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RESEARCH ARTICLE

Comparative study of bio-stimulants for improving the vase life of chrysanthemum cut flowers

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Abstract

Quality and longer vase life are the most important factors in cut flowers, while chemicals are the reliable ones for enhancing both. To prevent chemical usage, the study was undertaken to evaluate biostimulants for enhancing the vase life of cut flowers. Eleven different treatment combinations of bio-stimulants were used in this experiment to study their effect in enhancing the vase life of chrysanthemum, where extracts of aloe vera, *Ocimum gratissimum* leaves, lemon juice, *Mentha piperita* leaves and *Moringa olerifera* leaves were used at 2 % and 4 % concentration along with 2 % sucrose. The experiment was laid out in a completely randomized design replicated thrice and in each treatment, 10 flowers were used. Chrysanthemum var. Snowball was used in this experiment. Lemon juice at 4 % + Sucrose 2 % (B_7) recorded the maximum vase life (14.94, 15.15 and 15.05 days was observed in B_7 in Y_1, Y_2 and pooled respectively), the best antimicrobial activity by recording the minimum count of microbial colonies (43.33 × 10^4, 56.67 × 10^4 and 50.00 × 10^4 CFU/mL in B_7 in Y_1, Y_2 and pooled respectively), maximum total vase solution uptake (15.76 and 15.53 g in B_7 in Y_1 and pooled respectively) of chrysanthemum var. Snowball during the post-harvest period. A correlation study demonstrated that maintaining an acidic vase solution helped in suppressing microbial growth and this combined effect enhanced a longer vase life. From the study, it could be recommended that lemon juice at 4 % + sucrose 2 % effectively enhanced the post-harvest life of chrysanthemum.

Keywords: bio-stimulant; chrysanthemum; lemon juice; microbial activity; pH; postharvest; vase life

Introduction

Ensuring the vase life quality of cut flowers and enhancing vase life are vital concerns and pose a considerable challenge for florists worldwide. Main reasons for maintaining freshness with enhanced vase life in chrysanthemum cut flowers are premature leaf yellowing, wilting and poor water relations; buds fail to open fully after being harvested. As a result, many floriculture researchers concentrate on delaying flower ageing to extend vase life. The main goal of postharvest technology is to offer consumers flowers that are both high-quality and long-lasting (1). Cut flowers are particularly vulnerable to microbial contamination of *Pseudomonas* spp., *Enterobacter* spp., *Bacillus* spp., *Erwinia* spp., *Alcaligenes*, *Citrobacter* and *Klebsiella* at the stem base or in the vase solution, which can drastically shorten their vase life by occluding the xylem vessels (2-4). Floral preservatives are frequently recommended for cut stems and are extensively used by wholesalers, retailers and consumers to prolong flower longevity and to preserve quality (5-7). Preservatives like 8-HQC, 8-HQS and STS aid in maintaining water uptake by acidifying the vase solution and controlling microbial growth (8). They also provide the carbohydrates necessary for postharvest metabolic activities (9). Furthermore, floral preservatives can improve petal colour development, encourage flower opening and increase flower head size (7). Commonly used options include Aloe, *Moringa*, *Mentha*, *Ocimum* and lemon juice. Aloe contains various bioactive compounds such as aloin, emodin (anthraquinones), flavonoids, saponin and aloe-mannan, along with a range of amino acids and vitamins, which contribute to its antimicrobial, antifungal and preservative properties (10-12) and can potentially enhance the vase life of different flowers like carnations (13) and roses (14). Leaf extracts from *Moringa olifera*, *Mentha piperita*, *Ocimum basilicum* and lemon juice can also serve as floral preservatives in holding solutions (15, 16) as they possess antimicrobial activity and help maintain water balance in the cut stem (17). Although natural and eco-friendly preservatives are gaining popularity, more research is required to develop and validate new formulations. Due to the potential health risks of chemical preservatives, natural and eco-friendly preservatives are now becoming the alternatives for flower preservation and among these, bio stimulants are one of the important ones. The experiment was conducted to assess the effect of bio-stimulants for enhancing the vase-life of chrysanthemum.

