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Research Article

Enhancing the Growth and Flowering Attributes of *Polianthes tuberosa* L. through Environmentally Friendly Treatments

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Abstract:

Improving the growth and productivity of crops, including ornamental bulbs, via bio-stimulants is a very imperative topic since plant bio-stimulants have been recognized among the best agricultural practices. *Polianthes tuberosa* L. (Tuberose) is one of the most important flowering bulbs crop worldwide due to the excellent fragrance of spike florets. Seaweed and humic acid are among the multifunctional bio stimulants that improve the plant development. However, their impact on the production of flowering bulbs including tuberose is not well-investigated. Hence, the current study aimed to bridge this gap. The objective of this study was to investigate the effects of exogenous application of seaweed and humic acid on the growth and flowering characteristics of tuberose. Seaweed was applied at 0, 0.5, 1 and 2 mL L^{-1} however, humic acid was used at 0, 0.2, 0.4 and 0.8 g L^{-1}. The results showed that the leaf length, number of leaves, spike length, spike diameter, number of florets as well as fresh and dry weights of spikes were markedly enhanced as a result of applying seaweed or humic acid treatments compared to the control. Both applications decreased the period required until flower beginning relative to the control. Further, the total chlorophyll content, carotenoids, total carbohydrates and endogenous phytohormones were also improved due to seaweed or humic acid applications. Applying seaweed at 2 mL L^{-1} combined with humic acid at 0.4 g L^{-1} was the most effective treatment. Seaweed and humic acid may be applied as promise and ecofriendly bio-stimulants to enhance the growth and productivity of tuberose.

1. Introduction

Tuberose (*Polianthes tuberosa* L.) is very important tropical ornamental flowering bulbs belonging to the Agavaceae family cultivated for production of long-lasting flower spikes (Alan et al., 2007). Tuberose florets have attractive and elegant appearance with sweet fragrance (Patel et al., 2006). Therefore, this species has a considerable economic potential, principally in both cut flower market and volatile oil industry (Alan et al., 2007). Nutrition is the vital factor in enhancing the growth and increasing the yield and quality of spikes in tuberose since it requires a large quantity of fertilizers (Archana et al., 2019). However, the excessive application of chemical fertilizers causes serious impacts, such as polluting the agro-ecosystem, soil fertility deterioration and higher production costs (Kahil et al., 2017).

Recently, production of chemical-free crops has gained the attention of researchers worldwide to guarantee the safety and quality (Ali et al., 2018). Therefore, several applications have been established such as bio-stimulants and non-traditional fertilizers (Hassan and Fetouh, 2019 and Hassan et al., 2020). It has been found that bio-stimulants can promote plant growth and enhance the productivity in several plants, which supports their use as plant growth promoters (Mazrou et al., 2021). Seaweeds (SW) are among these environmentally friendly bio-stimulants. SW are macroscopic, multicellular organisms primarily found in marine environments. SW have been utilized in several forms such as compost, mulch, and extracts to enhance plant growth performance and productivity. SW can be used as fertilizer since it contains polysaccharides, micro-and macronutrients, sterols, N-containing compounds such as betaines, and hormones like gibberellin, cytokinin and auxins as potentially bio-active compounds and can act on plant and soil (De Clercq et al., 2023). Moreover, SW application is considered an economic and ecofriendly biostimulant (Abou El-Ghait et al., 2021). SW treatment increases the growth by stimulating the root growth, vegetative branches and also early flowering that reflected in increasing the yield (Battacharyya et al., 2015). It has been reported that SW markedly enhanced the growth and flowering of *Tagetes erecta* L. (Sridhar and Rengasamy, 2010), *Dahlia pinnata* L. (El-Alsayed et al., 2018) and *Freesia hybrid* L. (Abd Al-Karimjassim and Radhi, 2019).

Humic acid (HA) is another commercial product which is produced by decaying organic compounds and has been used as biostimulant. It contains essential elements which improve soil fertility and reduce soil nutrient deficiency. Furthermore, HA enhances root growth and increases water availability and nutrients by forming chelates of various elements (Pandya et al., 2023). HA treatments increase cation exchange capacity and in the same time enhance soil structure, aggregation, water permeability, fertility, aeration, moisture retention, and microbial activity (Mohamed, 2012). Nikbakht et al. (2008) reported that HA had beneficial impacts on nutrient uptake, and was particularly important for the availability and transport of nutrients. It has been reported that HA treatment markedly enhanced the growth traits and flowering characters in *Gazania splendens* L. (Khudair and Abdul Albbas, 2021). Additionally, foliar application of HA enhances vegetative and flowering traits, improved leaf pigments content and both photosynthesis rate and transpiration rate in *Gladiolus* (Baldotto and Baldotto, 2013), *Chrysanthemum* (Fan et al., 2014) and *Calendula officinalis* L. (El-Nashar, 2021).

