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Influence of Some Biostimulants on Growth, Yield and Quality of Grafted Watermelon "Citrullus lanatus" Using Cucurbita Maxima Rootstock

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Abstract
Bio-stimulants and grafting represent pivotal sustainable strategies to enhance crop productivity and quality while minimizing dependence on chemical fertilizers. Bio-stimulants activate plant defense mechani
sms against biotic and abiotic stresses while improving metabolic efficiency, whereas grafting serves as an eco-friendly alternative to pesticides, mitigating soil-borne diseases and boosting yield. The present study was carried out at El kottna area, El Dakahlia governorate, Egypt during the period from 2022 to 2023 to Study the effect of applying different sources of biostimulant (Condensed molasses soluble (CMS) and Algae extract) on grafted watermelon Seedlings on growth, yield and quality of watermelon. Two experiments were carried out; greenhouse and field experiment including one grafting method, i.e., as follows one cotyledon grafting method (Splice), and one scion of watermelon with rootstocks. Treatments were; 100% Control (recommendation R), 100% R + 10 ml/liter Algae extract, 100% R + 15 ml/liter Algae extract, 100% R + 20 ml/liter Algae extract, 100% R + 10 ml/liter CMS, 100% R + 15 ml/liter CMS, and 100% R + 20 ml/liter CMS. Results indicated that CMS at 20 mL/L significantly enhanced fruit weight (23% in 2022 and 31.4% in 2023) compared to control and TSS (31.2% in 2022 and 37.2% in 2023) over the control. Also, Algae extract at 20 mL/L also improved fruit weight by (22.2% in 2022 and 26.8% in 2023) over control and TSS by (15.5% in 2022 and 23.5 % in 2023) over the control. Biostimulants significantly increased yield per plant, CMS at 20 ml/L showing the highest yield per plant (24.2% in 2022, 31.5% in 2023), closely followed by algae extract (23.42% and 26.8% in 2022 and 2023, respectively). Highly recommend CMS at 20 ml/L as a biostimulant spray for grafted watermelon seedlings and crops. CMS improves production quality, increases yield, and enhances Brix and TSS of watermelons.
Keywords: Watermelon, Grafting, , Algae extracts, Condensed molasses soluble, Yield Enhancement. .

1. Introduction

Watermelon (*Citrullus lanatus*) is a variety of a family *Cucurbitacea.*It has been asserted that about 6.8% of the total area in the world dedicated to fruit production is for watermelon production.[1, 2]watermelon contains several bioactive compounds besides vitamins A and C, which are available in most fruits.. [1, 3] There is an urgent need to improve agricultural practices to ensure that food production is balanced with environmental sustainability, Formulations based on organic wastes, including Bio-stimulation, are considered fertilizers in organic and conventional agriculture. [2]

Grafting of vegetable Stared in the 1920s to control soil-borne diseases, and is now a common processing in Asia, parts of Europe, and the Middle East. Grafting has gained popularity and has the potential to be used in vegetable propagation to address problems that arise and limit vegetable output in agricultural systems. However, as food demand rises, growers are more likely to disregard crop rotation, which results in the cultivation of the same crop repeatedly or in the same growing season or area. These practices alter soil conditions, which can lead to a variety of physiological and pathological disorders that cause severe crop loss. [4][5, 6] Grafting can be a useful strategy in this situation to mitigate the negative impacts of the soil and shield the scions or crops from these unfavorable circumstances. One efficient method for managing biotic and abiotic challenges is grafting watermelon (*Citrullus lanatus*) onto resistant rootstocks. [5-7]According to Johnson, 2012, grafted watermelon can be 30–50% more productive than nongrafted plants. Grafted plants can offer the best results when planted at two-thirds of the non-grafted stock.