Materials and Methods

The experiment was conducted in the laboratory of Floriculture, Medicinal and Aromatic Plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India, during 2022-23 and 2023-24. Eleven different treatment combinations of bio-stimulants were used in this experiment to study the vase life namely, distilled water (control) + sucrose 2 % (B_1), aloe vera at 2 % + sucrose 2 % (B_2), aloe vera at 4 % + sucrose 2 % (B_3), *Ocimum gratissimum* leaf extract at 2 % + sucrose 2 % (B_4), *Ocimum gratissimum* leaf extract at 4 % + sucrose 2 % (B_5), Lemon juice at 2 % + Sucrose 2 % (B_6), Lemon juice at 4 % + Sucrose 2 % (B_7), *Mentha piperita* leaf extract at 2 % + sucrose 2 % (B_8), *Mentha piperita* leaf extract at 4 % + sucrose 2 % (B_9), moringa leaf extract at 2 % + Sucrose 2 % (B_{10}) and moringa leaf extract at 4 % + sucrose 2 % (B_{11}). Aloe vera gel was scooped out and blended in a mixer until it reached a liquid consistency. Freshly harvested leaves of *Ocimum*, *Moringa* and *Mentha* were ground using a mortar and pestle with distilled water. Lemon juice was obtained from freshly harvested lemons. All the extracts were purified through Whatman No. 1 filter paper and centrifuged at 1500 g for 5 min at room temperature. The supernatant was then diluted to 2 % and 4 % concentrations using distilled water and 50 mL solutions of each treatment were used for the vase solution. The experiment was laid out in a completely randomized design (CRD) with three replications, each consisting of 10 flowers per treatment. Chrysanthemum var. snowball was used in this experiment.

Statistical analysis

The mean ± Standard Error of the Mean (SEM) presents all values. Statistical analyses were performed using MS EXCEL, IBM SPSS Statistics 26 statistical package (SPSS Inc., USA) and MSTATC. The data collected was analyzed for one-way Analysis of Variance (ANOVA) and significant variations were at a probability value of 0.05 level of significance.

Results and Discussion

Vase life (days)

Vase life varied significantly among the different bio-stimulants added to the vase solution. The maximum vase life of 14.94, 15.15 and 15.05 days was observed in B_7 in Y_1, Y_2 and pooled respectively, while the minimum vase life of 9.81, 9.95 and 9.88 days was observed in B_1 in Y_1, Y_2 and pooled respectively (Table 1). Enhanced vase life might be due to lower microbial growth in the vase solution, which restricted the vascular blockage and maintained the relative fresh weight of the cut flowers (18). The positive effect of the sucrose and lemon juice solution on the vase life was likely due to the antimicrobial properties of citric acid, which acts as a biocide, increasing water uptake (16, 19, 20). Additionally, sucrose supplies carbohydrates to cut chrysanthemums, helping maintain fresh weight. The combination of sucrose and lemon juice may prevent vascular blockage, reduce fresh weight loss and enhance postharvest quality. Sour orange extract (*Citrus aurantium* L.) at 4 mL/L extended vase life to 30.33 days in cut Narcissus (21). Citric acid in different concentrations enhanced the vase life of different cut flowers up to 5 days (22-28).

pH of the solution

The pH ranged from 3.06 to 8.64 among the different vase solutions. B_7 and B_6 exerted highly acidic pHs, which consisted of lemon juice, while others were slightly alkaline in nature (Table 1). The most significant contribution of lemon juice to a vase solution is its ability to lower the pH. Lemon juice is rich in citric acid, which acts as a powerful acidifier. Lowering the pH of the vase water to a more acidic level (around pH 3.0-3.5) has two key benefits such as inhibiting microbial growth and improving water uptake. Most bacteria, including common vase water contaminants like *Pseudomonas*, *Erwinia* and *Bacillus*, thrive in a neutral to slightly alkaline pH. A highly acidic environment is outside their optimal growth range and can denature essential enzymes required for metabolism and reproduction, effectively slowing or stopping their proliferation. citric acid can penetrate the cell wall and once inside the more neutral cytoplasm, it dissociates and releases protons (H^+), which acidifies the cells' interior. This disrupts the internal pH balance and the proton motive force across the membrane, which is critical for energy production (ATP synthesis) and nutrient transport (29).