Recently, the use of SW and HA has emerged as a promising strategy, which may enhance plant growth and productivity, making them a valuable application to modern cultivation of tuberose. Despite the importance of both SW and HA in enhancing the growth and productivity of several ornamental species, their impact on *Polianthus tuberosa* L. has not been well investigated. Additionally, research on SW and HA application specifically on ornamental bulbs is limited. Therefore, the aim of this experiment was to evaluate the impact of SW and HA on growth performance and flowering characteristics of *Polianthus tuberosa* L.

2. Materials and Methods

2.1. Experimental site and soil analysis

In this study, two separate field experiments were conducted at the Experimental farm of the Faculty of Agriculture, Menoufia, University during two successive seasons of 2022 and 2023. This investigation aimed to study the impact of seaweed extract and humic acid applications on the growth, flowering and chemical constituents of *Polyanthus tuberosa* L. The soil texture was clay loamy and its physical and chemical characteristics were investigated according to the methodology of Jackson (1967) and were recorded in Table (A). The soil was prepared and a constant doses of calcium super phosphate 150 kg/ fed (15.5 % P_2O_5) was added during soil preparation in each growing season. The soil was divided into plots 2 × 2 m^2 and each plot contains three rows. Each row contained 6 plants and consequently each plot contained 18 plants.

Table A. Physical and chemical properties of the experimental soil.

Soil property	**Value**
Particle size distribution	
Coarse sand (%)	3.84
Fine sand (%)	27.40
Silt (%)	44.23
Clay (%)	23.20
Texture class	Clay loamy
Field capacity (%)	38.80
Chemical analysis	
EC (dS mt 25°C)	0.40
pH	7.90
Total $CaCO_3$ (%)	2.32
C.E.C (mg/100g)	25.60
Total N	0.12
Total P_2O_5 (%)	0.26
K^+ (mg/100g)	0.12
Ca^{++} (mg/100g)	0.42
Mg^{++} (mg/100g)	0.68
Na^+ (mg/100g)	0.62

2.2. Experimental setup and treatments

Tuberose bulbs about 80-90 g from a local cultivar were obtained from a commercial grower and transported to the experimental farm and cultivated on 28th of March in each growing season. After one month of cultivation, the treatments of seaweed extract and humic acid were begun. Plants were foliar sprayed with seaweed extract (SW) at 0, 0.5, 1.0 and 2.0 mL L^{-1} while humic acid (HA) was applied at 0, 0.2, 0.4 and 0.8 g L^{-1}. The treatments were arranged as a factorial experiment (4 × 4) in randomized complete block design. The exact of each concentration of SW and HA was prepared using distilled water and Tween-20 surfactant 0.1% (v/v) was added and the spraying was applied until run off point. The spraying was repeated three times at one-month intervals. Control plants were foliar sprayed with tap water contained the same surfactant. The other agricultural practices such as irrigation and weed control were applied as recommended by Agriculture Research Center, Ministry of Agriculture, Egypt.

2.3. Data collected

At the flowering stage in each season, the Vegetative growth parameters (leaf length and leaf number per plant) were recorded. The Flowering parameters (Spike length, Spik diameter, floret number per spike, spike FW, spike DW and days till flowering) were also investigated. In order to evaluate the dry weight, samples were oven dried at 70 ºC for 72 hours until constant weight and kept as powder for chemical analysis.

2.4. Determination of photosynthetic pigments

Samples of 0.2 g were used for chlorophyll extraction from tuberose leaves using acetone solvent (80%) as reported by Metzner *et al.* (1965). Then, the extracts were centrifuged at 15,000 *g* for 10 min and monitored at 663 and 645 nm using a spectrophotometer (ST150SA Model 7205, Cole-Parmer Ltd. Stone, Staffs, UK). The equations reported by Lichtenthaler (1987) were used to calculate the contents of chlorophyll a and b as follows:

$$\text{Chl a} = 12.25.A_{663} - 2.79.A_{647}$$

$$\text{Chl b} = 21.50.A_{647} - 5.10.A_{663}$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b}$$

Since A_{663} and A_{647} are the optical density at 663 and 647 nm wavelengths of, respectively. To calculate the total chlorophyll, both values were combined and reported as mg g^{-1} FW.

$$\text{Car} = 1000 \times A_{470} - 2.27 \times \text{Chl a} - 81.4 \times \text{Chl b}/227.$$

Where: A_{645}, A_{662} and A_{470} are the optical density at 645 nm, 662 nm and 470 nm wavelengths, respectively.

