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Evaluating Seaweed Extract Foliar Spray as a Substitute for Synthetic Cytokinin to Improve the Performance of Squash Plant

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ABSTRACT

Forchlorfenuron synthetic cytokinin CPPU, a synthetic cytokinin, is commonly used to enhance plant growth, but concerns about its potential environmental impact and residue accumulation have prompted the search for more sustainable alternatives. So, a field trial was conducted to assess the potential of seaweed extract (SWE) as a natural substitute for CPPU in compensating for its role. Foliar spraying with both SWE (0, 2, and 4 mg L^{-1}) and CPPU (0, 50, and 100 mg L^{-1}) was carried out either individually or in all combination. The evaluation included the assessment of vegetative growth performance, flowering traits, yield, and fruit quality. Results indicated that spraying SWE at the highest concentration (4 mg L^{-1}) demonstrated significant positive effects in all the studied characteristics. Similarly, CPPU at the highest concentration (100 mg L^{-1}) when applied as a foliar spray individually exhibited significant positive impacts on all studied traits. Furthermore, combining SWE at 4 mg L^{-1} with CPPU at 100 mg L^{-1} resulted in significant increases in most of the studied parameters. In conclusion, the findings suggest the potential use of foliar spraying with SWE as an effective alternative to CPPU for enhancing squash growth performance and yield in arid regions.

Keywords: SWE, CPPU, Squash performance

INTRODUCTION

Forchlorfenuron, commonly known as CPPU [N-(2-chloro-4-pyridyl)-N'-phenylurea], is a synthetic cytokinin used in the agricultural sector to promote cell division and stimulate plant growth (Chatsudthipong and Muanprasat, 2009). While CPPU has been widely employed for its positive effects on fruit development, it is essential to consider potential harmful aspects associated with its use. One of the primary concerns with CPPU is the possibility of residue accumulation in the harvested produce (Rademacher, 2015). Residues of synthetic growth regulators, including CPPU, may raise questions regarding food safety and adherence to regulatory standards. The environmental impact of synthetic cytokinins like CPPU is a topic of concern. Runoff from treated fields can potentially contaminate water sources and affect aquatic ecosystems (Gora *et al*., 2018). The persistence of CPPU in the environment may contribute to long-term ecological consequences. The long-term health effects of consuming crops treated with CPPU are not entirely clear. Limited research on the potential health risks for humans consuming fruits treated with synthetic cytokinins underscores the importance of comprehensive studies in this area. CPPU is designed to stimulate specific physiological responses in plants, but unintended effects on non-target organisms cannot be ruled out (El-Areiny *et al*.,2022). The impact on beneficial insects, soil microorganisms, and other components of the ecosystem should be thoroughly studied. Prolonged and extensive use of CPPU may lead to the development of resistance in plants. This resistance could reduce the effectiveness of the synthetic cytokinin over time, necessitating higher application rates or the development of alternative strategies. Excessive use of synthetic cytokinins may disrupt the natural balance within ecosystems (Pandey *et al*., 2023). This imbalance can affect nutrient cycling, microbial communities, and overall biodiversity. Compliance with regulatory standards and maximum residue limits is crucial. Misuse or overuse of CPPU without adhering to recommended guidelines may lead to legal and regulatory issues (dos Santos *et al*., 2024).

To mitigate these potential harms, it is important for the agricultural sector to adopt responsible and sustainable practices. This includes careful monitoring of pesticide applications, adherence to recommended dosage rates, and regular assessments of the environmental and health impacts associated with the use of synthetic cytokinins like CPPU.CPPU is commonly used to enhance plant growth, but concerns about its potential environmental impact and residue accumulation have prompted the search for more sustainable alternatives.

Seaweed extract is gaining attention as a potential substitute for synthetic cytokinins like CPPU in agriculture. This natural alternative, derived from seaweed, offers a range of bioactive compounds that can positively influence plant growth, flowering, and yield (Halpern *et al.,* 2015). As concerns about the environmental impact and residues associated with synthetic cytokinins grow, seaweed extract presents a sustainable and eco-friendly solution. Its application through foliar spraying has shown promising results in various crops, indicating its potential to enhance agricultural practices while minimizing adverse effects. Seaweed extract, rich in natural growth-promoting compounds, offers a potential solution (Stirk *et al.,* 2020).

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Research indicates that foliar spraying with seaweed extract can positively influence vegetative growth, flowering, and yield in various crops (Abd El-Hady et al., 2016; Shehata et al., 2019; El-Sherpiny *et al*., 2022; Doklega and Abd El-Hady, 2023). This alternative not only addresses environmental and health considerations but also aligns with the growing interest in eco-friendly and organic agricultural approaches.

Squash (*Cucurbita pepo* L.), a member of the Cucurbitaceae family, holds a prominent position among vegetable crops, cherished for its nutritional value both in Egypt and globally (Abd El-All *et al.,* 2013; Quintana *et al.,* 2018). Renowned for its health benefits, squash exhibits medicinal potential (Martínez-Valdivieso *et al.,* 2015). Fruits of squash are rich in essential nutrients such as manganese, potassium, phosphorus, copper, and magnesium, as well as protein and bioactive compounds including antioxidants, flavonoids, and vitamins (Youssef *et al.,* 2021). Notably, squash is characterized by its low calorie content (approximately 19 Kcal/100 g) and high fiber content (Tamer *et al.,* 2010).

