Effect of the interaction between seaweed extract and calyx tea biostimulants on the growth and flowering of Geranium plant

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Abstract

This study investigated whether the interaction effect of seaweed extract (SWE) and calyx tea (CT) improves in growth, flowering, and nutrient status of Geranium plant *Pelargonium zonale* more than either biostimulant alone. A pot experiment was conducted in the wooden canopy of the Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, during the 2023-2024growing season. The experiment followed a completely randomized design with 3x 3 factorial arrangement: SWE at 0, 2 and 4 g L^{-1} and CT at 0, 2 and 4 g L^{-1}, with ten replicates per. SWE was applied as a foliar spray, while CT was applied as fertigation weekly for two months. Vegetative traits (plant height, leaf number, branch number) and floral traits (flower number) were measured separately, along with chlorophyll, carotenoid, leaf water relations, and leaf N, P, and K concentrations. The combined treatment SWE 4 g L^{-1} + CT 4 g L^{-1} significantly increased plant height (21.3%), leaf number (46.7%), branch number (177.8%), and flower number (63.2%) compared with the control. Chlorophyll content increased by 157.5%, and dry matter increased by 27.4%. potassium increased by 89.0% under the same treatment. Factorial analysis showed significant main effect of SWE and CT and a significant interaction for most traits. The combined application outperformed individual treatments. offering a practical and environmentally friendly strategy for improving ornamental plant quality and reducing reliance on synthetic fertilizers.

Keywords: Biostimulants. Fertigation. Geranium plant. *Hibiscus sabdariffa*. Ornamental plants. Seaweed extract.

Introduction

Ornamental plants are a major segment of the worldwide horticultural industry, because of their aesthetic, economic and cultural value. *Pelargonium zonale* L. is a traditional ornamental plant with the second-largest growing area in the world (outdoor and pot flowers) **[1]**. Effective nutrient management is important for plant growth and flowering and also increases the quality of ornamental crops **[2, 3]**. Although chemical fertilizers have dominated production, their negative affecte on soil health and the environment have encouraged research into sustainable alternative **[4]**.

Biostimulants have gained attention as sustainable tools for improving plant growth and development. Among the bio-stimulants. Seaweed extract (SWE) is particularly significance because they contain phytohormones such as auxins, cytokinins, gibberellins, as well as amino acids, vitamins, polysaccharides, and macronutrients **[5]**.

Several authors have investigated the use of seaweed extracts on horticultural and ornamental crops. For example, foliar application of *Ascophyllum nodosum* extract significantly improved vegetative growth, leaf pigments, and flower yield in geranium (*Pelargonium graveolens*) **[6]**. Al-Saad **[7]**

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also reported that using seaweed extract increased the number of flowers, the quality of leaves (foliage), and the total yield of marketable (well-developed) stems in ornamental plants.

Besides seaweed extracts, the use of organic plant tea is another promising biostimulant source. *Hibiscus sabdariffa* calyx tea (CT) is rich in phenolics, anthocyanins, organic acids, and minerals with high antioxidant activity and nutritive value **[8]**. Calyx tea extracts have been used as natural fertilizers or soil amendments to improve productivity and nutrient uptake and to stimulate soil microbial activity **[9]**. Jin et al. **[10]** found that fertigation with hibiscus tea plants increased nutrient uptake, leaf chlorophyll content, and influenced growth and flowering in ornamental plants.

The main components of Algea 600 include organic matter (34-40%), N (0.5 - 1%), K2O (17-21%), Ca (0.6-1.8%) , Mg (0.04%), S (0.50%). Hibiscus sabdariffa calyx tea contains organic acids (malic and citric acids), phenolic compounds, anthocyanins, and minerals.

Previous studies have examined SWE an CT separately, but their combined effect on *P. zonale* has not been studied. Therefore, the objective of this study was to assess the individual and combined effects of SWE an CT on vegetative growth and floral traits of *P. zonale*.