2.5. Total Carbohydrates determination

The percentage of total carbohydrate in the tuberose dried leaves was assessed according to Herbert et al. (1971).

2.6. Mineral content

Leaf samples were oven dried at 70 ˚C for 48 h and then were milled to obtain suitable powder for NPK analysis. Samples were digested in sulphuric and perchloric acids method (Piper, 1967; Jackson, 1978) for nutrient analysis. The micro-Kjeldhl methodology was used to determine Nitrogen as reported by Black et al. (1965), phosphorus content was color metrically investigated at 660 nm as reported by Jackson (1978), while flame photometer was used to measure potassium content (Jackson, 1978).

2.7. Endogenous phytohormones determination

Gibberellic acid (GA_3), Indole-3-acetic acid (IAA), kinetin (Kin.) and Abscisic acid (ABA) assessment was implemented following the procedure described by Wasfy et al. (1974).

2.8. Statistical analysis

The collected results in each season were statistically analyzed and analysis of variance (ANOVA) was performed using Michigan Statistical Program Version C (MSTATC). Means were compared by Least significant difference (L.S.D.) at 0.05 probability level as reported by Snedecor and Cochran (1980).

3. Results and discussion

3.1. Vegetative growth

The leaf length and leaf number of tuberose plants were significantly increased due to SWand HA treatments compared to the control in both experimental seasons (Table 1). The leaf length was gradually increased with increasing SW level and recorded maximum values (58.33 and 44.66 cm) by applying the highest level in both seasons, respectively. Also, increasing HA level resulted in significant and gradual increase in leaf length and the tallest leaves were obtained by applying the treatment of HA_2 in both seasons. Additionally, the interaction between SW and HA treatments showed a positive effect on leaf length of tuberose plants. The tallest leaves were obtained by the treatment of SW_3HA_2 which recorded 59.33 and 52.66 cm in both seasons, respectively.

The impact of SW and HA on number of leaves showed a similar trend to leaf length since the leaf number was markedly increased as a result of SW and HA applications in both seasons relative to untreated plants (Table 1). The leaf number was gradually increased with increasing SW level and recorded highest number (21.06 and 24.66) by applying the highest level in both seasons, respectively. Similarly, increasing HA level resulted in significant and gradual increase in leaf number and the highest number of leaves were recorded by applying the treatment of HA_3 in both seasons. Furthermore, the combination between SW and HA treatments exhibited a positive impact on leaf number of tuberose plants. the treatment of SW_3HA_2 resulted in the highest leaf number (27.66 and 29.50) in both seasons, respectively.

The effective role of SW in enhancing the growth of tuberose plants could be explained through its vital role in promoting cell division due to its content of several hormones like cytokines, indole acetic acid and GA_3. Moreover, it contains also amino acids, vitamins and essential nutrient elements which able to enhance the vegetative growth (James, 1994 and Soliman et al., 2000). The current results are in agreement with those reported by El-Alsayed et al. (2018) on *Dahlia pinnata* L. and Abd Al-Karimjassim and Radhi (2019) on *Freesia hybrid* L. The beneficial impacts of SW on growth were reported in several ornamental species such as rose (Sumangala et al., 2019), and hydrangea (De Clercq et al., 2023) which support the current findings. It is well known that HA application increases the nutrient uptake as well as the availability and transport of essential elements (Nikbakht et al., 2008 and Pandya et al., 2023) which may reflect in growth promotion of tuberose plants. In accordance with our results, Khudair and Abdul Albbas (2021) reported that HA treatment markedly enhanced the growth traits in *Gazania splendens* L. Additionally, the positive impact of HA was also reported in *Tulipa gesneriana* (Ali et al., 2014), *Chrysanthemum* (Fan et al., 2014) and *Calendula officinalis* L. (El-Nashar, 2021).

Table 1. Effect of seaweed (SW) and humic acid (HA) applications on leaf length and number of leaves of *Polianthes tuberosa* L.