The present study aims to explore the potential of employing seaweed extract foliar spraying as a substitute for the synthetic cytokinin Forchlorfenuron (CPPU). The objective is to enhance the squash cultivar "Azid F1" grown on sandy soil under Aswan conditions.

MATERIALS AND METHODS

Location site

This research work was conducted during the autumn seasons of 2022 and 2023 at the experimental farm of Aswan University's Faculty of Agriculture and Natural Resources in Aswan, Egypt. The site is positioned at approximately 23°59′56″N latitude and 32°51′36″E longitude, with an average altitude of 85 meters above sea level.

Soil sampling

Prior to sowing, soil samples were randomly collected from various locations within the planting field, reaching a depth of 0-30 cm. Essential chemical and physical properties of the soil were analyzed using the methods outlined by Page et al. (1982) and Jackson (1973). The detailed results of these analyses are presented in Table 1.

Table1. Soil properties

Properties	Season of 2022	Season of 2023
	Values	
Sand,%	96.8	96.3
Silt, %	0.0	0.0
Clay, %	3.2	3.7
Soil texture	Sandy	
pH	8.45	8.88
Electric conductivity,dSm^{-1}	3.01	3.00
Nitrogen, mg kg^{-1}	19	15
Phosphorus, mg kg^{-1}	3.41	3.44
Potassium, mg kg^{-1}	153	152

Squash seeds

The seeds of the "Azid F1" cultivar of squash were imported by Gaara Company in Cairo, Egypt, and were produced by the Japanese company Sakata. This particular cultivar is known for its extensive cultivation in the study area, showcasing traits such as high productivity, early maturation, and cold tolerance.

Substances studied

The Acadian SWE, produced by the Canadian Acadian Sea Plants Company, was imported by Chema Industries Company in Egypt. The composition of Acadian SWE includes 100% SWE of *Ascophyllum nodosum* L., 45-55% organic matter, 10% Alginic acid, Manitol, 4.4% Amino acid, 6.5% moisture, 1% Nitrogen (N), 1% phosphorus (P_2O_5), 17% potassium (K_2O).

Additionally, Forchlorfenuron (CPPU), the synthetic cytokinin, was procured from El-Gomhouria Company for Trading Drugs, Chemicals, and Medical Supplies in Alexandria, Egypt.

Treatments

The treatments comprised two independent variables used as foliar applications. These included three concentrations of SWE (0, 2 and 4 mg L^{-1}), and three concentrations of CPPU (0, 50 and 100 mg L^{-1}), administered both separately and in all combinations under split plot design with three replicates.

Experimental set up

The experimental soil underwent ridging, forming rows of one-meter width divided into plots. Each experimental unit consisted of four rows measuring 3.6 meters in length, resulting in a plot area of 14 meters. On September 10th, squash seeds were directly sown in the field for both seasons, spaced 60 cm apart on one side of the row. Following germination, plants were thinned to maintain one plant per hill. All agricultural practices such as irrigation (drip irrigation), fertilization, weed control, and pest and disease management were conducted as needed, following the guidelines recommended for commercial squash production by the Egyptian Ministry of Agriculture under the specific circumstances of the experimental site. Throughout the growing seasons, squash plants received three rounds of treatment spraying: the first at 20 days post-planting, the second 10 days after the initial application, and the third at the onset of flowering. All foliar sprays were meticulously applied to cover the entire plant foliage, with runoff occurring early in the morning.

Harvesting

Fruit harvesting commenced for early yield 45 days after sowing, followed by subsequent harvesting cycles occurring every 4 days over a period of 60 days during both seasons.

Experimental data collection

From each treatment within each replication, plants were randomly chosen for the collection of data on growth, yield and fruit quality.

1. Vegetative growth characteristics, including plant height (cm), leaf area (cm^2), shoot fresh and dry weights, and leaf chlorophyll content measured through the SPAD indicator (Minolta, 1989; Yadava, 1986), were recorded 45 days after planting.
2. Flowering characteristics, encompassing the count of male, female, and total flowers per plant, the sex ratio, and the percentage of fruit setting were documented during the study.
3. Parameters related to fruit yield, including the count of fruits per plant, average fruit weight (g), total yield per plant and feddan (kg&ton), were recorded. Additionally, early yield per feddan (ton) was assessed, representing the weight of all harvested fruits during the initial four pickings.
4. Morphological characteristics of the fruit, including fruit diameter and length (cm) were measured. The fruit shape

index was then calculated as the ratio of fruit length to fruit diameter.

5. The chemical composition was analyzed, encompassing plant foliage N, P,K as well as their uptake (g/plant). Additionally, concentrations of plant $N\text{-}NO_3$ and $N\text{-}NO_2$ (mg/kg), along with total protein (mg/g, DW) and total soluble amino acids (mg/g, DW), were determined using methods outlined by Singh *et al.* (2005) and AOAC (2006).

$$\textbf{Nutrient uptake}\left(\frac{\textbf{g}}{\textbf{plant}}\right) = \frac{\textbf{Nutrient content }(\%) \times \textbf{plant dry matter}(\textbf{g/plant})}{\textbf{100}}$$

Statistical analysis

The data from the current study underwent statistical analysis using the design prescribed by the COSTAT. The revised least significant difference test at a 0.05 level of probability was applied, following the methodology outlined by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Vegetative growth and photosynthetic pigment

