 ($=2 \times 8 \times 3 \times 4$) treatments. Before conducting the Shapiro-Wilk test, the data was assessed for normality and log-transformed if needed. The analysis of variance and comparison of means based on the least significant difference (LSD) test with a significance level of $P < 0.05$ were performed using SAS software Ver. 9.4 (SAS Institute, Cary, NC, USA). The hierarchical cluster analysis (HCA) was conducted using Ward's method and Euclidean distance. R (4.1.3) was used for HCA, and the "d3heatmap" package in R was used to produce the heatmap. Statgraphics software Ver. 19 was used to do principal component analysis (PCA).

3. Results

3.1. Morphological traits

The application of NB and NSi resulted in a significant increase in peduncle length, with NB showing a 12.5 % increase and NSi showing a 5.21 % increase compared to the control group ($P < 0.001$). Compared to the control group, applying NSi treatment to 'Samurai' roses resulted in a 9.48 % increase in the height of the flowering stem, a 12.7 % increase in the number of 3-leaflets, and a 15.8 % increase in the fresh weight of the flowering stem (Table 3). Meanwhile, the peduncle length

of roses of the 'Jumilia' variety treated with SWE grew by 2.69 %, and the leaf area increased by 4.85 % (Table 4). The interaction between NB and NSi resulted in a 2.70 % increase in the diameter of the flower stem and a 5.93 % rise in the number of 7 leaflets. The interaction effects of NB \times SWE resulted in a 4.32 % increase in the height of the blooming stem and an 11.4 % increase in the fresh weight of the flowering stem compared to the control (Fig. 2). The interaction between NSi \times SWE resulted in an 8.51 % increase in the fresh weight of the flowering stem compared to the control.

3.2. Leaf area

The 'Samurai' had an 8.16 % increase in leaf area compared to the 'Jumilia' (Table 5). The application of NSi treatments resulted in a 16.3 % increase in leaf area compared to the control treatment, as shown in Table 5. In addition, the application of SWE resulted in a 10.9 % decrease in the leaf area of cut rose flowers compared to the control plants, as shown in Table 5. The 'samurai' had the greatest leaf area when subjected to SWE treatment, while the lowest leaf area was observed in the same cultivar under control conditions (Table 4)

3.3. Leaf pigment content

The application of NSi in plants resulted in a 10.9 % rise in total chlorophyll content and an 18.9 % increase in carotenoid content (Table 5). In addition, the application of SWE resulted in a 10.7 % increase in total chlorophyll content and a 25.7 % rise in carotenoid content compared to the control treatments, as shown in Table 5. The 'Jumilia' showed the greatest rise in carotenoid content when it was treated with both NB and SWE at the same time. Applying NB, NSi, and SWE together resulted in a significant increase in chlorophyll a, b, total chlorophyll, and carotenoid levels compared to the control plants. The increases were 33.9 %, 85.9 %, 49.2 %, and 94.3 %, respectively (data not shown).

3.4. Petals total anthocyanins content

The application of various nutritional treatments did not have significant effect on the concentration of anthocyanin in both cultivars of cut roses, as shown in Fig. 4B. The 'Samurai' exhibited a greater anthocyanin content in comparison to the 'Jumilia' (Table 5). The 'Samurai' variety exhibited a 2.83 % increase in anthocyanin concentration compared to the control when the interaction effect of NB and