Table 1. Effect of bio-stimulants on vase life (days) of chrysanthemum variety Snowball and pH of the vase solution

Treatment	Vase life (days)			pH of the solution		
	Y_1	Y_2	Pooled	Y_1	Y_2	Pooled
B_1	9.81 ± 0.65	9.95 ± 0.46	9.88 ± 0.51	7.59 ± 0.02	7.59 ± 0.02	7.59 ± 0.01
B_2	12.04 ± 0.61	11.81 ± 0.54	11.93 ± 0.53	8.31 ± 0.02	8.31 ± 0.02	8.31 ± 0.01
B_3	10.68 ± 0.54	10.48 ± 0.48	10.58 ± 0.47	8.56 ± 0.02	8.55 ± 0.02	8.56 ± 0.01
B_4	12.73 ± 0.64	12.48 ± 0.58	12.61 ± 0.56	8.47 ± 0.04	8.51 ± 0.03	8.49 ± 0.03
B_5	13.59 ± 0.69	13.33 ± 0.61	13.46 ± 0.60	8.64 ± 0.02	8.63 ± 0.02	8.64 ± 0.01
B_6	14.15 ± 0.54	14.20 ± 0.66	14.17 ± 0.54	4.35 ± 0.01	4.35 ± 0.01	4.35 ± 0.01
B_7	14.94 ± 0.56	15.15 ± 0.41	15.05 ± 0.45	3.06 ± 0.01	3.07 ± 0.01	3.06 ± 0.02
B_8	12.41 ± 0.63	12.17 ± 0.56	12.29 ± 0.55	8.39 ± 0.02	8.38 ± 0.02	8.39 ± 0.01
B_9	11.09 ± 0.56	10.87 ± 0.50	10.98 ± 0.49	8.62 ± 0.02	8.61 ± 0.02	8.62 ± 0.01
B_{10}	11.77 ± 0.59	11.54 ± 0.53	11.65 ± 0.52	8.16 ± 0.02	8.16 ± 0.02	8.16 ± 0.01
B_{11}	11.43 ± 0.57	11.21 ± 0.52	11.32 ± 0.50	8.49 ± 0.02	8.48 ± 0.02	8.49 ± 0.01
SEM (±)	0.346	0.309	0.232	0.006	0.005	0.004
CD (P = 0.05)	1.015	0.906	0.662	0.019	0.015	0.011

Total microbial colonies after 12, 24 and 36 hr at 10^5 dilutions (10^4 CFU/mL)

Total microbial colony count after 12 hr at 10^5 dilutions varied significantly among the treatments studied. The maximum microbial colony count after 12, 24 and 36 hr at 10^5 dilution was observed in B_1 in Y_1, Y_2 and pooled respectively while minimum was observed in B_2 after 12 hr, B_7 after 24 and 36 hr (Fig. 1). The accumulation of microorganisms and their decay products within the vascular tissues is a primary cause of vessel blockage in cut flowers (30), which reduces water uptake, hinders transport and thereby decreases fresh weight and vase life. Citric acid at concentrations of 0.5 - 0.7 % in holding solutions has been shown to promote flower growth and preserve the quality of cut tuberose spikes (31). 20 % lemon juice along with 10 % sugar extended vase life and improved relative fresh weight, number of open florets, membrane stability index and minimized bacterial growth in gladiolus cultivars (32).

Correlation between vase life, pH and microbial population

The correlation study demonstrated that both lower pH and lower microbial colony counts were critical for extending the vase life of the flowers (Fig. 2, Fig. 3). The microbial count appears to be the single most influential factor, but it is closely linked to pH. The pH and microbial colonies in the vase solution were negatively correlated with the vase life with r-vales -0.72 (significant at 5 % level of significance) and - 0.80 (significant at 1 % level of significance) respectively which indicated that lower pH and microbial growth enhanced the vase life of chrysanthemum (Fig. 4). On the other hand, pH was positively correlated (r = 0.41) with microbial growth of the vase solution. In essence, maintaining an acidic vase solution helps to suppress microbial growth and this combined effect was the key to achieving a longer vase life (Fig. 5). Multiple regression has been conducted in both ways i.e., effect of pH (Adj. R^2 = 0.46) (Fig. 2) and microbial activity (Adj. R^2 = 0.61) (Fig. 3) individually and to analyse the combined effect of both criteria on the vase life (Adj. R^2 = 0.61 and 0.78 respectively) (Fig. 5).

Total solution uptake (g/flower)

Significant differences were found in the total vase solution uptake. Maximum solution uptake was observed in B_7, followed by B_4, whereas the minimum was observed in B_1, followed by B_3 (Fig. 6). Citric acid was reported to prevent microbial growth and accumulation in vase solutions, which resulted in improved water uptake (33-35).