Table 2 illustrates the impact of varying levels of seaweed extract (SWE) and CPPU cytokinin, along with their interactions, on various squash characteristics throughout the 2022 and 2023 seasons. This includes measurements of plant height, leaf area, plant fresh and dry weights, as well as chlorophyll content. The findings indicate that the highest concentration of seaweed extract (SWE) at 4 mg L^{-1} significantly enhanced all vegetative growth parameters, namely plant height (69.60 and 74.07 cm), leaf area (382.85 and 360.62 cm^2), plant fresh weight (494.21 and 774.55 g), plant dry weight (38.59 and 43.81g), and SPAD chlorophyll content (91.86 and 85.01), across both seasons. In contrast, the plants of control group displayed the lowest mean values. This observed improvement in growth characteristics with the application of SWE may be attributed to its capacity to enhance and support nutritional status and plant growth by providing essential molecules, including both macro- and micronutrients. These results are in an agreement with those obtained by Alhadede and Abdula (2020) on squash, Al-Barbary *et al.* (2022) on artichoke, Hassan *et al.* (2021) on cucumber, Madian (2020) and Ghazi (2020) on hot pepper, Shahen *et al.* (2019) on sweet pepper, Alkharpotly *et al.* (2017) on strawberry, Carrasco-Gil *et al.* (2018); Zodape *et al.* (2011), on tomato. Regarding chlorophyll content, some researchers have noted that seaweed extracts often enhance leaf color by either stimulating chlorophyll biosynthesis or inhibiting its degradation (Khan *et al*., 2009; Abbas and Akladious, 2013).

All measured vegetative growth parameters of squash plants exhibited significant and progressive increases with higher levels of CPPU in both seasons. The highest mean values were observed with the application of the highest CPPU level (100 mgL^{-1}), resulting in increased plant height (70.26 and 73.37 cm), leaf area (327.72 and 305.49 cm^2), plant fresh weight (439.08 and 631.35 g), plant dry weight (39.26 and 43.11 g), and SPAD chlorophyll content (87.98 and 88.75). Conversely, all investigated vegetative growth characteristics significantly decreased, showing the lowest mean values in control plants during both seasons. These outcomes can be attributed to the diverse functions of cytokinins as mentioned by Te-Chato and Lim (2000).

Table 2 displays the interaction influences of SWE levels and CPPU levels on various vegetative growth characteristics during both the 2022 and 2023 growth seasons. All aforementioned parameters exhibited significant increases with the successive rise in CPPU levels at each SWE level. Notably, the combination of 4 mg L^{-1} SWE and 100 4 mg L^{-1} CPPU resulted in the highest significant mean values for all vegetative growth traits in both seasons. Conversely, the untreated plants displayed the lowest mean values during both seasons.

Table 2. Effect of exogenous application of seaweed extract (SWE) and forchlorfenuron (CPPU) on squash growth characters during 2022 and 2023 seasons

Treatments		Plant height, cm		Leaf area, cm^2		Plant fresh weight, g		Plant dry weight, g		Chlorophyll, SPAD reading	
		2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
						SWE (mg .l^{-1})					
0		67.64 b	68.72 c	218.26 c	196.03 c	324.00 c	401.19 c	36.63 b	38.57 c	57.67 c	55.20 c
2		67.47 b	70.64 b	291.80 b	269.57 b	397.54 b	564.50 b	36.46 c	40.27 b	78.11 b	75.94 b
4		69.60 a	74.07 a	382.85 a	360.62 a	494.21 a	774.55 a	38.59 a	43.81 a	91.86 a	85.01 a
						CPPU (mg.l^{-1})					
0		65.35 b	68.95 c	268.44 c	246.24 c	374.18 c	536.16 c	34.34 c	38.92 c	72.55 b	75.34 c
50		69.09 a	71.10 b	296.75 b	274.52 b	402.49 b	572.73 b	38.06 b	40.62 b	67.11 b	81.87 b
100		70.26 a	73.37 a	327.72 a	305.49 a	439.08 a	631.35 a	39.26 a	43.11 a	87.98 a	88.75 a
						SWE (mg.l^{-1})× CPPU (mg.l^{-1})					
SWE	CPPU										
	0	66.03 g	67.00 f	183.30 h	161.07 h	289.04 i	351.01 i	35.03 h	37.74 h	44.17 i	51.47 i
0	50	66.93 f	66.76 f	218.18 g	195.95 g	323.92 h	410.96 h	35.93 g	35.84 i	62.77 g	52.80 h
	100	69.95 c	72.40 bc	253.30 f	231.07 f	359.04 g	441.61 g	38.95 c	42.14 d	66.07 f	61.33 g
	0	63.07 h	68.49 e	263.41 f	241.18 f	369.15 f	530.91 f	32.07 i	37.90 g	89.17 c	80.63 c
2	50	71.36 b	72.98 b	283.50 e	261.27 e	389.24 e	560.95 e	40.36 b	42.72 c	53.40 h	70.80 f
	100	67.97 e	70.46 d	328.50 d	306.27 d	434.24 d	601.66 d	36.97 e	40.20 f	91.77 b	76.40 d
	0	66.94 f	71.38 cd	358.61 c	336.38 c	464.35 c	726.58 c	35.94 f	41.12 e	84.30 e	72.70 e
4	50	68.99 d	73.56 b	388.58 b	366.35 b	494.32 b	746.29 b	37.99 d	43.30 b	85.17 d	87.40 b
	100	72.86 a	77.27 a	401.37 a	379.14 a	523.96 a	850.79 a	41.86 a	47.01 a	106.10 a	94.93 a

Flowering and fruit setting characteristics

Table 3 illustrates the impact of varied SWE and CPPU levels, along with their interactions, on the flowering and fruit setting characteristics of squash plants throughout the 2022 and 2023 seasons.