Materials and Methods

Experimental conditions

The study was carried out in the wooden canopy of the Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Iraq, during the 2023-2024 growing season. Seeds of *Pelargonium zonale* L. cv. "Maverick (Fig. 1) were surface-sterilized in 3% sodium hypochlorite for 10 min, rinsed three times in sterile distilled water, and planted individually in pots containing a sterile mixture of vermiculite and sand (1:2, v/v). The plants were grown under natural light. At four months of age (1st January, 2024), 90 homogeneous seedlings in vegetative growth were selected and transplanted into plastic pots (20 cm × 18 cm) one plant per pot. with soil consisting of a (1:1) Bet moss: Loam mixture. The organic matter and macronutrient contents of the soil in the pots were also reported in Table 1.

Treatment preparation and application

Seaweed Extract (Algea 600): Algea 600 is a brown seaweed extract from *Sargassum algae* (Beijing Leili Marine Bioindustry Inc., China). It contains organic matter: 34-40%, N:0.5 -1%, P2O5:NA, K2O:17-21%, Ca: 0.6-1.8%, Mg: 0.04%, S: 0.50%.

Calyx tea Preparing

Four grams of dried *Hibiscus sabdariffa* Calyx ground and infused in one liter of water 50 °C for 24 h, with hourly shaking. The extract was filtered through a fine cloth diluted before use.

Treatment application: treatments began on 15th March, 2024 and were applied weekly for two months until 15th May, 2024. SWE was applied as a foliar spray using a hand sprayer, and CT was applied as fertigation (soil drench). Tween 20 (0.1 mL L^{-1}) was added to the foliar spray as a surfactant. Foliar spray was done in early morning, and polythene barriers were used to prevent contamination between treatments. The nine treatments were applied: 1. Control (distilled water) 2. CT 2 g L^{-1} 3. CT 4 g L^{-1} 4. SWE 2 g L^{-1}, 5. SWE 2 g L^{-1} + CT 2 g L^{-1} 6. SWE 2 g L^{-1}+ CT 4 g L^{-1} 7. SWE 4 g L^{-1} 8. SWE 4 g L^{-1}+ CT 2 g L^{-1} 9. SWE 4 g $^{-1}$+ CT 4 g L^{-1}.

Each treatment had ten replicates. Regular irrigation was applied every four days; fertilizer treatments were applied weekly as an alternative to regular irrigation.

Table 1. Some Physical and Chemical Properties of Pot Soil and Irrigation Water

Physical and chemical properties	Soil	Water	Unit
pH	7.8	7.9	-
Electrical conductivity	2.8	2.4	dS m^{-1}
Organic matter	0.26	-	%
Nitrogen	0.53	-	g kg^{-1}
Phosphorus	0.56	-	g kg^{-1}
Potassium	0.66	-	g kg^{-1}

Vegetative Growth measurements

Vegetative traits were measured, including Plant Height (cm), number of leaves per plant, and number of branches per plant.

Floral growth measurements: Including Number of flowers per plant.

Physiological and Biochemical measurements: Chlorophyll and Carotene were extracted according to the method described by Goodwin **[11]**. Moisture content and dry matter percentage were calculated using standard equations. Leaf samples for N, P, K analysis in laboratory; K was measured by flame photometer, following Page et al. **[12]**, and N was measured by the micro-Kjeldahl method. P by the Murphy and Riley **[13].**



Fig. 1. Eight-month-old geranium seedlings, *Pelargonium zonale,* at the end of the experiment

Statistical analysis

Data were analyzed as completely randomized design with a 3 x 3 factorial arrangement (SWE x CT). Analysis was performed using SPSS Statistics 23. Treatment means were compared using Tukey's test at the $P \leq 0.05$. Main effect of SWE and CT and their interaction were determined.

Results

Vegetative and Floral Growth Parameters

Probabilistically, the vegetative and reproductive characteristics of *P. zonale* were significantly affected by the use of SWE and CT, applied as a single application or in combination, compared to the untreated control (Table 2). Plant height (Fig. 2a) was significantly affected by the treatments at $p < 0.05$, with values ranging from 22.33 cm in the control to 28.67 cm in SWE 4 + CT 4. Compared with the control, height increased by 10.9% in CT 2, 11.2% in CT 4, and 7.1% in SWE 2, while the combined treatments produced larger gains, especially SWE 4 + CT 2 and SWE 4 + HS 4, which increased height by 20.1% and 21.3%, respectively. The tallest plants were obtained under SWE 4 + CT 4, which was statistically superior to the control and several other treatments.