Relative fresh weight (%)

Maintaining relatively fresh weight during the vase life is crucial to enhancing vase life. During post-harvest, relative fresh weight is expected to decrease gradually, but light weight reduction triggered improved vase life (36). The maximum relative fresh weight was observed with lemon juice treatments (B_7 and B_6) while minimum occurred with *Ocimum* treatments (B_5 and B_4) (Fig. 7). Lemon juice, being rich in ascorbic and citric acids, may enhance the antimicrobial properties of the vase solution, thereby improving vessel conductivity and water uptake resulting in better relative fresh

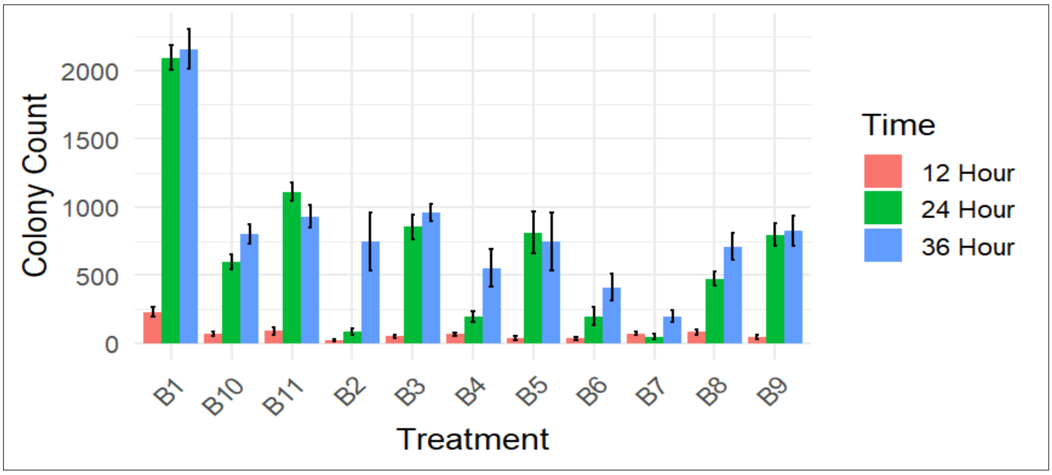

Fig. 1. Effect of bio-stimulants on total microbial colony count of vase solutions of chrysanthemum var Snowball after 12, 24 and 36 hr of inoculation at 10^5 dilutions (CFU ´10^4 /mL).

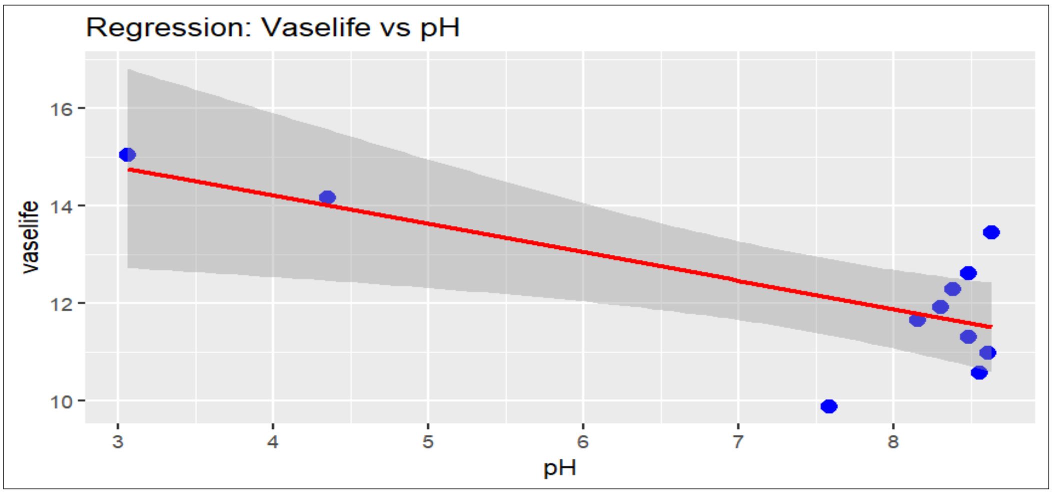

Fig. 2. Regression of vase life on pH.