Treatments	1st season		2nd season	
	Leaf length(cm)	Leaf number/plant	Leaf length(cm)	Leaf number/plant
SW_0	40.33	14.66	40.66	15.62
SW_1	50.66	16.33	41.33	18.43
SW_2	56.66	19.66	43.56	22.33
SW_3	58.33	21.06	44.66	24.66
LSD 5%	1.174	0.4707	0.749	0.3699
HA_0	40.33	14.66	40.66	15.62
HA_1	45.66	17.33	43.33	19.36
HA_2	46.40	19.33	44.33	20.63
HA_3	41.33	20.33	37.88	21.87
LSD 5%	2.266	0.8140	0.492	0.475
SW_0HA_1	48.66	17.33	43.33	19.36
SW_0HA_2	40.38	19.33	44.46	22.20
SW_0 HA_3	41.33	20.33	37.34	22.63
SW_1 HA_1	54.15	21.66	45.66	22.16
SW_1 HA_2	4622	23.76	44.66	24.66
SW_1 HA_3	43.39	22.66	42.66	24.40
SW_2 HA_1	53.66	23.66	41.33	25.33
SW_2 HA_2	56.60	25.2	46.24	26.86
SW_2 HA_3	49	25.86	48.66	26.30
SW_3 HA_1	52.33	25.33	46.25	27.86
SW_3 HA_2	59.33	27.66	52.66	29.50
SW_3 HA_3	46.66	26.66	38.61	29.31
LSD 5%	4.532	1.628	0.984	0.95

SW_0, SW_1, SW_2 and SW_3 means seaweed extract at 0, 0.5, 1.0 and 2.0 mL L^{-1} while HA_0, HA_1, HA_2 and HA_3 means humic acid at 0, 0.2, 0.4 and 0.8 g L^{-1}, respectively.

3.2. Flowering traits

Data presented in Table (2) clearly show that all SW levels significantly enhanced spike length and spike diameter compared to control plants in both experimental seasons. Moreover, SW application markedly reduced the period required until flowering in comparison with untreated plants. The spike length and spike diameter were gradually increased with increasing SW level and recorded their maximum values (73.66 and 0.68 cm) in the first season and (72.30 and 0.73 cm) in the second one, respectively by applying the highest level (SW_3). This treatment also resulted in the minimum days required for the flower beginning (79 and 82 days) compared to the control that recorded (87 and 103 days) in both seasons, respectively. Similarly, increasing HA level resulted in significant and gradual increases in spike length and spike diameter and the highest values were obtained by applying the treatment of HA_2 in both seasons. Increasing the level from HA_2 to HA_3 had no beneficial impact on both parameters (Table 2). The minimum days required until flower beginning (79 and 91 days) in both experimental seasons, respectively. Otherwise, the combination between SW and HA treatments showed a positive impact on flowering traits of tuberose plants. The best interaction treatment in this respect was SW_3HA_2 which markedly increased both spike length and its diameter compared to the other treatments in both seasons. Further, this treatment also induced the early flowering and markedly minimized the days until flower beginning in both seasons.

Table 2. Effect of seaweed (SW) and humic acid (HA) on Spike length, spike diameter and days till flowering of *Polianthes tuberosa* L. plant.

Treatments	1st season			2nd season		
	Spike length (cm)	Spike diameter (cm)	Days till flowering	Spike length (cm)	Spike diameter (cm)	Days till flowering
SW_0	64.73	0.55	87	55.66	0.55	103
SW_1	67.87	0.59	87	58.53	0.61	99
SW_2	71.66	0.63	87	65.06	0.68	87
SW_3	73.66	0.68	79	72.30	0.73	82
LSD 5%	0.915	0.0059	1.56	1.209	0.017	1.64
HA_0	64.73	0.55	87	55.66	0.55	103
HA_1	74.73	0.58	87	59.10	0.61	101
HA_2	78.93	0.66	79	64.83	0.66	91
HA_3	79.26	0.65	82	65.33	0.67	92
LSD 5%	0.658	0.0066	1.44	0.920	0.0223	1.59
SW_0HA_1	74.73	0.58	87	59.10	0.61	103
SW_0HA_2	78.93	0.66	79	64.83	0.66	103
SW_0 HA_3	79.66	0.65	87	65.33	0.67	90
SW_1 HA_1	76.56	0.63	73	64.66	0.68	90
SW_1 HA_2	78.33	0.71	72	69.33	0.73	74
SW_1 HA_3	80.66	0.70	73	70.27	0.74	83
SW_2 HA_1	78.33	0.68	73	68.53	0.74	80
SW_2 HA_2	82.33	0.75	71	76.66	0.78	77
SW_2 HA_3	81.66	0.74	87	77.33	0.78	79
SW_3 HA_1	81.20	0.69	70	74.10	0.79	82
SW_3 HA_2	83.33	0.78	68	86.33	0.86	75
SW_3 HA_3	83.43	0.79	71	87.37	0.86	80
LSD 5%	1.317	0.133	2.11	1.84	0.446	2.34

SW_0, SW_1, SW_2 and SW_3 means seaweed extract at 0, 0.5, 1.0 and 2.0 mL L^{-1} while HA_0, HA_1, HA_2 and HA_3 means humic acid at 0, 0.2, 0.4 and 0.8 g L^{-1}, respectively.