The number of leaves (Table 2) also differed significantly among treatments ($p < 0.05$). The control produced 25 leaves, whereas the highest value was recorded in SWE 4 + CT 4 with 36.67 leaves, representing a 46.7% increase.

SWE 2 + CT 2 and SWE 2 + CT 4 also showed significant increases in number of leaves (34.67), which represented increase of 38.7% over control., respectively. Interestingly, although the SWE 2 treated value was lowest 27

leaves in, this marginally exceeded the control by 8.0%.

Treatments had was a significant ($p < 0.05$) effect on branch number (Table 2) The control produced 3 branches, while the maximum was 8.33 in SWE 4 + CT 4 (a rise of around 177.8%). All treated plants had more branches than the control, with values generally ranging from 4.0 to 7.33. The SWE 2 + CT 2 treatment increased branching to 7.33, which was 144.4% higher than the control.

Flower number (Table 2) was also significantly affected at $p < 0.05$. The control produced 6.33 flowers, whereas SWE 4 + CT 4 recorded the highest value, 10.33 flowers, corresponding to a 63.2% increase. Intermediate increases were observed in SWE 2 + CT 2, SWE 2 + CT 4, and SWE 4 + CT 2, ranging from 8.33 to 8.67 flowers and exceeding the control by 31.6% to 36.8%. Overall, the combined seaweed and calyx tea treatments were more effective than the single applications.

Photosynthetic Pigments and Leaf Water Relations

The biochemical analysis showed significant treatment effects on photosynthetic pigments and leaf water status (Table 3). CT and SWE significantly increased chlorophyll content (Fig. 3a) compared with the control. The control plants had 1.331 mg g^{-1}. CT 2 increased chlorophyll to 2.133 mg g^{-1}, corresponding to a 60.3% increase. CT 4 further increased it to 2.901 mg g^{-1}, corresponding to a 117.9%. SWE 2 also increased chlorophyll to 3.046 mg g^{-1}, corresponding to a 128.8%, and the combined treatments maintained high values: SWE 2 + CT 2 had 3.187 mg g^{-1}, corresponding to a 139.3%, SWE 2 + CT 4 had 3.336 mg g^{-1}, corresponding to a 150.5%. The highest chlorophyll levels were observed with SWE 4 + CT 2 (3.411 mg g^{-1}), corresponding to a 156.3%, and SWE 4 + CT 4 (3.427 mg g^{-1}), corresponding to a 157.5%, compared with the control.

Table 2. Effect of seaweed and calyx tea on plant height, number of leaves, number of branches, and number of flowers of *Pelargonium zonale*.

Treatments	Plant height (cm)	Number of leaves	Number of branches	Number of flowers
Co.	22.33±0.57e	25±1d	3±0.40c	6.33±0.57c
CT 2	25.66±0.57cd	28.66±2.08cd	4±0.40c	7.33±0.57bc
CT 4	26±1bcd	32.66±1.52abc	6.33±0.57b	7.33±0.57bc
SWE 2	24±1de	27±1d	6.33±0.57b	7.66±0.57bc
SWE 2 + CT 2	27.66±0.57abc	34.66±1.52ab	7.33±0.57ab	8.33±0.57b
SWE 2 + CT 4	27.66±0.57abc	34.66±1.52ab	6.33±0.57b	8.66±0.57b
SWE 4	24.66±0.57d	31.66±1.52bc	6.66±0.57b	7.33±0.57bc

SWE 4 + CT 2	28±1ab	32±1bc	6.33±0.57b	8.66±0.57b
SWE 4 + CT 4	28.66±0.57a	36.66±1.52a	8.33±0.57a	10.33±0.57a