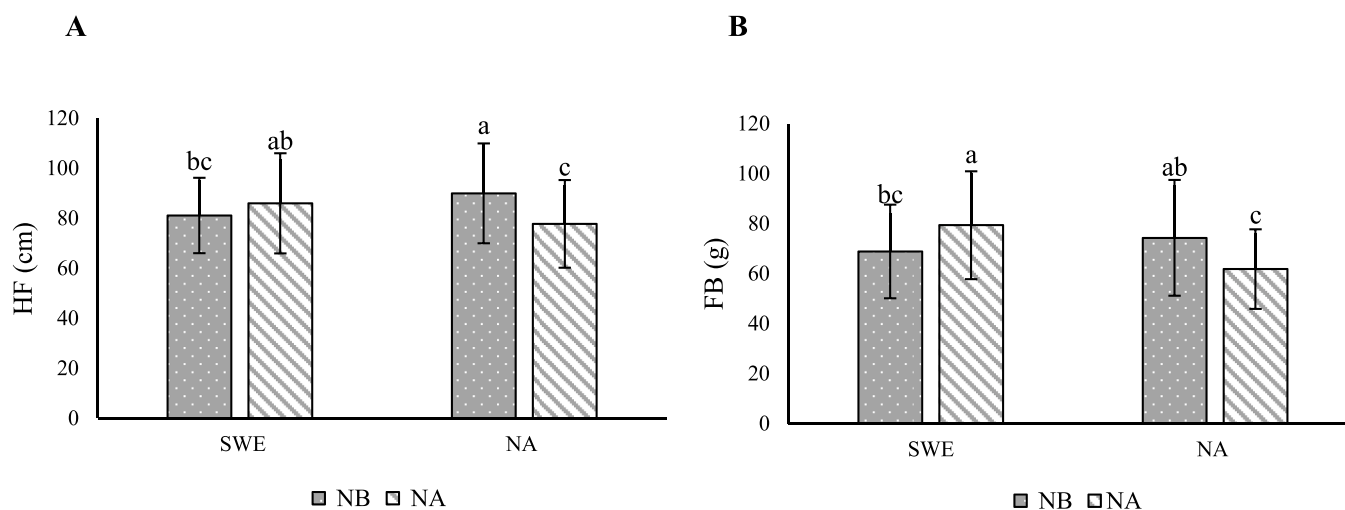


Fig. 2. The effect of oxygen nanobubble (NB) and seaweed extract (SWE) on (A) the height of the flowering stem (HF), (B) the fresh weight of the flowering branch (FB) of two cut rose flowers (cv. 'Jumilia' and 'Samurai'). Vertical bars (mean \pm SD) not connected with the same letter represent a significant difference between treatments according to the LSD test ($P < 0.05$). NA (non-application).

SWE was used. For 'Jumilia', we applied only SWE.

3.5. MDA and H₂O₂

The levels of MDA and H₂O₂ in the 'Samurai' were found to be 95.7 % and 41.4 % higher, respectively, compared to the Jumilia cultivar (Table 5). The use of NSi reduced the concentration of H₂O₂ in the 'Samurai' and 'Jumilia' by 27.9 % and 10.8 %, respectively, as shown in Fig. 3B. Furthermore, the utilization of NB resulted in a reduction of the MDA concentration in the 'Samurai' and 'Jumilia' by 16.5 % and 27.3 % respectively, as indicated in Fig. 3A.

3.6. Vase life

The combined application of NB, NSi, and SWE resulted in a significant increase in the vase life of cut rose 'Samurai' and 'Jumilia' flowers (Table 2 and Fig 4). The vase life of 'Samurai' flowers increased by 21.3 % and 'Jumilia' flowers increased by 26.9 % compared to the control plants, as shown in Table 2. The 'Jumilia' showed the greatest improvement (47.6 %) in the longevity of cut rose flowers when treated with NB, while the 'Samurai' had the lowest improvement under control conditions (Fig 4).

3.7. Nutrient concentrations

The nutrient concentrations of leaves of two cut rose cultivars were influenced by the cultivar, NB, NSi, and SWE, and their interactions (Table 2). The combined application of NB, NSi, and SWE resulted in a significant increase in the N, P, Ca, and Mg concentrations of cut rose 'Samurai' and 'Jumilia' (Table 2). The results showed that the highest increase in Mn concentration (252 $\mu\text{g}\cdot\text{kg}^{-1}$ DW) was obtained in the NB treatment of 'Jumilia' while the maximum increase Mn concentration (248 $\mu\text{g}\cdot\text{kg}^{-1}$ DW) was observed in 'Samurai' under combination treatment of NB and NSi (Table 2). Also, the highest increase in Si concentration (932 $\mu\text{g}\cdot\text{kg}^{-1}$ DW) was obtained in the combination treatment of NSi and SWE in both cultivars (Table 2). However, for the other nutrients no constant trend was noticed and the response to various treatments was largely dependent on the cultivar. The levels of all leaf nutrients in the 'Jumilia' increased (except for N) as compared to the 'Samurai' (data not shown). The utilization of NB, increased N, K, Mg, and B concentrations by 16.5 %, 10.4 %, 27.3 %, and 12.8 %, respectively, compared to control plants. The application of NSi treatment resulted in a significant increase in the N (11.5 %), P (4.56 %), Mn (2.78

%), and Si (15.4 %) concentrations compared to the absence of this treatment, while reducing the other nutrients. The utilization of SWE resulted in a 3.82 % rise in N and a 46.4 % increase in Zn, as compared to the control group where this treatment was not applied (data not shown).

3.8. Heatmap and PCA analysis

The relationships between rose-treated cultivars (treatments) and measurement characteristics (traits) were analyzed using hierarchical cluster analysis (HCA) and principal component analysis (PCA) as multivariate methods. The cultivars that were subjected to NB treatment, NSi application, and SWE supplementation were indicated on the labels. A two-way cluster analysis (HCA) was conducted to group the variables (characteristics and treatments), and the results were presented as a heatmap. The heatmap represents the intensity values of traits within treatment groups, with red indicating high intensity and blue indicating low intensity (Fig. 5). Traits were grouped using a vertical dendrogram, and treatments were grouped using a horizontal dendrogram. There were two distinct clusters created from the treatments. The upper cluster comprises the treatments for the first cultivar (Jumilia), while the other cluster includes the treatments for the second cultivar (Samurai). Indeed, the analysis of measured traits among different cultivars revealed that the Samurai cultivar had the greatest values for bud diameter (BD), bud height (BH), the fresh weight of the flowering branch (FB), Fe, Mn, anthocyanin (Antho), and total-chlorophyll. In addition, the Samurai cultivar exhibited greater levels of flower quality (FQ), H₂O₂, P, Mg, MDA, Ca, K, Si, N, and leaflet count compared to the Jumilia-treated cultivars.