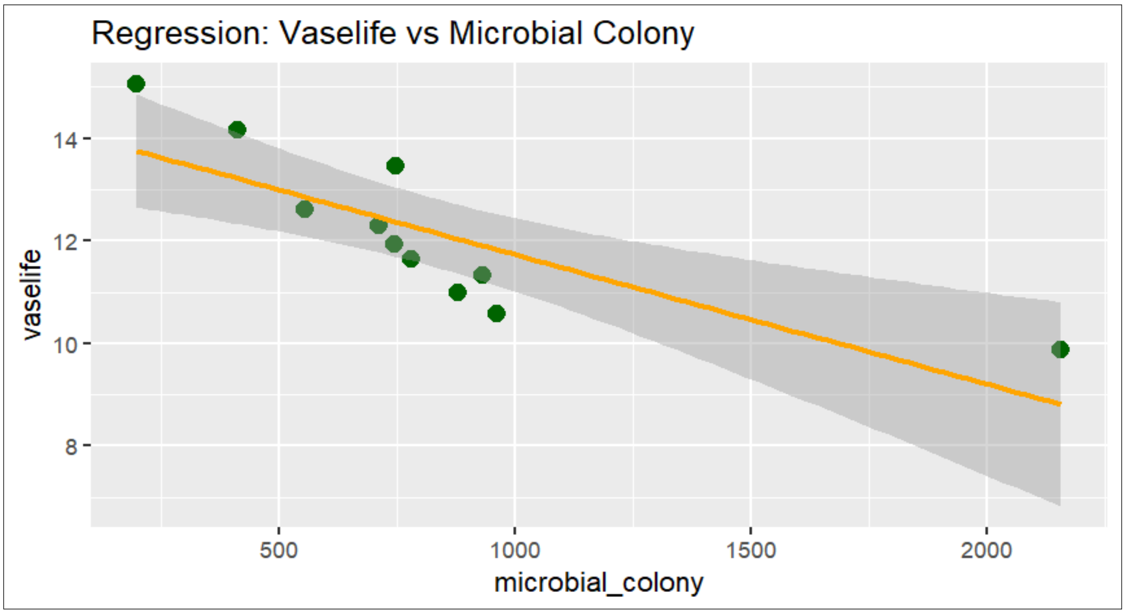

Fig. 3. Regression of vase life on microbial colony in vase solution.

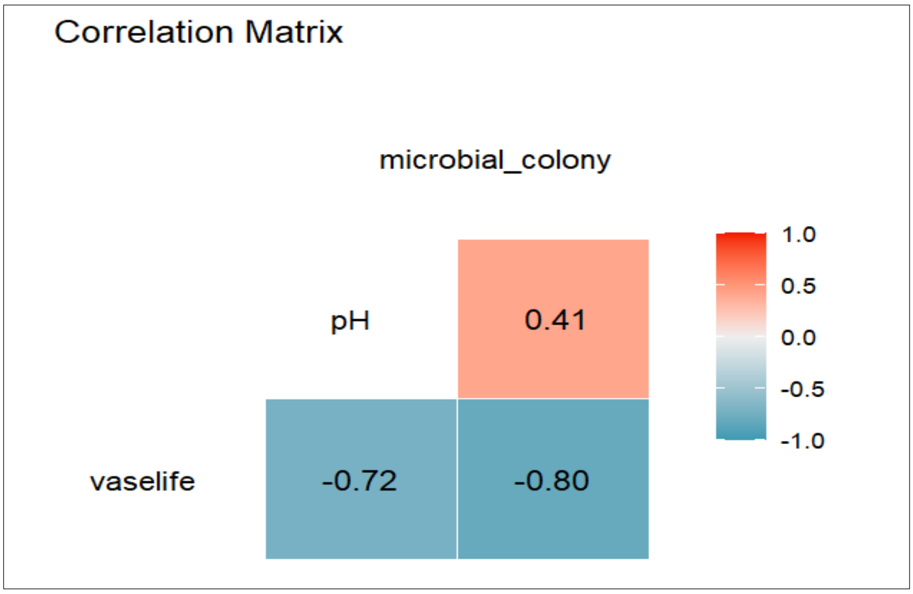

Fig. 4. Correlation matrix between vase life, pH and microbial colony of the vase solution.