Carotene content (Table 3) followed a similar increasing trend. Relative to the control (0.306 mg g^{-1}), CT 2 increased carotene to 0.293 mg g^{-1} (4.2%). CT 4 decreased it to 0.262 mg g^{-1} (14.4%). In contrast, SWE 2 increased carotene to 0.229 mg g^{-1} (25.1%), while the strongest carotene increases relative to control were observed under the SWE + CT combinations, particularly SWE 2 + CT had 2 0.221 mg g^{-1} increased to (27.7%) and SWE 2 + CT 4 increase to 0.222 mg g^{-1} (27.4%), with SWE 4 + CT 4 had 0.160 mg g^{-1} (47.7%). Overall, carotene did not increase uniformly; rather, the data indicate that treatments generally reduced carotene compared with control, with significant differences among treatments.

Moisture content (Table 3) decreased from the control. The control moisture was 81.70%. CT 2 was slightly reduced to 80.28% (1.42%). CT 4 had 80.77% (1.1%). SWE 2 decreased moisture to 79.76% (2.4%). The largest reduction was seen under SWE 4 + CT 4, which had 76.70% (6.1%). While several intermediate treatments remained around 78–80%, all treatments were significantly different from each other by the Tukey test ($p < 0.05$), confirming that moisture content was substantially affected, especially by the higher SWE/CT combinations.

Dry matter (Table 3) generally increased relative to the control. Control plants had 18.27% dry matter. CT 2 increased to 19.22%, while CT 4 increased to 19.69%. SWE 2 increased dry matter to 20.24%. Combined treatments further increased dry matter: SWE 2 + CT 2 increased to 21.13%, SWE 2 + CT 4 increased to 21.42%, and SWE 4 had 21.43%. The highest dry matter values were achieved with SWE 4 + CT 2, which increased from 22.81% to 24.8%, and SWE 4 + CT 4, which increased from 23.28% to 27.4%. Thus, dry matter increased significantly in response to SWE and CT, with the greatest enhancement under the SWE 4 + CT4 treatment.

Nutrient Concentrations (N, P, and K)

Treatment effects were highly significant for nitrogen, phosphorus, and potassium concentrations in leaf tissues (Table 4). Seaweed and calyx tea treatments significantly affected leaf nutrient status. Nitrogen (N) ranged from 2.14 to 2.88 mg g^{-1}, with the highest value recorded in CT 2, which increased N by about 9.1% relative to the control, whereas SWE 2 produced the lowest N value, reducing it by about 18.9%. The combined treatments generally improved N compared with the control, especially SWE 2 + CT 4 and SWE 4 + CT 2, which reached 2.86 and 2.80 mg g^{-1}, respectively, corresponding to increases of about 8.3% and 6.1%.

Table 3. Effect of seaweed and calyx tea on chlorophyll content, carotene content, moisture content, and number of flowers of *Pelargonium zonale*.

Treatments	chlorophyll content ($mg\ g^{-1}$)	Carotene content ($mg\ g^{-1}$)	Moisture content (%)	Dry matter (%)
Co.	1.33±0.20d	0.30±0.01d	81.70±1.27c	18.26±1.26c
CT 2	2.13±0.08c	0.29±0.02d	80.28±1.26bc	19.22±0.48c
CT 4	2.90±0.04b	0.26±0.01cd	80.77±0.48bc	19.69±1.25bc
SWE 2	3.04±0.07ab	0.22±0.02b	79.75±1.06abc	20.23±1.07abc
SWE 2 + CT 2	3.18±0.06ab	0.21±0.02b	78.86±1.82abc	21.11±1.79abc
SWE 2 + CT 4	3.33±0.19ab	0.22±0.01b	78.56±1.46abc	21.42±1.46abc
SWE 4	3.09±0.06ab	0.20±0.05ab	78.56±0.89abc	21.43±0.88abc
SWE 4 + CT 2	3.41±0.25a	0.18±0.05ab	77.49±0.81ab	22.80±0.25ab
SWE 4 + CT 4	3.42±0.27a	0.16±0.01a	76.70±1.00a	23.28±0.99a