In Table 3, it is evident that the increment in the applied level of seaweed extract up to 4 mg L^{-1} resulted in a significant rise in flowering characteristics, including the number of male, female, and total flowers, as well as the sex ratio, during both seasons. Conversely, control plants exhibited the lowest mean values for these traits throughout the study. While the percentage of fruit setting was not significantly affected by foliar spraying with SWE, as the treatments did not differ significantly from the control plants during both seasons. The notable increase in flowering characteristics observed in Table 3 with the application of the 4 mg L^{-1} concentration of seaweed extract may be attributed to its content of plant hormones such as auxins and cytokines, activating physiological processes in the plant that influence flowering traits. Battacharyya *et al.* (2015) asserted that cytokines and auxins play a role in enhancing flower initiation and inducing more flowering.

Table 3 outlines the primary effects of CPPU levels on the flowering characteristics of squash. The results reveal that the foliar application of CPPU at the 100 mg L^{-1} level led to significant increases in flowering traits, including the number of male, female, and total flowers, as well as the sex ratio, in both seasons. In contrast, control plants exhibited the lowest mean values for these traits during both seasons of the study. However, the percentage of fruit setting was not significantly affected by foliar spraying with CPPU, as the treatments did not significantly differ from the control plants in both seasons. Notably, while individual concentrations of seaweed extract and cytokinin did not exhibit a significant impact on the fruit setting trait compared to the control, their combined use at the highest concentration resulted in the highest percentage of fruit set compared to other treatments.

Table 3. Effect of exogenous application of seaweed extract (SWE) and forchlorfenuron (CPPU) on squash flowering characters during 2022 and 2023 seasons.

Treatments		No. male flower		No. female flower		Total No. flower		Sex ratio		Fruit setting (%)	
		2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
SWE (mg .l^{-1})											
0		3.34 b	2.15 b	15.80 c	15.02 c	19.14 c	17.32 c	0.21 b	0.14 b	58.95 a	66.46 a
2		4.23 a	2.98 a	16.66 b	16.09 b	20.90 b	19.08 b	0.25 a	0.19 a	59.08 a	66.87 a
4		4.54 a	3.29 a	17.03 a	16.46 a	21.58 a	19.75 a	0.27 a	0.20 a	60.05 a	68.89 a
CPPU (mg.l^{-1})											
0		3.52 c	2.34 b	16.01 c	15.21 c	19.54 b	17.71 b	0.34 b	0.15 b	59.35 a	67.30 a
50		4.12 b	2.87 a	16.58 b	16.03 b	20.70 a	18.90 a	0.25 a	0.18 ab	59.24 a	67.16 a
100		4.46 a	3.21 a	16.91 a	16.33 a	19.54 b	19.61 a	0.26 a	0.20 a	59.50 a	67.76 a
SWE (mg .l^{-1})× CPPU (mg.l^{-1})											
SWE	CPPU										
	0	2.40 d	1.33 d	14.88 d	13.62 e	17.28 d	15.44 e	0.16 e	0.11 c	59.27 bc	67.09 bc
0	50	3.62 c	2.37 c	16.06 c	15.53 d	19.68 c	17.90 d	0.23 d	0.15 bc	59.00 cd	66.49 cd
	100	3.99 bc	2.74 bc	16.48 bc	15.89 cd	20.47 bc	18.63 cd	0.24 cd	0.17 ab	58.57 d	65.80 d
	0	4.03 bc	2.78 bc	16.51 bc	15.93 c	20.54 bc	18.71 b-d	0.24 cd	0.17 ab	59.37 bc	67.36 bc
2	50	4.22 b	2.97 bc	16.63 b	16.08 c	20.86 b	19.05 bc	0.25 b-d	0.18 ab	59.06 b-d	66.84 cd
	100	4.45 ab	3.20 ab	16.84 ab	16.28 bc	21.30 ab	19.48 a-c	0.26 a-c	0.20 ab	58.82 cd	66.41 cd
	0	4.16 bc	2.91 bc	16.64 b	16.08 c	20.80 bc	18.98 b-d	0.25 b-d	0.18 ab	59.40 bc	67.46 bc
4	50	4.53 ab	3.28 ab	17.04 ab	16.48 ab	21.57 ab	19.76 ab	0.27 ab	0.20 ab	59.65 b	68.14 b
	100	4.95 a	3.70 a	17.41 a	16.82 a	22.36 a	20.52 a	0.28 a	0.22 a	61.11 a	71.08 a

This observation may be attributed to the presence of a high concentration of natural cytokinins in seaweed extract, enhancing the efficacy of the high concentration of synthetic cytokinins, which exhibited a significant effect on fruit setting during both growing seasons. The outcomes presented in Table 3 demonstrate that the combined treatment involving the application of 4 mg L^{-1} SWE with either 50 or 100 mg L^{-1} of CPPU resulted in a significant increase in the flowering traits of squash plants, including the number of male, female, and total flowers, as well as the sex ratio, in both seasons. Conversely, control plants exhibited the lowest mean values for these characteristics during both seasons of the study. Regarding fruit setting, the highest significant mean values were observed when 4 mg L^{-1} SWE was applied in combination with 100 mg L^{-1} of CPPU during both seasons. In contrast, the lowest significant mean values were recorded for the untreated (control) plants in both seasons.