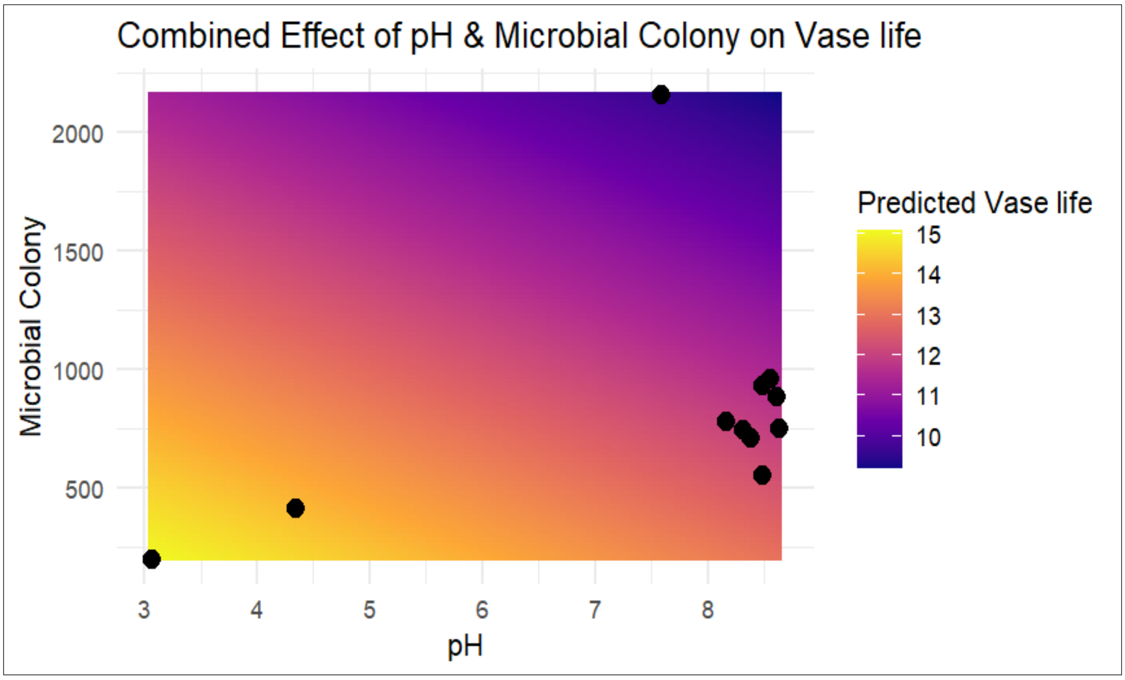

Fig. 5. Combined effect of pH and microbial colony on vase life of chrysanthemum.

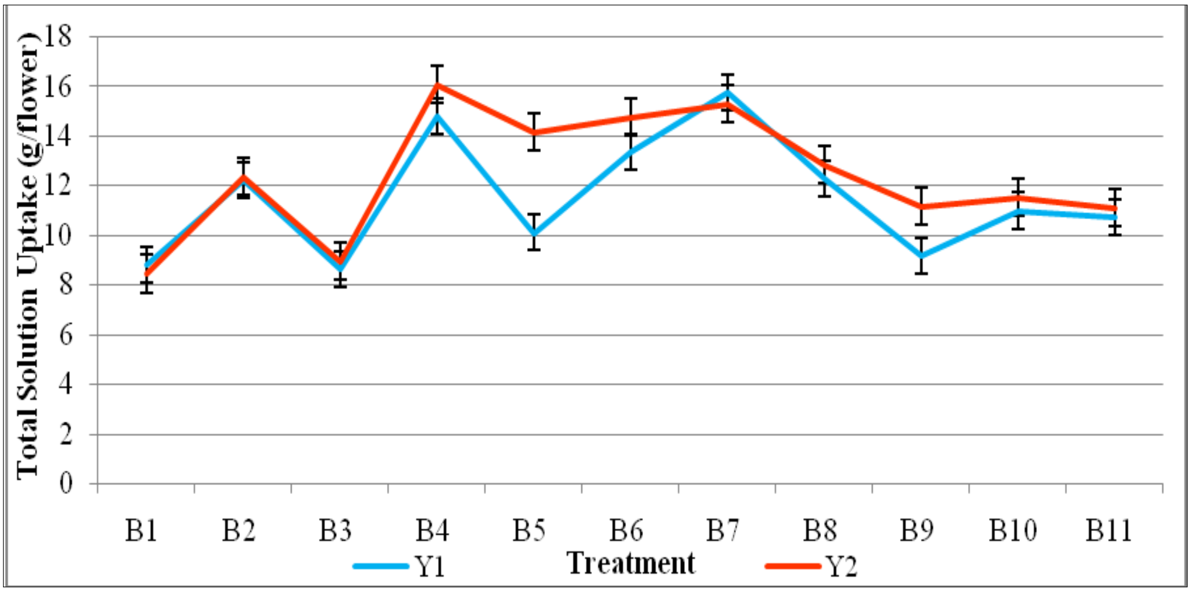

Fig. 6. Effect of bio-stimulants on total solution uptake (g/flower) of vase solutions, chrysanthemum var snowball.

weight (37). Furthermore, the high acidity of the vase solution affects its pH, impacting microbial communities and water uptake. Vascular blockages in xylem vessels, caused by air emboli, microbial accumulation, physiological plugs, tyloses and gels, are common causes of reduced solution uptake (38). Organic acids like citric acid and ascorbic acid restricted the blockage of cut sections of flower stalks in the vase solution and thus increased water uptake and maintained the relative fresh weights of cut flowers (39). Additionally, suberin and tannin deposition in conducting tissues stimulated by increased polyphenol oxidase (PPO) enzyme activity can decrease water uptake capacity in cut flowers (38) and citric acid improved relative fresh weight in different cut flowers (40-42).