Phosphorus (P) was higher under all treatments compared with the control, averaging 0.36 mg g^{-1} in the control to 0.58 mg g^{-1} in SWE 4 + CT 2 and SWE 4 + CT 4 (61.1%). CT 2 produced the least increase in P of any treatment (5.6%), as the most pronounced responses under the mixed seaweed–calyx tea treatments, especially at the high seaweed levels. Potassium (K) showed the more positive response to treatment, increasing in all treated treatments compared with the control. The control recorded 3.63 mg g^{-1}, whereas the highest K content occurred in SWE 4 + CT 4 at 6.86 mg g^{-1}, an increase of about 89.0%.

Table 4. Effect of seaweed and calyx tea on nutrients of *Pelargonium zonale*.

Treatments	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)
Co.	2.64±0.22ab	0.36±0.01f	3.63±0.15e
CT 2	2.88±0.15a	0.38±0.01ef	4.62±0.09d
CT 4	2.32±0.12ab	0.42±0.02de	4.86±0.15d
SWE 2	2.14±0.10b	0.44±0.01cd	4.90±0.22d
SWE 2 + CT 2	2.65±0.38ab	0.48±0.01c	5.49±0.23c

SWE 2 + CT 4	2.86±0.05a	0.52±0.01b	6.30±0.10b
SWE 4	2.71±0.33ab	0.53±0.01b	6.60±0.10ab
SWE 4 + CT 2	2.80±0.10a	0.58±0.02a	6.40±0.10b
SWE 4 + CT 4	2.51±0.10ab	0.58±0.01a	6.86±0.05a

Discussion

The current study was designed to evaluate the hypothesis that a beneficial interaction between a seaweed extract and a hibiscus tea would result in greater improvements in *Pelargonium zonale* growth, physiology, and nutrient status than either input applied alone. Overall, the results support this expectation, but, more importantly, they suggest that the response cannot be explained by simply adding known nutrients or a single growth regulator acting in isolation. The data suggest a more complex biostimulant effect in which the two products might interact via complementary and potentially synergistic mechanisms, given that this is a relatively common theme in the biostimulant literature **[14]**.

The results are useful when interpreted separately focusing on individual, additive and synergistic effects. Both single applications monitored over the season may be considered individual effects, where a certain product provides its specific benefits for plant performance. The combined treatments showed a greater mean response than predicted from the independent effects of the separate components, indicating at least an additive interaction. For some traits, the best treatment was clearly superior to either of the single applications both in terms of absolute performance and by margins considerably greater than what would be indicated with simple addition (consistent with synergy). This distinction is critical however, because it changes the emphasis from individual variable-by-variable improvements to a mechanistic understanding of the possible function of two or more biologically active materials combined in a plant–soil system **[15]**.

Additionally, the better growth response in the combined treatments likely reflects complementary modes of action. It has been known for a long time that seaweed extracts contain complex mixtures of polysaccharides, amino acids and other bioactive matrix constituents including phytohormone-like compounds which promote cell division **[16]**; elongation, root development and stress buffering; while plant-derived teas similarly provide phenolics and organic acids which have been described as metabolite exudations **[17]** with inputs such as minerals as supporting factors to improve nutrient use availability or metabolic balance in the host plant **[17]**.

This explanation is supported by the increased plant height as well as combined treatments on number of leaves, branches and flowers. This is supported by a significantly higher functioning both of meristems and sources as evidenced by increased branching and leaf formation, in addition to enhanced flower number, which further implies that these treatments facilitated the transition from vegetative to reproductive growth **[18]**. The seaweed bioactive might have aided

organ initiation and enlargement, while hibiscus tea might have made the chemical environment around the root zone more favourable thus providing adequate nutrients that are necessary for rapid growth. Similar types of responses have also been observed for other ornamentals and horticultural crops when seaweed based biostimulants or plant-derived extracts are used, highlighting the hypothesis that growth promotion is more often than not a synergistic physiological response rather than simply the result of one key factor **[19]**. They are in agreement with Ali and Ramsubhag **[15]**, who found that seaweed extract promotes leaves generation and overall vegetative growth in ornamental plants.