The biplot in PCA justified 53.9 % of the data's information. The positive coefficients of the variables height of the flowering stem (HF), flower branch diameter (FD), 3 and 7 leaflets, the fresh weight of the flowering branch (FB), flower quality (FQ), anthocyanin, and MDA indicated a positive correlation with PC1. Conversely, the negative coefficients of bud diameter (BD) and bud height (BH) showed a negative correlation with PC1. The variables assessed for PC2 include peduncle length (PL), total chlorophyll content, and the concentrations of chlorophylls a and b. Additionally, these traits showed a positive correlation with iron (Fe) levels. The biplot displays the treatments located in the top-right quarter of the plot, which have high values for all attributes and positive coefficients. Both PCs have had a positive performance this quarter. The quantities of characteristics bud diameter (BD) and bud height (BH) were lower in the first quarter. We can extend these

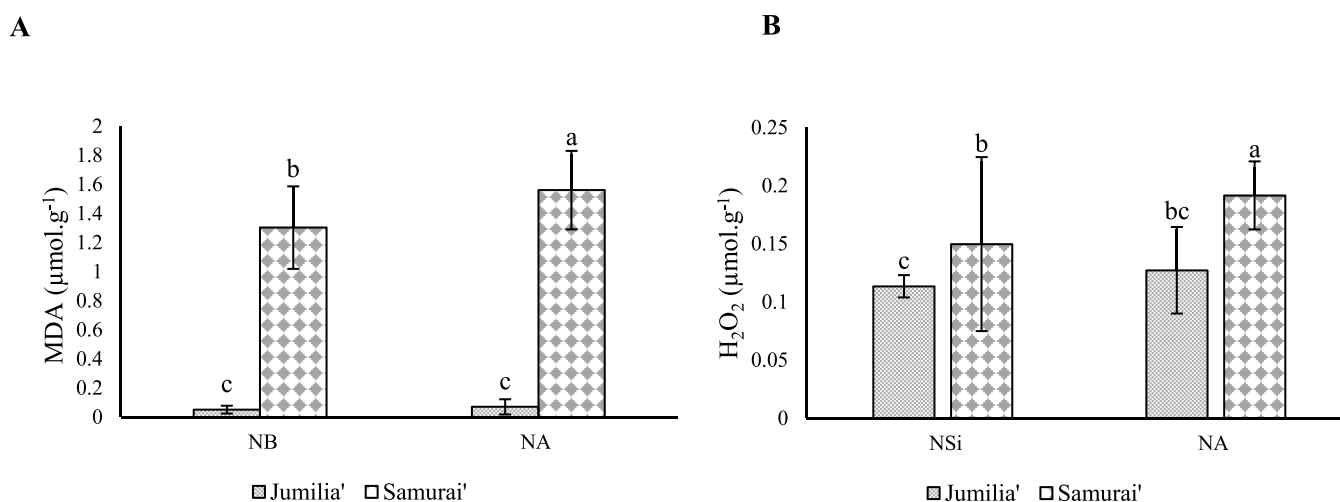


Fig. 3. (A) The effect of oxygen nanobubble (NB) on malondialdehyde (MDA), and (B) the effect of nano-silicon (NSi) on hydrogen peroxide (H₂O₂) of two cut rose flowers (cv. 'Jumilia' and 'Samurai'). Vertical bars (mean \pm SD) not connected with the same letter represent a significant difference between treatments according to the LSD test ($P < 0.05$). NA (non-application).