The results of this study also indicate that all SW, HA and their interaction treatments significantly enhanced floret number per spike, spike fresh and dry weights in comparison with the control in both seasons (Table 3). A gradual and significant increase was observed in these flowering characters due to increasing SW level and the maximum values were obtained by SW_3 treatment (2.0 mL L^{-1}). By applying this treatment the floret number per spike, spike fresh and dry weights were increased by 43.65, 37.34 and 59.62 % in the first season and by 35.55, 28.13 and 50.97 % in the second one, respectively relative to the control. Similarly, increasing HA levels resulted in a gradual increase in floret number per spike, spike fresh and dry weights in comparison with the control in both seasons. The treatment of HA_2 (0.4 g L^{-1}) recorded the highest values of these traits in both seasons. Otherwise, increasing HA level to 0.8 g L^{-1} had no impact on improving these characters relative to 0.4 g L^{-1} level (Table 3). Relative to the control, applying HA_2 treatment increased the floret number per spike, spike fresh and dry weights by 37.24, 20.16 and 35.28 % in the first season and by 35.60, 23.55 and 45.33 % in the second one, respectively. A positive impact on floret number per spike, spike fresh and dry weights was detected due to the combination between SW and HA treatments. The highest values of these traits were obtained SW_3HA_2 treatment in both seasons.

Table 3. Effect of seaweed (SW) and humic acid (HA) on floret number/spike, spike fresh weight (FW) and spike dry weight (DW) of *Polianthes tuberosa* L. plant.

Treatments	1st season			2nd season		
	Floret number/spike	Spike FW (g)	Spike DW (g)	Floret number/spike	Spike FW (g)	Spike DW (g)
SW_0	14.66	38.83	5.30	21.29	38.63	5.14
SW_1	16.33	43.96	6.70	18.43	42.83	5.86
SW_2	19.66	47.70	7.50	24.53	46.33	6.63
SW_3	21.06	53.33	8.46	28.86	49.50	7.76
LSD 5%	0.54	0.43	0.050	2.26	0.35	0.101
HA_0	14.66	38.83	5.30	21.29	38.63	5.14
HA_1	17.33	42.63	5.66	23.97	43.4	5.95
HA_2	20.12	46.66	7.83	28.87	47.73	7.47
HA_3	19.87	46.33	7.17	27.97	47.50	6.52
LSD 5%	0.53	0.68	0.07	2.09	0.61	0.13
SW_0HA_1	17.33	42.63	5.66	23.97	43.4	5.95
SW_0HA_2	19.33	46.33	5.83	28.87	47.50	6.47
SW_0 HA_3	20.33	46.66	5.87	23.97	48.53	6.52
SW_1 HA_1	21.66	47.50	6.95	22.63	46.56	5.52
SW_1 HA_2	23.77	53.80	7.84	29.77	52.43	7.82
SW_1 HA_3	22.66	54.33	7.82	24.07	53.43	7.86
SW_2 HA_1	23.66	50.8	7.8	27.53	51.30	7.60
SW_2 HA_2	25.20	62.70	9.1	23.97	58.36	8.91
SW_2 HA_3	25.87	61.33	9.10	26.63	59.23	8.93
SW_3 HA_1	25.33	54.60	8.18	23.35	55.03	8.25
SW_3 HA_2	27.66	68.66	9.60	26.30	65.30	9.62
SW_3 HA_3	26.54	68.11	9.52	22.17	64.46	8.98
LSD 5%	1.07	1.37	0.15	4.53	1.23	0.23

SW_0, SW_1, SW_2 and SW_3 means seaweed extract at 0, 0.5, 1.0 and 2.0 mL L^{-1} while HA_0, HA_1, HA_2 and HA_3 means humic acid at 0, 0.2, 0.4 and 0.8 g L^{-1}, respectively.