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Grafting is generally considered a novel and promising technique in Egypt and Sudan's vegetable agriculture. Consequently, there are many nurseries interested in producing grafted vegetable transplants, and they have to choose between several grafting techniques, such as splice, tongue approach, hole insertion, and side graft, in addition to a variety of rootstock cultivars or hybrids from the genus *Cucurbita maxima, Cucurbita moschata.*

Algae extracts, categorized into green, brown, and red, have been found to positively impact various vegetable crops. [8] Algae extracts have been shown to enhance plant growth, blooming, and fruit setting by acting as natural growth regulators, preventing leaf fall, flowering, fruiting, yellowing, retaining chlorophylls, encouraging cell division, and promoting root growth. Additionally, algae include natural organic elements and a growth stimulant that raise soil efficiency and promote the growth of microorganisms in the soil, which enhances nutrient and water absorption. By accelerating the process of photosynthesis, the application of algal extracts to the leaf enhances its internal metabolism; they are natural bio active materials rich in minerals, protein, lipids, carbohydrates, vitamins and microelements. [9, 10] And it is safe to humans, animals and the environment. [11]Algae also include natural phenols like tannins and lignin, which makes them more disease tolerant. Additionally, because they include alginic acid, a naturally occurring chelating agent that chelates nutrients with a soil solution, they aid in accelerating the absorption of nutrients. They also include some vitamins, including C, B1, B2, and B12. Because algae contain free amino acids that support healthy, balanced plant development and improve the plant's response to fertilization, they act as natural growth regulators and antibiotics inside the plant. [12] There are many studies on the use of algae extract sprays that recorded the best results of obtaining the highest vegetative growth parameters, yield, and chemical components.[13-15]

Decreasing of chemical fertilizers became more significant due to increasing their prices, which ever reduce ground water pollution as well as for human health.[16]In compare to chemical fertilizers and biostimulants as algae extracts are safe and non-toxic, decomposable, and safe for both people and other living things. [17] They may be used as soil conditioning agents and biofertilizers to increase soil fertility and plant productivity. [18-21]

Condensed molasses soluble (CMS) is a byproduct of various fermentation processes involved in yeast production. Molasses serves as a nutritional reactant in numerous fermentations manufacturing processes, including yeast production. In this process, the sugar content of the molasses is used by microbiological activity. The output fluid waste has very little residual sugar content and this waste is condensed, further processed and commercial as CMS. [22] It has motivational influences on plants. [23, 24] is one of the affluent reason amino acids like lysine, tryptophan etc., include the macro and micronutrients and the best reason of the B-complex vitamins such as B1, B2, B6 and B12. Its beneficial function during both vegetative and reproductive phases is evident, as it enhances flower emergence and their eventual setting in certain plants due to the increase in auxin and cytokinin levels, as well as improved carbohydrate accumulation. [25]It was noted that it promotes effects on cell division and growth, the synthesis of proteins and nucleic acids, and the composition of chlorophyll., [26] On the other way, using CMS in organic farming as biofertilizer and biostimulants is 'zero impact' on the environment. [27]CMS, a rich source of mineral nutrients and organic matter, has the potential to be a new organic fertilizer for crops, but its full potential remains unexplored(Li et al., 2020).It has several benefits for plant growth, including lowering plant parasitic nematodes and changing the C: N ratio, which benefits the soil microbial community, cleanses soil and enhances nitrogen fixation..[28, 29] Molasses aids in rebuilding soil structure and reduces surface crusting in soils that are susceptible to becoming hard. [30] Compared to artificial fertilizers, studies have shown that CMS increases the availability of nitrogen (N), phosphorous (P), potassium (K), and other organic matter in the soil, improving crop output overall. When compared to artificial fertilization, the CMS alteration in sugarcane enhanced yield, chlorophyll content, surfacing rate, average root weight, root yield and polar sugar content and tillering rate. It also helped to improve the soil's chemical and physical characteristics.[31, 32]

The study aims to highlight the value of biosimulate as a modern agricultural practice, offering an economical and eco-friendly solution to enhance grafted watermelon quality and yield. This ultimately benefits nurseries, farmers, and consumers alike.