Yield and its components parameters

Table 4 depicts the effects of seaweed extract and CPPU levels, as well as their interactions, on various yield-related parameters such as the number of fruits per plant, average fruit weight (g), total yield per plant (kg), total yield per feddan (ton), and early yield per feddan (ton).

The application of the highest seaweed extract level (4 mg L^{-1}) resulted in significant increases in all yield-related parameters compared to untreated plants, which exhibited the lowest significant mean values for yield and its components in both seasons (Table 4). While there was no significant difference observed between the two concentrations of seaweed extracts (2 and 4 mg L^{-1}) for average fruit weight (g), total yield per plant (kg), total yield per feddan (ton), and early yield per feddan (ton) traits in the first season only, a significant difference was noted between these concentrations and the control plants. The observed enhancement in total yield and its components parameters associated with the application of seaweed extract may be attributed to the abundance of natural plant hormones, particularly cytokinins, gibberellins, and, to some extent, auxins and brassinosteroids (Stirk *et al.,* 2020). These compounds have the potential to stimulate the development of a more extensive root mass, facilitating improved uptake of water and minerals and their efficient translocation within the plant. Consequently, this contributes to the cultivation of healthier and more resilient plants capable of withstanding stress. Furthermore, the role of seaweed extract extends to the augmentation of physiological activities such as photosynthesis and the promotion of growth parameters (refer to Table 2), ultimately fostering an increase in the number of flowers and influencing the sex ratio (refer to Table 3).

Table 4. Effect of exogenous application of seaweed extract (SWE) and forchlorfenuron (CPPU) on squash yield characters during 2022 and 2023 seasons.

Treatments		No. fruits/plant		Average fruit weight (g)		Yield/plant (kg)		Early yield/fed. (Ton)		Yield/fed. (Ton)	
		2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
SWE ($mg.l^{-1}$)											
0		11.48 c	11.93 c	90.16 b	95.07 c	1.03 b	1.13 c	1.84 b	2.00 c	6.89 b	7.50 c
2		12.34 b	12.76 b	104.98 a	109.65 b	1.29 a	1.39 b	2.31 a	2.47 b	6.63 a	9.25 b
4		12.71 a	13.16 a	105.49 a	142.31 a	1.34 a	1.85 a	2.39 a	3.30 a	8.94 a	12.35 a
CPPU ($mg.l^{-1}$)											
0		11.69 c	12.10 b	101.79 a	116.19 a	1.19 b	1.40 c	2.12 b	2.50 c	7.95 b	9.36 c
50		12.26 b	12.72 a	99.19 a	113.89 a	1.22 ab	1.44 b	2.17 ab	2.57 b	8.12 a	9.61 b
100		12.59 a	13.04 a	99.65 a	116.95 a	1.26 a	1.52 a	2.24 a	2.71 a	8.38 a	10.13 a
SWE ($mg.l^{-1}$)× CPPU ($mg.l^{-1}$)											
SWE	CPPU										
0	0	10.56 d	10.98 e	95.05 g	99.06 de	1.00 a	1.08 f	1.78 c	1.93 f	6.68 c	7.23 f
	50	11.74 c	12.22 d	87.26 i	92.35 e	1.02 a	1.12 ef	1.82 c	2.00 ef	6.83 c	7.47 ef
	100	12.16 bc	12.60 cd	88.18 h	93.82 e	1.07 a	1.17 e	1.91 c	2.08 e	7.15 c	7.81 e
2	0	12.19 bc	12.55 cd	103.02 e	105.28 cd	1.26 a	1.31 d	2.24 b	2.33 d	8.37 b	8.72 d
	50	12.31 b	12.75 b-d	107.71 a	108.31 bc	1.32 a	1.37 d	2.36 ab	2.43 d	8.83 ab	9.09 d
	100	12.52 ab	12.98 e	104.20 d	115.36 b	1.30 a	1.49 c	2.32 b	2.66 c	8.69 b	9.95 c
4	0	12.32 b	12.76 b-d	107.30 b	144.25 a	1.32 a	1.82 b	2.35 ab	3.24 b	8.80 ab	12.13 b
	50	12.72 ab	13.18 ab	102.58 f	141.01 a	1.31 a	1.84 ab	2.33 b	3.27 ab	8.71 b	12.27 ab
	100	13.09 a	13.53 a	106.58 c	141.68 a	1.40 a	1.90 a	2.49 a	3.38 a	9.31 a	12.64 a

The results presented in Table 4 reveal that the highest level of CPPU (100 mg L^{-1}) led to significant increases in all yield-related parameters, except for average fruit weight (g), compared to untreated plants, which exhibited the lowest significant mean values for yield and its components in both seasons. However, there was no significant difference observed between the two concentrations of seaweed extracts (2 and 4 mg L^{-1}) for the number of fruits per plant in the second season only, total yield per plant (kg), total yield per feddan (ton), and early yield per feddan (ton) traits in the first season only. Nevertheless, there was a significant difference between these concentrations and the control plants. In the case of the average fruit weight character, no significant difference was observed among all CPPU treatments during the two growing seasons. These results may be attributed to the functions of CPPU, promoting cell division, cell enlargement, and delaying senescence. Additionally, CPPU enhances growth parameters (Table 2), resulting in an increase in the number of flowers and the sex ratio (Table 3), ultimately leading to an increase in yield and its components.