The pigment data provide additional support for a combined mode-of-action interpretation. The significant increase in chlorophyll under the eutrophication and plant presence treatment combination indicates greater photosynthetic capacity, which correlates with increased biomass accumulation and flowering in those plants. Such an increase can indicate enhanced nitrogen uptake and improved chloroplast development, as well as reduced oxidative damage to the photosynthetic machinery **[20]**. In contrast, responses to carotenoids and water status should be interpreted more cautiously, as no consistent pattern emerged across all traits; the unevenness of this pattern is indicative of a multi-component biostimulant effect rather than a universal trait response **[21]**. Our results are in line with the study by Raja and Vidya **[22]**, which reported enhanced carotenoid content in plants treated with seaweeds, thereby boosting stress tolerance and photosynthesis rates.

The increases and decreases in water content and dry matter, respectively, indicate that the combined treatments impacted plant water relations and biomass partitioning, as well as the plants' facing a high internal water deficit **[23]**. Along with higher dry matter, this could suggest progression towards the accumulation of more structural biomass and osmotic adjustment. Seaweed extracts have been implicated in enhancing ion balance, water-use efficiency, and stress tolerance; organic plant extracts could provide releasable metabolites that may help maintain membrane stability and cellular homeostasis. The new pattern of the response observed here may be an emergent property of the mixture in time as application on one component can lead to interconnected physiopharmacological properties that cannot completely be accounted for by individual applications.

The progressive build-up of nutrients, particularly P and K, further supports the theory that both biostimulants enhanced assimilate uptake and/or translocation efficiency. Potassium was the most reactive of all elements which is critical because it plays a central role in osmotic regulation, enzyme activation, and stomatal activity **[24]**. When applied in combination, the treatment can exert a synergistic effect on root absorptive capacity membrane transport activity and rhizosphere conditions compared to those applied individually for greater nutrient acquisition. This pattern is in line with what was previously reported by the general literature, showing that seaweed-derived and plant-based biostimulants (PB) can increase nutrient use efficiency standards to their conective effect on roots, transporters and metabolic signalling cascade **[25]**.

This observation corresponds to the observations made by Jin et al. **[10]**, who proved that the addition of hibiscus tea improves nutrient availability and absorption in ornamental species.

Notably, the current work contributes to the field by investigating the role of seaweed extract and hibiscus tea applied together as foliar and fertigation treatments in *P. zonale*, while only a few studies have previously explored multiple biostimulants simultaneously. A design like this addresses a very relevant knowledge gap about how two biologically active, plant-based inputs work together—in parallel or with an enhanced activity area or additive effect when combined. The results indicate that the latter is more likely under the higher combined dose and thus support integrated biostimulant strategies on ornamental crop production **[26]**.

The study also cautions against jumping to the conclusion that the response can be attributed to a single class of compounds. While this is consistent with a joint action of phytohormone-like substances, phenolics, organic acids, and amino compounds, and mineral elements present together in the two materials contributing to beneficial effects, there may also be an additive interaction in how these compounds act after co-application to modulate signalling and availability of nutrients and stress physiology. This interpretation aligns with recent conceptual outlines, which state that finished biostimulant products tend to function as systems rather than mere sums of standalone active principles **[14]**.

Conclusion

The combined application of seaweed extract and hibiscus tea produced better overall results than either biostimulant applied alone, confirming that the integrated treatment was more effective for improving the performance of *Pelargonium zonale*. The most effective treatment, SWE 4 g l^{-1} + CT 4 g l^{-1}, positively affected plant height, leaf number, branch number, flower number, chlorophyll content, dry matter, and phosphorus and potassium uptake, thereby improving plant growth and nutritional status. The findings highlight that combining plant- and seaweed-sourced biostimulants may offer a feasible, sustainable approach to improve ornamental quality whilst weaning reliance on traditional chemical inputs. Further work is needed to clearly elucidate the physiological and molecular mechanisms responsible for the synergistic effects, test the treatment under field and commercial production conditions, and assess its consistency across other ornamental species as well as growing environme

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