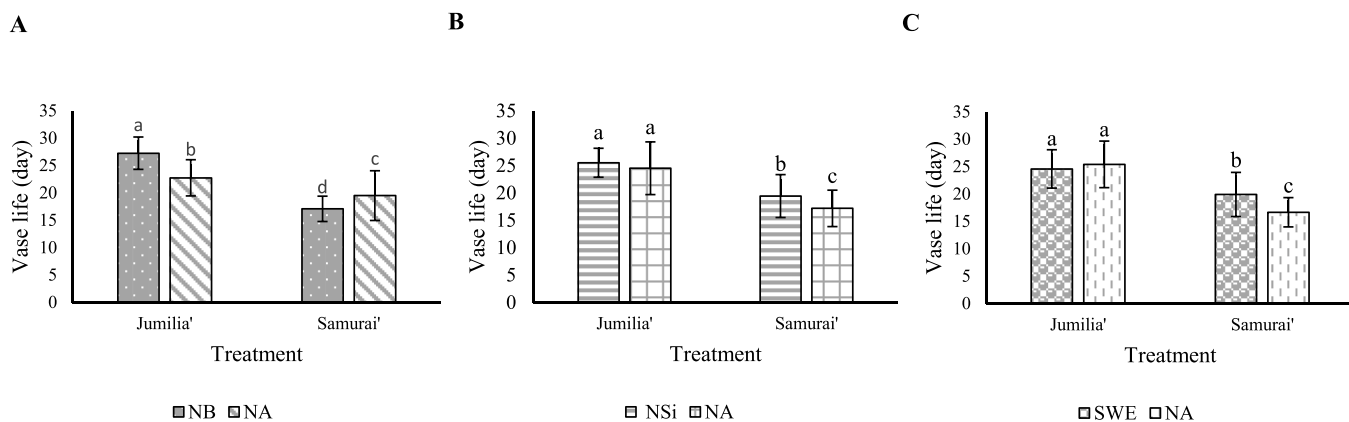


Fig. 4. The effect of oxygen (A) nanobubble (NB), (B) nano-silicon (NSi), and (C) seaweed extract (SWE), on vase life of two cut rose flowers (cv. ‘Jumilia’ and ‘Samurai’). Vertical bars (mean ± SD) not connected with the same letter represent a significant difference between treatments according to the LSD test ($P < 0.05$). NA (non-application).