Furthermore, Table 4 shows that, in both seasons, the combination of 4 mg L^{-1} of seaweed extract with either 50 or 100 mg L^{-1} CPPU resulted in the highest mean values for squash yield and its components (except the yield per plant character in the first season, where no significant difference was found between all treatments). In contrast, control plants yielded the lowest significant mean values in both seasons.

Fruit physical characteristics

Table 5 presents the fruit morphological characteristics of squash influenced by seaweed extract and CPPU levels, along with their interaction, during the 2022 and 2023 growth seasons.

Notably, significant increases in the fruit length and diameter of squash occurred with the escalating levels of seaweed extract up to 4 mgL^{-1}, compared with control plants which exhibited the lowest values for squash fruit length and diameter in both seasons. These results may be attributed to the content of seaweed extract, rich in natural plant hormones, which could enhance cell division and elongation. However, the fruit shape index character was not significantly affected by foliar spraying with seaweed extract during the two growing seasons.

Table 5 also indicates that CPPU levels exerted profound and significant effects on fruit length, diameter, and shape index characters in both growing seasons. Application of CPPU at 50 or 100 mg L^{-1} resulted in significant increases in fruit length, diameter, and shape index during both seasons. These outcomes align with similar results reported by dos Santos *et al*. (2024) on squash fruit.

Table 5. Effect of exogenous application of seaweed extract (SWE) and forchlorfenuron (CPPU) on squash fruit morphological characters during 2022 and 2023 seasons.

Treatments		Fruit length (cm)		Fruit diameter (cm)		Fruit shape index	
		2022	2023	2022	2023	2022	2023
SWE ($mg.l^{-1}$)							
0		12.76 c	12.72 c	2.31 c	2.31 c	5.52 a	5.50 a
2		13.61 b	13.56 b	2.42 b	2.43 b	5.62 a	5.57 a
4		13.97 a	13.93 a	2.50 a	2.49 a	5.59 a	5.59 a
CPPU ($mg.l^{-1}$)							
0		12.95 b	12.91 b	2.38 b	2.39 b	5.44 b	5.39 b
50		13.54 a	13.50 a	2.40 b	2.41 ab	5.64 a	5.60 a
100		13.85 a	13.81 a	2.46 a	2.43 a	5.64 a	5.67 a
SWE ($mg.l^{-1}$)× CPPU ($mg.l^{-1}$)							
SWE	CPPU						
0	0	11.82 e	11.77 e	2.27 e	2.30 d	5.22 b	5.12 c
	50	13.05 d	13.01 d	2.30 de	2.30 d	5.67 a	5.66 ab
	100	13.42 cd	13.37 cd	2.37 cd	2.33 d	5.67 a	5.73 a
2	0	13.45 b-d	13.41 b-d	2.40 bc	2.40 c	5.61 a	5.59 ab
	50	13.58 b-d	13.54 b-d	2.40 bc	2.43 bc	5.66 a	5.56 ab
	100	13.78 bc	13.74 bc	2.47 ab	2.46 ab	5.59 a	5.56 ab
4	0	13.58 b-d	13.54 b-d	2.47 ab	2.46 ab	5.51 a	5.48 b
	50	13.98 ab	13.94 ab	2.50 a	2.50 a	5.59 a	5.57 ab
	100	14.35 a	14.31 a	2.53 a	2.50 a	5.66 a	5.72 a

Furthermore, the highest applied level of seaweed extract at 4 mgL^{-1}, combined with either of the CPPU levels (50 or 100 mgL^{-1}), led to a significant increase in fruit length and diameter compared with control plants, which displayed the lowest values during both seasons. In the case of fruit shape index, no significant differences were found among all treatments, except for control plants, which exhibited the lowest significant fruit shape index in both seasons.

Chemical composition

Tables 6 and 7 present the chemical constituents of squash influenced by seaweed extract and CPPU levels, along with their interaction, during the 2022 and 2023 growth seasons.

The findings in Tables 6 and 7 reveal that foliar application of seaweed extract at the highest concentration (4 mgL^{-1}) resulted in the highest mean values for squash chemical constituents, including N, P, K (%) foliage nutrient content, N, P, K foliage nutrient uptake (g/plant), total protein (mg/g, DW), and total soluble amino acids (mg/g, DW.). In comparison, control plants exhibited the lowest significant mean values during both seasons. These results are likely attributed to the high levels of natural plant hormones, especially cytokinins, gibberellins, and to some extent auxins and brassinosteroids found in seaweed extracts. These compounds could stimulate the production of a greater root mass, leading to improved uptake of water and minerals such as N, P, and K, and facilitating their translocation. This ultimately results in healthier plants with higher NPK content.

Table 6. Effect of exogenous application of seaweed extract (SWE) and forchlorfenuron (CPPU) on squash chemical characters during 2022 and 2023 seasons.