The current findings indicate that SW application is effective in supplying tuberose plants with adequate levels of essential nutrients and hormones (auxins, cytokines, and gibberellins) for biosynthesis (Soliman et al., 2000), which promote the growth and enhancing the flower characteristics. It has been reported that hormones like cytokinin is essential for the generation of more inflorescence meristematic cells (Blanchard and Runkle, 2008). In the same direction with current results, El-Alsayed et al. (2018) on *Dahlia pinnata* L. and Abd Al-Karimjassim and Radhi (2019) on *Freesia hybrid* L. reported similar findings. Additionally, the flowering attributes have been enhanced in several ornamental species due to SW application (Sumangala et al., 2019 on rose and De Clercq et al., 2023 on hydrangea). The positive impacts of SW on the flowering traits and inducing early flowering were previously observed in roses (Khan et al., 2009) and *Iris tingitana* cv. Wedgewood (Abdel-said et al., 2018). Similarly, the positive effect of HA on enhancing the flowering characters may be ascribed to the enhancement of essential nutrient uptake as well as the availability and transport of nutrients (Nikbakht et al., 2008). Hence, enhanced the vegetative growth which may reflected in improving the flowering attributes of tuberose plants which align with the reports of Ahmad et al. (2013) and Baldotto and Baldotto (2013) on gladiolus and Khodakhah et al. (2014) on tuberose. In agreement with current results, Pandya et al. (2023) found that solely application of HA or combined with SW enhanced the flowering attributes of *Callistephus chinensis* L. and induced the early flowering. The current results support the previous reports of Khudair and Abdul Albbas (2021) on *Gazania splendens* L., Ali et al. (2014) on *Tulipa gesneriana* and Ibrahim et al. (2016) on *Limonium sinuatum,* L. plants.

3.3. Chemical constituents

3.3.1. Photosynthetic pigments and total carbohydrates

The total chlorophyll, carotenoids and total carbohydrates were significantly enhanced due to SW and HA treatments compared to the control in both experimental seasons (Table 4). The total chlorophyll and carotenoids were gradually increased with increasing SW level and recorded maximum chlorophyll (1.99 and 2.09 mg g^{-1} FW) and carotenoids (0.46 and 0.47 mg g^{-1} FW) values by applying the highest SW level in both seasons, respectively. Similarly, increasing HA level resulted in a significant increase in total chlorophyll and carotenoids and the highest values were obtained by applying the treatment of HA_2 in both seasons. The current results also revealed that the total carbohydrate percentages were significantly increased as a result of SW and HA applications. The maximum carbohydrate percentages were recorded when tuberose plants were foliar sprayed with SW at 2.0 mL L^{-1} or HA at 0.4 g L^{-1} in both seasons. Furthermore, the interaction between SW and HA treatments showed a positive effect on total chlorophyll, carotenoids and total carbohydrates of tuberose plants. The highest values were obtained by the treatment of SW_3HA_2 in both seasons (Table 4).

The beneficial effects of SW in enhancing total chlorophyll and carotenoids in current investigation may be attributed to a reduction in chlorophyll degradation (Whapham et al., 1993). It has been found that the betaines in SW enhance the chlorophyll content in leaves (Blunden et al., 1996). Additionally, the adequate levels of essential nutrients particularly Mg and hormones (auxins, cytokines, and gibberellins) in SW may be able to motivate the plant metabolism and therefore increasing the photosynthetic pigments and the biosynthesis of carbohydrates. In agreement with current findings, Abdel-said et al. (2018) on *Iris tingitana* cv. Wedgewood and De Clercq et al. (2023) on hydrangea observed similar trend. The positive impact of SW on enhancing the photosynthetic pigments was previously observed in *Dahlia pinnata* L. (El-Alsayed et al., 2018) and *Iris tingitana* cv. Wedgewood (Abdel-said et al., 2018). Enhancing total chlorophyll, carotenoids and total carbohydrates due to HA treatment may be attributed to the fact that HA enhances the nutrient uptake as well as transport (Nikbakht et al., 2008) and therefore resulted in vegetative growth promotion which may participate in biosynthesis of more chlorophyll, carotenoids and carbohydrates. Khodakhah et al. (2014) on tuberose found that HA application markedly increase the photosynthetic pigments which in accordance with current results. Furthermore, Pandya et al. (2023) found that solely application of HA or combined with SW enhanced the photosynthetic pigments of *Callistephus chinensis* L.

Table 4. Effect of seaweed (SW) and humic acid (HA) application on total chlorophyll, carotenoids and total carbohydrates of *Polianthes tuberosa* L.