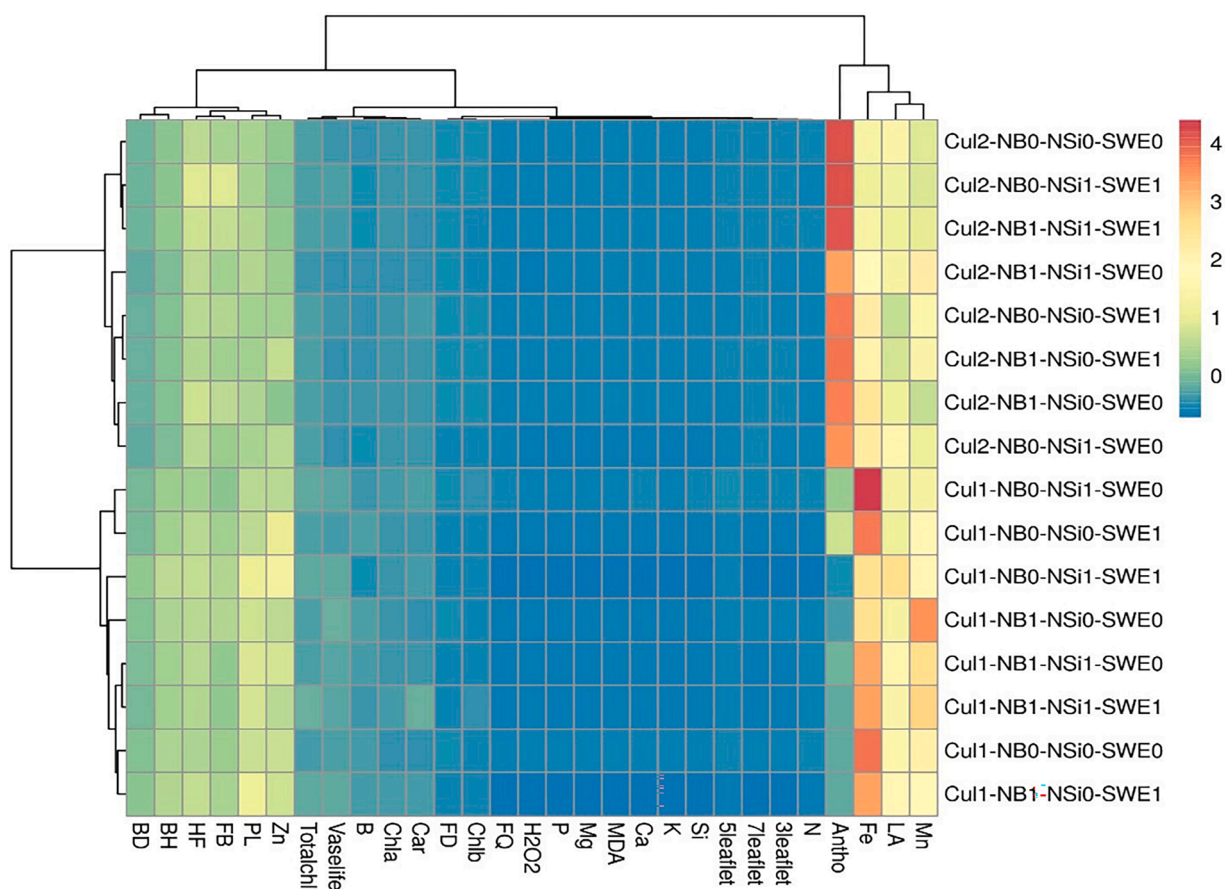


Fig. 5. Hierarchical cluster analysis (HCA) of cut rose flowers of CV Samurai and Jumilia treated with oxygen nanobubble (NB), nano-silicon (NSi) and seaweed extract (SWE). Jumilia (Cul1), Samurai (Cul2), non-application oxygen nanobubble (NB0), oxygen nanobubble (NB1), non-application nano-silicon (NSi0), nano-silicon (NSi1), manganese (Mn), leaf area (LA), iron (Fe), anthocyanin (Antho), nitrogen (N), number of 3, 5 and 7-leaflets leaves, silicon (Si), potassium (K), Calcium (Ca), malondialdehyde (MDA), magnesium (Mg), phosphor (P), hydrogen peroxide (H₂O₂), flower quality (FQ), chlorophyll b (chl_b), flower branch diameter (FD), carotenoid (Car), chlorophyll a (Chla), boron (B), Vase life, total chlorophyll (Total Chl), zinc (Zn), peduncle length (PL), fresh weight of the flowering branch (FB), height of the flowering stem (HF), bud height (BH), bud diameter (BD).

appropriate, and aeration is adequate, aerobic microorganisms in the soil become more active and can release more nutrients in a form that plants can absorb, resulting in an improvement in visible morphological characteristics (Gyaneshwar et al., 2002). Soil oxygenation treatments have been shown to greatly boost tomato growth by increasing stem diameter and plant height in the early stages of development (Wu et al.,

2019). Also, watering of cucumber plants with NB-containing water (after 12 weeks) resulted in increased leaf number, leaf size, and stem length compared to control plants (Dahrazma et al., 2019). Jiang et al. (2016) found that NB in water may enhance the development and quality of lettuce (*Lactuca sativa* L.).

NSi enhances water absorption, regulates phytohormones, increases

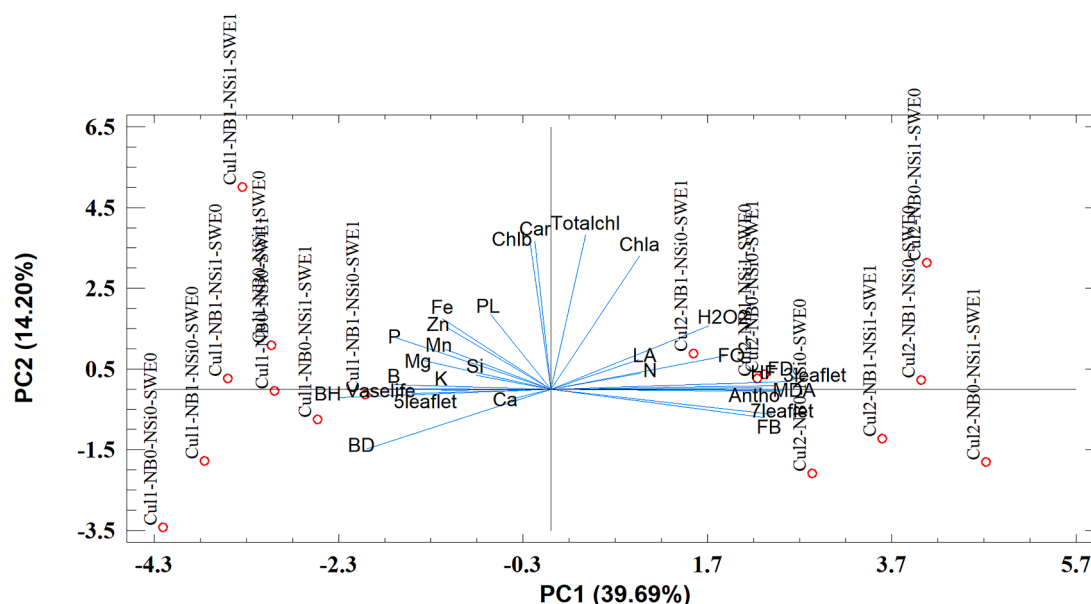


Fig. 6. Principal component analysis (PCA) on the characteristics of cut roses of Cv Samuraj and Jumilia treated with oxygen nanobubble (NB), nano-silicon (NSi), and seaweed extract (SWE). height of the flowering stem (HF), flower branch diameter (FD), bud diameter (BD), bud height (BH), peduncle length (PL), the number of 3, 5 and 7-leaflets leaves, the fresh weight of the flowering branch (FB), flower quality (FQ), and leaf area (LA), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl), carotenoid (Car), and anthocyanin (Antho) contents, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), vase life, nitrogen (N), phosphor (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), boron (B) and silicon (Si).

cell length, and provides nutrients. It has beneficial effects on the growth, development, physiology, and protection of plants, thereby influencing their morphological characteristics (Attia and Elhawat, 2021). According to Attia and Elhawat (2021), applying NSi to marigold plants through foliar spraying resulted in an increase in the quantity of the flowers and the diameter of the plants.