Biochemical analysis of bio-stimulants of different concentrations

The heat map illustrates the clustering of biochemical profiles for bio-stimulants at 2 % and 4 % concentrations, comparing each extract across several parameters. Aloe Vera and lemon juice extracts (especially at 4 %) are highly enriched in sugars, vitamin C and proline, shown by deep red shades on those columns. *Ocimum gratissimum*, *Mentha piperita* and *Moringa oleifera* extracts at higher concentrations (4 %) show high levels (deep red shades) of phenolic compounds, flavonoids, antioxidant enzymes (Superoxide Dismutase, catalase, peroxidase) and amino acids (Fig. 8). The trend aligned with previous research studies (43-46). High concentration of sugar in lemon juice provides a viable exogenous carbohydrate source for cut flowers, while its primary efficacy stems more from its acidity than a significant role as a proline-based osmo protectant (47). Cut flowers must rely on stored carbohydrates to sustain metabolic processes after being cut off from their photosynthetic energy source. The natural sugars included in lemon juice, such as fructose and glucose, are easily absorbed by the cut stem and supply the energy required for cellular respiration. Lemon juice's higher levels of vitamin C, citric acid and other bioactive compounds reduced oxidative stress, stabilized membranes and altered metabolism and hormone-pathogen signaling (48). It improved the water balance of cut stems, decreased fresh-weight loss and enhanced solution uptake by preventing microbial proliferation. Membrane stability and senescence-related enzyme activity may be impacted by citric acid's ability to bind metal ions and alter the ionic equilibrium at the cut stem (49). Organic acids that chelate divalent cations can change the availability of ions that impact cell wall stability and membrane permeability, as well as inhibit oxidative Fenton-type processes locally. Citric acid helps preserve cell walls and membrane integrity by interacting with calcium salts (e.g., in formulations with $CaCl_2$), which is associated with less electrolyte

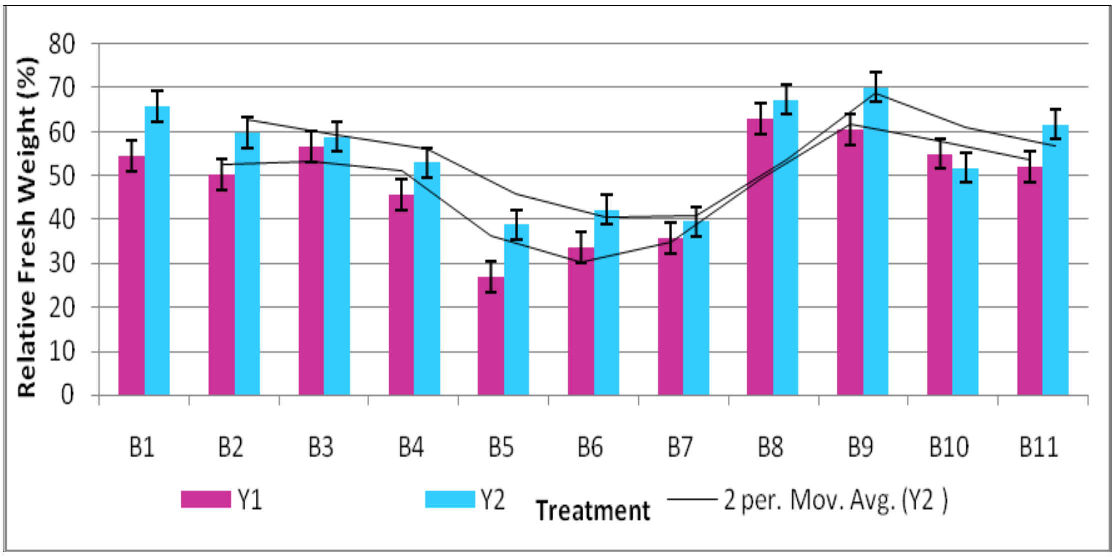

Fig. 7. Effect of bio-stimulant on relative fresh weight (%) of chrysanthemum var snowball.