Treatments		Foliage nutrient content									
		N%		P%		K%		$N\text{-}NO_3$ (mg/kg)		$N\text{-}NO_2$ (mg/kg)	
		2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
SWE ($mg.l^{-1}$)											
0		1.89 c	2.03 c	0.132 c	0.182 c	1.37 c	1.48 c	163.13 a	162.60 a	1.25 a	1.35 a
2		1.99 b	2.14 b	0.148 b	0.198 b	1.67 b	1.76 b	138.30 b	134.68 a	1.23 a	1.39 a
4		2.08 a	2.19 a	0.153 a	0.202 a	1.73 a	1.84 a	132.21 c	138.12 b	1.12 b	1.20 b
CPPU ($mg.l^{-1}$)											
0		1.90 c	2.05 b	0.137 c	0.188 c	1.50 c	1.61 c	133.12 c	129.37 c	0.95 c	1.13 c
50		1.99 b	2.14 a	0.145 b	0.195 b	1.59 b	1.69 b	139.66 b	144.61 b	1.16 b	1.29 b
100		2.06 a	2.17 a	0.151 a	0.200 a	1.68 a	1.78 a	160.87 a	161.42 a	1.48 a	1.52 a
SWE ($mg.l^{-1}$)× CPPU ($mg.l^{-1}$)											
SWE	CPPU										
	0	1.88 d	1.96 e	0.128 g	0.178 i	1.32 i	1.44 i	147.17 bc	144.53 de	1.00 de	1.15 c
0	50	1.87 d	2.10 bcd	0.133 f	0.183 h	1.37 h	1.47 h	151.43 b	156.20 b	1.18 c	1.34 b
	100	1.93 c	2.04 d	0.136 e	0.186 g	1.41 g	1.53 g	190.80 a	187.07 a	1.58 a	1.57 a
	0	1.80 e	2.06 cd	0.138 e	0.191 f	1.56 f	1.65 f	122.17 e	117.93 h	0.98 ef	1.15 c
2	50	2.05 b	2.13 b	0.148 c	0.198 d	1.67 d	1.76 d	128.77 e	136.50 f	1.20 c	1.38 b
	100	2.12 a	2.22 a	0.157 a	0.206 b	1.78 b	1.88 b	145.70 bc	149.60 c	1.51 a	1.64 a
	0	2.02 b	2.12 bc	0.146 d	0.195 e	1.63 e	1.73 e	130.03 de	125.63 g	0.87 f	1.09 c
4	50	2.06 b	2.19 a	0.153 b	0.203 c	1.73 c	1.83 c	138.77 cd	141.13 ef	1.11 cd	1.14 c
	100	2.15 a	2.25 a	0.159 a	0.209 a	1.84 a	1.95 a	146.10 bc	147.60 cd	1.37 b	1.36 b

Table 7. Effect of exogenous application of seaweed extract (SWE) and forchlorfenuron (CPPU) on squash chemical characters during 2022 and 2023 seasons.

Treatments		Foliage nutrient uptake (g/plant)						Total protein(mg/g, DW.)		Total soluble amino acids (mg/g, DW)	
		N		P		K					
		2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
SWE ($mg.l^{-1}$)											
0		0.69 c	0.78 c	0.049 c	0.071 c	0.50 c	0.57 c	12.02 a	11.47 c	1.22 c	1.27 c
2		0.73 b	0.68 b	0.054 b	0.078 b	0.61 b	0.71 b	11.97 a	11.54 b	1.31 b	1.38 b
4		0.80 a	0.96 a	0.059 a	0.090 a	0.67 a	0.81 a	12.80 a	12.45 a	1.37 a	1.44 a
CPPU ($mg.l^{-1}$)											
0		0.65 c	0.80 c	0.047 c	0.072 c	0.51 c	0.63 c	9.70 b	10.03 c	1.09 c	1.20 c
50		0.76 b	0.78 b	0.055 b	0.080 b	0.61 b	0.69 b	13.11 a	11.97 b	1.36 b	1.38 b
100		0.81 a	0.94 a	0.059 a	0.087 a	0.66 a	0.77 a	13.98 a	13.46 a	1.45 a	1.51 a
SWE ($mg.l^{-1}$)× CPPU ($mg.l^{-1}$)											
SWE	CPPU										
	0	0.66 f	0.74 g	0.045 f	0.067 d	0.461 g	0.543 g	9.13 a	9.89 h	1.02 e	1.12 i
0	50	0.67 f	0.75 g	0.048 e	0.067 d	0.492 f	0.527 h	12.68 a	10.80 g	1.26 c	1.27 f
	100	0.75 d	0.86 e	0.053 d	0.080 c	0.550 e	0.643 e	14.24 a	13.72 b	1.39 b	1.43 d
	0	0.58 g	0.78 f	0.044 f	0.070 d	0.499 f	0.627 f	9.76 a	9.32 i	1.11 d	1.23 h
2	50	0.83 b	0.91 c	0.060 b	0.083 c	0.675 b	0.753 c	12.69 a	12.49 e	1.39 b	1.40 e
	100	0.78 c	0.89 d	0.058 c	0.080 c	0.659 c	0.757 c	13.47 a	12.81 c	1.43 b	1.52 b
	0	0.73 e	0.87 de	0.053 d	0.080 c	0.584 d	0.710 d	10.20 a	10.90 f	1.14 d	1.26 g
4	50	0.78 c	0.95 b	0.058 c	0.090 b	0.657 c	0.793 b	13.96 a	12.62 d	1.44 b	1.48 c
	100	0.90 a	1.06 a	0.066 a	0.100 a	0.770 a	0.913 a	14.23 a	13.85 a	1.55 a	1.59 a

Additionally, seaweed extract's amino acid content plays a crucial role in nitrogen metabolism, enhancing the absorption and concentration of nitrogen (Han *et al.*, 2024). These findings align with similar studies conducted on squash by Shareef *et al.* (2022) and on cucumber by Hassan *et al.* (2021).