The growth of cells is influenced by SWE, which is caused by plant growth regulators like gibberellins, auxins, betaines, and cytokinins (Tavakoli and Asadi-Gharneh, 2020). The presence of gibberellin in SWE, according to Tavakoli and Asadi-Gharneh (2020), enhanced the flower diameter of gerbera. The maximum blossom diameter and stem length in rose cv Charisma were recorded when the plants were sprayed with SWE at a rate of 2 ml L^{-1} (Parinita et al., 2022). The application of SWE in chrysanthemum cv. Poornima White and pot marigold (*Calendula officinalis* L.) resulted in enhanced vegetative growth characteristics in these plants, as observed in comparison to the control plants (Tirki and Gantait, 2021; Taih et al., 2023).

The utilization of NSi and SWE resulted in an increase of leaf area in two cut rose cultivars, as indicated in Table 5. Li et al. (2023) found that the application of NSi can enhance the leaf area of wheat leaves in comparison to the control plants, which is consistent with our findings. Additionally, the deposition of Si in the cell wall of the leaves rectifies leaf positions, so enhancing light interception and consequently improving photosynthesis (Inanaga and Okasaka, 1995). Praveen et al. (2021) found that using SWE leads to an increase in the leaf area of the floribunda rose cv. Mirabel, which is consistent with the findings of our study. The growth enhancement caused by SWE may be attributed to the existence of cytokinin and auxin precursors, as well as macro and micronutrients that promote cell division and cell enlargement (Parinita et al., 2022). According to Al-khuzayyeh and Al-Asadi (2019), the presence of K and micronutrients in SWE was likely responsible for the increase in leaf area seen at high concentrations of SWE. The application of SWE to ornamental plants leads to an increase in leaf area. This increase is attributed to the presence of natural phytohormones in the extract, particularly cytokinins (Mahmoud et al., 2019; Kularathne et al., 2021).

Chlorophyll and carotenoid contents in two cut rose flowers were significantly elevated in comparison to the control plants when NB, NSi,

Table 5

Mean comparison cultivar (Cul), nano-silicon (NSi) and seaweed extract (SWE) of height of leaf area (LA), anthocyanin (Antho), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2), vase life, chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl) and carotenoid (Car) content for two cut rose flowers (cv. 'Jumilia' and 'Samurai').

CUL	LA cm ²	Antho	MDA	H_2O_2	Vase life
		(μmol. g ⁻¹ FW)			Day
Jumilia	129 ^b	36.5 ^b	0.060 ^b	0.120 ^b	25 ^a
Samurai	139 ^a	349 ^a	1.43 ^a	0.170 ^a	18.2 ^b
NSi	LA cm ²	Chl a	Chl b	Total Chl	Car
		mg. g ⁻¹ FW			
AP	144 ^a	17.3 ^a	8.72 ^a	26.03 ^a	19.3 ^a
NA	124 ^b	16.01 ^b	7.46 ^b	23.4 ^b	16.2 ^b
SWE	LA cm ²	Chl a	Chl b	Total Chl	Car
		mg. g ⁻¹ FW			
AP	126 ^b	17.08a	8.92 ^a	26.01 ^a	19.79 ^a
NA	142 ^a	16.2a	7.26 ^b	23.5 ^b	15.7 ^b

Within each column means followed by the same letters do not express significant differences according to LSD at $P < 0.05$.

and SWE were applied concurrently (Table 2). NSi has been found to enhance chlorophyll and ATP biosynthesis indirectly, as demonstrated by Pavlovic et al. (2021). This, in turn, results in elevated photosynthetic rates (Li et al., 2023). Si enhances the formation of photosynthetic pigments by protecting chloroplastic enzymes. Si likely functions as cofactors in numerous enzymatic activities that are part of the metabolic pathways within the chloroplast (Cao et al., 2015). SWE includes a significant concentration of mineral components, including nitrogen (N), potassium (K), magnesium (Mg), and iron (Fe). Magnesium plays a crucial role in the production of chlorophyll, while potassium has a beneficial impact on photosynthesis and the growth of meristems (Chrysargyris et al., 2018). Furthermore, cytokinin in SWE promotes the transcription of the chloroplast gene, which speeds up the creation of chlorophyll, and inhibits the breakdown of chlorophyll. Cytokinins are

also efficient in inhibiting carotenoid breakdown. In contrast, the existence of auxin and betaine in SWE enhances the process of chlorophyll production and stimulates the growth of grana (Nabti et al., 2018). The results of this study are consistent with those of previous research that found SWE to be effective in increasing the chlorophyll content of lettuce (*Lactuca sativa* L.) (Asadi et al., 2022), celery (*Apium graveolens* L. var. rapaceum) (Shehata et al., 2011), and red radish (*Raphanus sativus* L.).