Treatments	1st season			2nd season		
	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Total carbohydrates (%)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Total carbohydrates (%)
SW_0	1.25	0.24	13.68	1.29	0.27	14.33
SW_1	1.32	0.29	14.92	1.38	0.33	15.55
SW_2	1.76	0.37	15.37	1.48	0.39	16.67
SW_3	1.99	0.46	17.36	2.09	0.47	18.25
HA_0	1.25	0.24	13.68	1.29	0.27	14.33
HA_1	1.3	0.26	13.73	1.32	0.29	14.62
HA_2	1.35	0.27	13.87	1.4	0.30	14.86
HA_3	1.33	0.27	13.84	1.37	0.31	14.83
SW_0HA_1	1.3	0.26	13.73	1.32	0.29	14.62
SW_0HA_2	1.35	0.27	13.87	1.4	0.30	14.83
SW_0 HA_3	1.33	0.27	13.84	1.37	0.31	14.86
SW_1 HA_1	1.38	0.32	14.97	1.44	0.36	15.74
SW_1 HA_2	1.43	0.34	15.04	1.51	0.38	16.32
SW_1 HA_3	1.4	0.33	15.02	1.51	0.38	16.29
SW_2 HA_1	1.82	0.38	15.61	1.93	0.44	16.89
SW_2 HA_2	1.88	0.41	15.93	1.97	0.47	17.11
SW_2 HA_3	1.85	0.40	16.11	1.95	0.45	17.03
SW_3 HA_1	2.07	0.47	17.49	2.17	0.53	18.67
SW_3 HA_2	2.17	0.49	17.54	2.29	0.57	18.79
SW_3 HA_3	2.12	0.48	17.53	2.23	0.56	18.7

SW_0, SW_1, SW_2 and SW_3 means seaweed extract at 0, 0.5, 1.0 and 2.0 mL L^{-1} while HA_0, HA_1, HA_2 and HA_3 means humic acid at 0, 0.2, 0.4 and 0.8 g L^{-1}, respectively.

3.3.2. N, P and K percentages

The application of all SW and HA treatments enhanced the percentages of N, P and K in tuberose leaves relative to untreated plants in both seasons (Table 5). Plants treated with SW_3 (2.0 mL L^{-1}) resulted in the highest percentages of these elements since it recorded 2.26, 0.38 and 2.34 % in the first season and 2.18, 0.42 and 2.23 % in the second one, respectively. HA at the level of 0.8 g L^{-1} (HA_2) resulted in the highest N, P and K percentages in both seasons compared to the other levels. When the application of SW was combined with HA treatment the effect was better than solely application. Therefore, the maximum N, P and K percentages were observed when tuberose plants were foliar sprayed with SW at 2.0 mL L^{-1} and combined with HA at 0.4 g L^{-1} in both seasons. On the other hand, control plants recorded the lowest values in this respect in both seasons.

The beneficial effect of SW in increasing the nutrient content of tuberose plants may be ascribed to its content of several essential elements which able to enhance the endogenous nutrient content (James, 1994 and Soliman et al., 2000) and motivate the absorbance and translocation of elements, hence improving the growth that may motivate more nutrients. The same effect of SW in improving the nutrient content has been previously reported in *Iris tingitana* cv. Wedgewood (Abdel-said et al., 2018) and hydrangea (De Clercq et al., 2023). The positive impact of SW on enhancing the photosynthetic pigments was previously observed in *Dahlia pinnata* L. (El-Alsayed et al., 2018) and *Iris tingitana* cv. Wedgewood (Abdel-said et al., 2018). It is widely accepted that HA application increases the nutrient uptake as well as the availability and transport of essential elements (Nikbakht et al., 2008 and Pandya et al., 2023) which may reflect in endogenous nutrient content in plants. The application of humic substances might interact with the phospholipid structures of cell membranes, acting as carriers for elements, which enables their transport into the plant cells (Ulukan, 2008). In accordance with our results, Khudair and Abdul Albbas (2021) reported that HA treatment markedly enhanced the nutrient content in *Gazania splendens* L. Additionally, the positive impact of HA was also reported in *Tulipa gesneriana* (Ali et al., 2014), *Chrysanthemum* (Fan et al., 2014) and *Calendula officinalis* L. (El-Nashar, 2021).

Table 5. Effect of seaweed (SW) and humic acid (HA) application on N, P and K percentage of *Polianthes tuberosa* L.