Contrastingly, the foliage nutrient content of $N\text{-}NO_3$ (mg/kg) and $N\text{-}NO_2$ (mg/kg) decreased with the highest seaweed extract levels (4 mgL^{-1}) and increased at the lowest seaweed levels (2 mgL^{-1}) and in control plants during both seasons. The results presented in Tables 6 and 7 indicate that the application of CPPU levels significantly led to the highest squash chemical constituents during both seasons compared to control plants. Specifically, the highest level of CPPU (100 mg.l-1) resulted in the highest mean values for foliage nutrient content of N, P, K (%), $N\text{-}NO_3$ (mg/kg), N-

NO_2 (mg/kg), N, P, K uptake (g/plant), total protein (mg/g, DW.), and total soluble amino acids (mg/g, DW.).

Moreover, the combination of the highest seaweed extract rate (4 mgL^{-1}) with the highest CPPU level (100 mgL^{-1}) brought about a significant increase in shoot nutrient concentrations (%) and uptake (g/plant), total protein (mg/g, DW.), and total soluble amino acids (mg/g, DW) compared to control plants, which exhibited the lowest significant mean values during both seasons. Regarding the foliage nutrient content of N-NO_3 (mg/kg) and N-NO_2 (mg/kg), the application of seaweed extract at 0 mgL^{-1}combined with the highest applied level of CPPU (100 mgL^{-1}) resulted in the highest significant mean values in both seasons.

CONCLUSION

The results demonstrated significant positive effects when SWE was applied at the highest concentration (4 mg L^{-1}), showing improvements in all assessed characteristics. Similarly, CPPU, when individually applied as a foliar spray at the highest concentration (100 mg L^{-1}), exhibited significant positive impacts across all studied traits. Moreover, combining SWE at 4 mg L^{-1} with CPPU at 100 mg L^{-1} resulted in substantial increases in most of the evaluated parameters.

Based on the positive outcomes observed, it is recommended to consider foliar spraying with SWE at a concentration of 4 mg L^{-1} for maximizing the growth, flowering, and yield of squash in arid regions. The synergistic effects observed with the combined application of SWE and CPPU suggest the potential for an integrated approach. Future studies could explore and fine-tune the optimal ratios for achieving the best results. To enhance the understanding of the mechanisms involved, further research is encouraged to investigate the physiological and biochemical aspects underlying the positive responses to SWE and CPPU. This would contribute to a more comprehensive understanding of their interactions and potential synergies. Considering the practical implications, farmers and agricultural practitioners in arid regions may benefit from adopting foliar spraying with SWE as a sustainable and cost-effective alternative to CPPU for enhancing squash productivity.

In summary, the findings not only emphasize the promising potential of seaweed extract but also underscore the significance of exploring eco-friendly alternatives to synthetic cytokinins for sustainable agricultural practices in challenging environments.

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تقييم الرش الورقي بمستخلص الطحالب البحرية كبديل للسيتوكينين الصناعي لتحسين أداء نبات قرع الكوسة

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الملخص

يستخدم السيتوكينين الصناعي بشكل شائع لتعزيز نمو النبات، ولكن المخاوف بشأن تأثيره البيئي المحتمل وتأثيره المتبقي دفعت إلى البحث عن بدائل أكثر استدامة. لذلك، تم إجراء تجربة حقلية لتقييم إمكانية استخدام مستخلص الطحالب البحرية كبديل طبيعي للسيتوكينين الصناعي في تعويض دوره. تم إجراء الرش الورقي بكل من مستخلص الطحالب البحرية (بمعدل ٠، ٢، و٤ ملجم لتر-١) والسيتوكينين الصناعي (بمعدل ٠، ٥٠، و١٠٠ ملجم لتر-١) إما بشكل فردي أو مجتمع لكلا المادتين المدروستين. وتضمن التقييم أداء النمو الخضري وصفات التزهير والمحصول وجودة الثمار. أشارت النتائج إلى أن رش مستخلص الطحالب البحرية بأعلى تركيز (٤ ملجم لتر-١) أظهر تأثيرات إيجابية معنوية في جميع الصفات المدروسة. وبالمثل، أظهرت السيتوكينين الصناعي بأعلى تركيز (١٠٠ ملجم لتر-١) عند رشها ورقياً بشكل فردي تأثيرات إيجابية معنوية على جميع الصفات المدروسة. علاوة على ذلك، أدى الجمع بين مستخلص الطحالب البحرية بجرعة ٤ ملجم لتر-١ مع السيتوكينين الصناعي بجرعة ١٠٠ ملجم لتر-١ إلى زيادات كبيرة في معظم المعايير المدروسة. ختاما، تشير النتائج إلى إمكانية استخدام الرش الورقي بـ مستخلص الطحالب البحرية كبديل فعال للسيتوكينين الصناعي لتعزيز أداء نمو قرع الكوسة والمحصول في المناطق القاحلة.