The concentration of anthocyanin in both cultivars of cut roses was different (Table 5). The observed differences between the cultivars are more related to the natural color of the flower of the genotypes (Rezaei et al., 2023). Indeed, the synthesis of anthocyanin in plants is typically linked to the accumulation of sugar. Consequently, every element that enhances the sugar content promotes the production of anthocyanins in plant tissues (Tavakoli and Asadi-Gharneh, 2020). According to Tavakoli and Asadi-Gharneh (2020), SWE provides amino acids, protein, and carbohydrates that can raise anthocyanin by increasing the sugar level in the petals.

Compared to alkaline solvents, anthocyanins have greater stability in acidic environments with lower pH values. The vibrant blue/red colorations observed in plant vacuoles, where the pH is approximately 7.0, are believed to be caused by the interaction of anthocyanins with other flavonoids, phenolics, and/or metal ions (Dangles et al., 1994). Elevated concentrations of Si appeared to increase pH, which subsequently influenced the pH of vacuoles and induced the manifestation of various hues (Reezi et al., 2009).

The use of NB resulted in a drop in MDA content, with the 'Jumilia' showing the highest reduction of 27 % compared to the control (Fig 3A). Furthermore, the utilization of NSi resulted in a reduction of H₂O₂ concentration, with the most significant decrease found in the 'Samurai' (28 % compared to the control) (Table 5).

The use of Si substantially improves the activity of antioxidant enzymes (Farooq et al., 2019; Song et al., 2022). Then, antioxidant enzymes that are stimulated by Si are likely to be responsible for the sharp drop in ROS, H₂O₂, as well as MDA quantities (Song et al., 2022). The longer postharvest longevity of cut flowers and the subsequent delay in senescence can be attributed to the enhancement of the antioxidant defense system facilitated by Si. Si could mitigate ROS-induced damage, according to studies on rose (*Rosa hybrida* cv 'Vega') (Geerdink et al., 2020), celeriac (*Apium graveolens* L. var. rapaceum) (Shahzad et al., 2021), and bamboo (*Indocalamus barbatus* L.) (Li et al., 2012).

One of the oldest and most popular theories concerning aging is the free radical theory. This theory postulates that aging is due to the cumulative metabolic damage suffered by a living system over its entire life span through the action of reactive oxygen species (ROS) (Kirkwood and Kowald, 2012). Si plays a crucial role in regulating cell membrane stability and enhancing the accumulation of osmolytes. This helps in eliminating ROS and H₂O₂, as demonstrated by Abdelaal et al. (2020). Additionally, Si has a protective function by preventing the production of excessive ROS under stressful conditions, as shown by Kim et al. (2016). Furthermore, Si not only decreases the creation of reactive oxygen species (ROS), but also provides protection to fatty acids. As a result, plants that are treated with Si experience less damage, as demonstrated by Kim et al. (2016).

Individual application of NB, NSi, and SWE or their interactions resulted in an extended vase life for the cut rose 'Samurai' and 'Jumilia', as shown in Table 2. Our research findings indicate that using NSi prolongs the postharvest life of cut rose flowers by delaying the breakdown of chlorophyll. In addition, El-Serafy (2019) observed a significant improvement in the longevity of cut rose flowers when treated with Si, resulting in a 39.9 % increase in vase life compared to the control group. Several studies have indicated that the use of Si helps maintain the overall chlorophyll levels, which in turn can extend the vase life of cut roses (Asgari and Diyanat, 2021), lisianthus (Kazemi et al., 2012), and lilies (Sanchez-Navarro et al., 2021). Si can change the factors that determine the lifetime of flowers. When Si enters plants, it is deposited

in the structures responsible for transpiration and undergoes a transformation into insoluble crystals (Souri et al., 2021). In this scenario, the cell walls undergo reinforcement, resulting in increased durability and less cell wall degradation, thus extending the postharvest life.

Also, applying SWE, which contains auxin and cytokinin, to flowers can extend their vase life by boosting antioxidant defenses and strengthening their resilience to senescence. The use of SWE may extend the vase life of flowers by increasing metabolic activity and limiting the C:N ratio through considerable carbohydrate buildup (Parinitha et al., 2022). According to Parinitha et al. (2022), the longest period of time that flowers in the rose cv. Mirabel can remain fresh is achieved by treating the plants with SWE. Tavakoli and Asadi-Gharneh (2020) found that the application of SWE can enhance the amount, quality, and longevity of cut gerbera flowers. This is attributed to the presence of plant hormones and nutrient elements in SWE (Tavakoli and Asadi-Gharneh, 2020). On the other hand, insufficient oxygen concentration leads to the buildup of abscisic acid in leaves, resulting in reduced stomatal density and opening, inhibition of stem and leaf growth, decreased net photosynthesis rate, ultimately resulting in a decrease in carbohydrate accumulation and vase life (Li et al., 2016).