Treatments	1st season			2nd season		
	N %	P%	K%	N %	P%	K%
SW_0	1.88	0.27	1.97	1.76	0.28	1.78
SW_1	1.97	0.32	1.99	1.89	0.35	1.86
SW_2	2.13	0.36	2.26	2.09	0.39	1.98
SW_3	2.26	0.38	2.34	2.18	0.42	2.13
HA_0	1.88	0.27	1.97	1.76	0.28	1.78
HA_1	1.93	0.29	1.99	1.83	0.30	1.83
HA_2	1.96	0.30	2.03	1.89	0.33	1.85
HA_3	1.96	0.30	2.02	1.94	0.31	1.19
SW_0HA_1	1.93	0.29	1.99	1.83	0.30	1.83
SW_0HA_2	1.96	0.30	2.03	1.89	0.33	1.85
SW_0 HA_3	1.96	0.30	2.02	1.94	0.31	1.19
SW_1 HA_1	2.04	0.35	2.09	1.96	0.37	1.93
SW_1 HA_2	2.09	0.37	2.12	2.01	0.38	1.98
SW_1 HA_3	2.07	0.36	2.11	2.03	0.38	1.96
SW_2 HA_1	2.21	0.37	2.29	2.13	0.42	2.03
SW_2 HA_2	2.28	0.39	2.35	2.19	0.45	2.08
SW_2 HA_3	2.25	0.39	2.34	2.20	0.44	2.05
SW_3 HA_1	2.29	0.40	2.36	2.25	0.44	2.19
SW_3 HA_2	2.31	0.44	2.39	2.37	0.48	2.26
SW_3 HA_3	2.30	0.42	2.38	2.35	0.46	2.25

SW_0, SW_1, SW_2 and SW_3 means seaweed extract at 0, 0.5, 1.0 and 2.0 mL L^{-1} while HA_0, HA_1, HA_2 and HA_3 means humic acid at 0, 0.2, 0.4 and 0.8 g L^{-1}, respectively.

3.3.3. Endogenous phytohormone content

Foliar application of SW and HA enhanced the endogenous contents of GA_3, IAA and Kin. and reduced in the same time the content of ABA in tuberose leaves compared to the control (Table 6). The impact of SW in this respect was higher than SW treatment. Application of SW_3 treatment recorded 1202.41, 523.96 and 2341.78 µg/100g FW for GA_3, IAA and Kin., respectively. Otherwise, this treatment also recorded 26.49 µg/100g FW for ABA. Additionally, the interaction between SW and HA treatments showed a positive effect in this respect. The interacted treatment of SW_3HA_2 resulted in the highest values of these plant growth promotion substances (GA_3, IAA and Kin.) and the lowest value of plant growth retardant (ABA). Foliar application with SW or HA in current study increased the phytohormone levels in the tuberose leaves, and this result is novel. This result might be ascribed to the induction of mineral nutrients caused by SW and HA which are needed for both protoplasm and phytohormone formation (Semida et al., 2019). Furthermore, SW itself is rich in phytohormones and therefore it may induce the endogenous phytohormone biosynthesis (James, 1994 and Soliman et al., 2000). In agreement with these results, other biostimulants have been reported to enhance the endogenous phytohormone levels in several species (Semida and Rady, 2014; Moussa et al., 2024). Therefore, we suggest that the promoted endogenous hormones efficiently enhanced tuberose growth and flower productivity observed in this research. Our findings are supported by the fact that growth regulators exhibited a promotional impact in tuberose (Amin et al., 2017; Harshita and Vijay, 2024). Based on the above, we suggest that elevated phytohormone levels in response to SW or HA treatment largely contributed to the observed enhancement in the tuberose growth and spike productivity.

Table 6. Effect of seaweed (SW) and humic acid (HA) application on Gibberellic acid (GA_3), Indole-3-acetic acid (IAA), kinetin (Kin.) and Abscisic acid (ABA) content of *Polianthes tuberosa* L.

	GA_3	**IAA**	**KIN.**	**ABA**
	µg/100g FW			
Control	930.72	355.68	1840.28	48.46
SW_3	1202.41	523.96	2341.78	26.49
HA_2	1089.55	412.32	2197.46	42.40
SW_1HA_2	962.84	369.42	1942.21	44.97
SW_2HA_2	1229.30	603.26	2457.82	25.78
SW_3HA_2	1278.82	660.47	2520.98	22.23

SW_1, SW_2 and SW_3 means seaweed extract at 0.5, 1.0 and 2.0 mL L^{-1} , respectively while HA_2 means humic acid at 0.4 g L^{-1}.

5. Conclusions

The results of current investigation revealed that growth and flowering traits of tuberose were markedly enhanced as a result of applying seaweed or humic acid treatments compared to the control. This promotion effect of both biostimulants was accompanied by improving total chlorophyll content, carotenoids, total carbohydrates and endogenous phytohormones. Therefore, seaweed and humic acid treatments may be applied as promise and ecofriendly bio-stimulants to enhance the growth and productivity of tuberose and may be for various flowering bulbs as well.

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