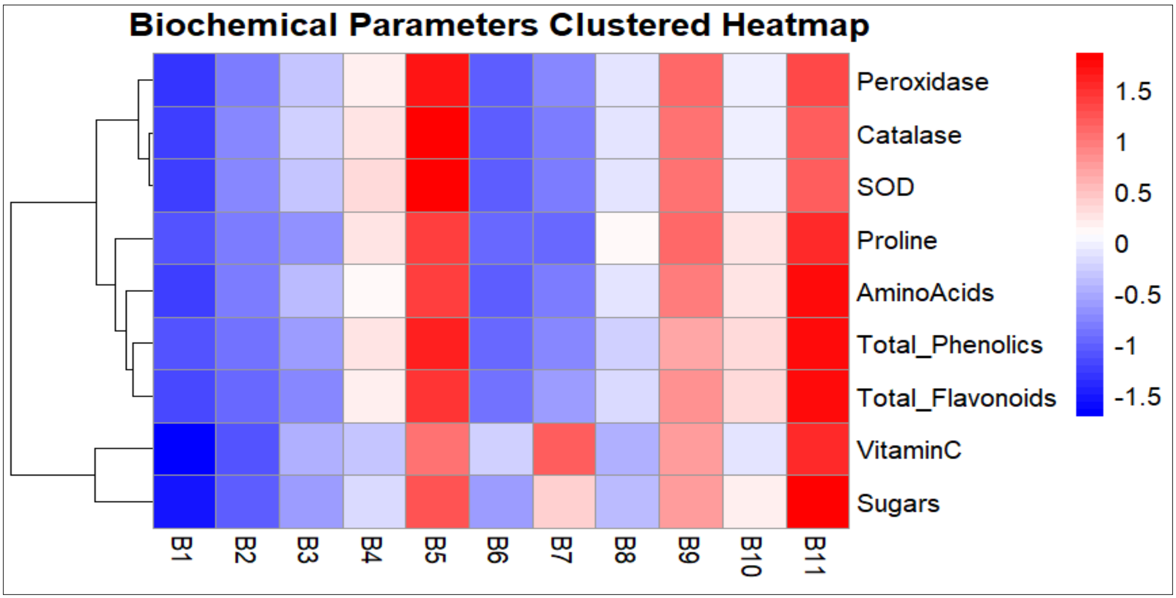

Fig. 8. Heat map with hierarchical clustering visualizing biochemical analysis for different treatments.

leakage and extended vase life. Additionally, ascorbic acid directly scavenges reactive oxygen species (ROS) like superoxide and H_2O_2, reducing lipid peroxidation and the buildup of malondialdehyde (MDA), which indicates membrane damage and senescence (50). Vitamin C and citrus phenolics prevent the oxidative breakdown of membrane lipids and proteins by lowering ROS levels and chelating catalytic metal ions. This reduces electrolyte leakage and maintains cellular compartmentation, which is necessary for petal turgor and colour. The longevity of cut flowers is determined by several factors, with water relations and microbial control frequently being more immediately critical than managing oxidative stress alone. Therefore, the high antioxidant content in extracts of *Ocimum* (basil) and *Mentha* (mint) might not translate to a better vase life compared to the acidic lemon juice. Even beneficial compounds might have negative effects when present in high doses (51).

Conclusion

In this study, the effects of different bio-stimulants on enhancing the vase life of chrysanthemum were examined. It was observed that acidic pH restricted the microbial growth in the vase solution and enhanced the vase life of chrysanthemum. Based on the findings, it is recommended that lemon juice at 4 % + Sucrose 2 % effectively enhanced the post-harvest life of chrysanthemum. Further studies are needed to investigate the physiological changes and mechanisms of action of bio-stimulants on enhancing vase life and quality of cut flowers.

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Authors' contributions

DS conceptualised the study, conducted the investigation, performed the formal analysis and data curation and prepared the original draft. PS handled data curation and formal analysis. RS and RC[1] contributed to data curation. IS contributed to conceptualisation and investigation and assisted in writing, review and editing. RC[2] contributed to conceptualisation, investigation, data curation, writing, reviewing and editing the draft. SK assisted with investigation and data curation. SO handled data curation. RPM contributed to writing the review and editing. All authors read and approved the final manuscript. [RC[1]-Riya Chakraborty; RC[2]-Ranjit Chatterjee].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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