The application of NB, NSi, and SWE resulted in a significant increase in the N, P, Ca, and Mg concentrations of cut rose 'Samurai' and 'Jumilia' (Table 2). The utilization of NB, increased N by 16.5 % compared to control plants. The application of NSi treatment resulted in a significant increase in the N by 11.5 % compared to the absence of this treatment.

There are a few possible reasons why N increased in this study. Biological decomposition processes are essential for the release of plant-available nutrients, including N and P, from organic matter. The effectiveness of these processes is heavily influenced by the soil's oxygen content and hydration level. By simultaneously providing the soil with oxygen and water, oxygenation can increase the amount of N and P that is available to plants, as is the case with ventilation treatment (Xiao et al., 2015). In comparison to the control group, tomato plants treated with NB had a greater total nitrogen content, according to Wu et al. (2019).

The foliar application of NSi enhances the levels of total nitrogen and phosphorus in *Indocalamus barbatus* (Li et al., 2012). NSi, when utilized, has the potential to enhance gas exchange within plants, thereby promoting an expansion of the plants' photosynthetic capacity and growth (Siddiqui et al., 2014). The application of NSi to enhance the photosynthetic capacity of cut lily flowers has resulted in improved flower quality. This can be attributed to the increased concentrations of magnesium, phosphorus, and sulfur in the flowers (Sanchez-Navarro et al., 2021).

The highest increase in Mn concentration was obtained in the NB treatment of 'Jumilia' while the maximum increase Mn concentration was observed in 'Samurai' under combination treatment of NB and NSi (Table 2). The presence of redox conditions and soil pH have a significant impact on the accessibility of manganese (Mn) to plants. An increase in soil pH leads to a decrease in MnO₂ complex formation, which reduces Mn availability, whereas soil compaction and flooding increase Mn availability under reduced O₂ conditions (Ijaz et al., 2021). Si exhibited a greater increase in manganese net accumulation and concentration in shoots compared to roots. This indicates that Si facilitated Mn translocation to the shoot to a greater extent than Mn absorption by the plant roots. Si elevated the manganese (Mn) content in carrot (*Daucus carota* cv. Nantaise), lettuce (*Lactuca sativa* L. cv. Amerikanischer brauner), and maize (*Zea mays* L. cv. Reduta) plants (Greger et al., 2018). The natural occurrence of silicon as a constituent of cellular walls was demonstrated by Datnoff et al. (2001). Si deposition in the cell wall facilitates the transportation of nutrients across the membrane, according to a recent research by Mandlik et al., 2020.

The utilization of SWE resulted in a 46.4 % increase in Zn, as compared to the control group where this treatment was not applied. SWE, which is composed primarily of marine algae, has gained

significant popularity because of its rich composition of micronutrients (Co, Bo, Mo, Zn, Cu, Mg, and N). These micronutrients inhibit aging processes and enhance root nutrient absorption and growth. (Al Hatem et al., 2023). According to Asadi et al. (2022), an increase in the concentration of SWE applied to lettuce (*Lactuca sativa* L.) resulted in a greater accumulation of most of minerals in the stems. *Rajanus sativus* L. (red radish) foliar application of SWE was associated with an increase in zinc content, according to a study by Mahmoud et al. (2019).

5. Conclusion

NB enhanced the physiological characteristics and vase life of cut roses of the 'Samurai' and 'Jumilia' by enhancing nutrient release and uptake. Irrespective of the cultivar, the concurrent use of all treatments resulted in improved flower quality and vase life. However, the majority of growth characteristics exhibited a positive impact from NSi. Si may be recommended as a supplementary ingredient that effectively enhances the quality of cut rose flowers and prolongs their postharvest life. Regarding the effects of NB, NSi, and SWE on the pre-harvest and post-harvest attributes of two rose cultivars, Samurai and Jumilia, significant interactions were observed. In summary, the results indicated that the two cultivars exhibited similar reactions to the utilization of NB, NSi, and SWE when grown under hydroponic conditions. However, certain treatments elicited a marginally stronger response from the 'Samurai' in comparison to the 'Jumilia'. The 'Jumilia' and 'Samurai' exhibited the greatest increase in the postharvest life of cut rose flowers when treated with NB and control treatments respectively. To clarify how the mechanisms of NB and NSi effect the postharvest longevity of cut roses, additional research may be required.

CRedit authorship contribution statement

Roghayeh Hossein Hashemi: Writing – review & editing, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Ali Nikbakht:** Visualization, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Hamed Aalipour:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

All data generated and analyzed during this study are included in this paper.

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