2. Material sand Methods

The present study was carried out at El kottna area El Dakahlia governorate during the period from 2022 to 2023. Study the effect of applying different sources of biostimulant for grafted watermelon variety and rootstock on growth, yield and quality of watermelon. The experiment included one grafting method, i.e., as follows one cotyledon grafting method (Splice)[33] and one scions of watermelon with rootstocks.

2.1. 1. Plant material

Watermelon (*Citrullus lanatus*) 'Aswan F1' produced by (Sakata Company, Japan) was used, it is widely grown in commercial production of watermelon in Egypt. This variety of watermelon grafted on Rootstock (*Cucurbita maxima*), produced in India as local variety of bottle gourd was obtained from seed department, agriculture research center, Egypt. The grafting method was by the one cotyledon grafting method (Splice). (Figure 1)

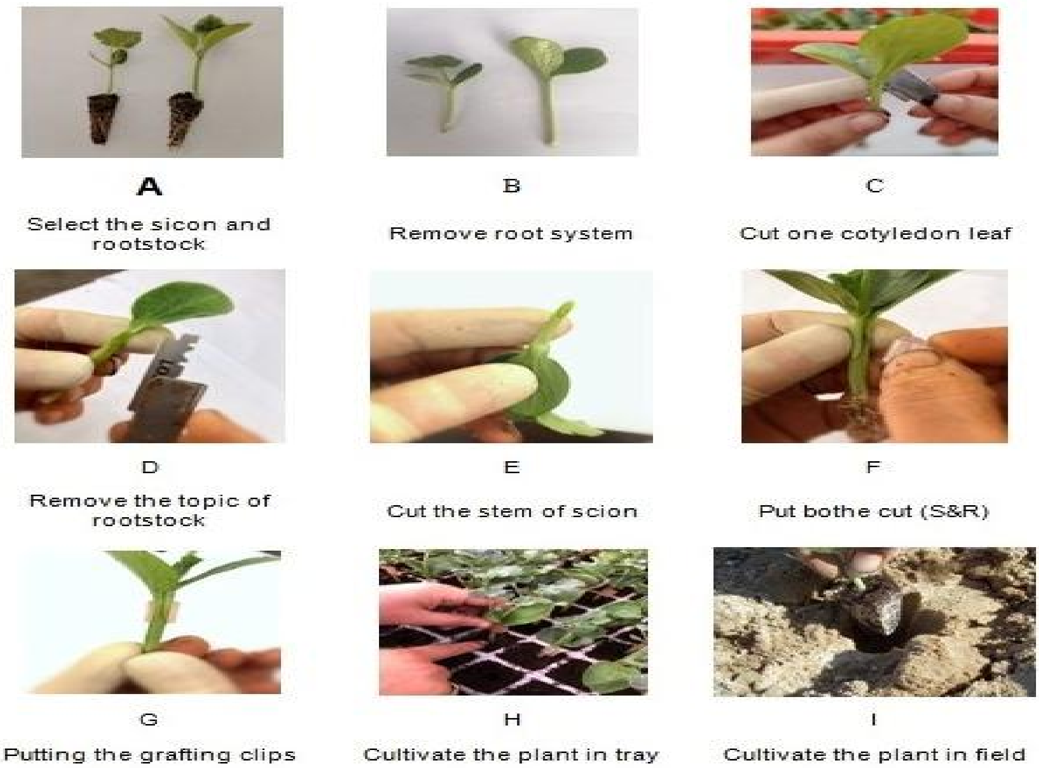

Figure 1: The one cotyledon grafting method (Splice)Steps. (Elkersh *et al.* 2016) [34]

2.2. Biostimulant Materials

Algae extract: The source of algae was the Algal Biotechnology Unit, NRC., Egypt. The chemical composition, mineral content, HPLC chromatogram hormones, and amino acid content of the algae extract are available in Table 1 and Table 2.

By-product of yeast production (CMS): Condensed molasses soluble (CMS) are by-products of various fermentation processes in yeast production. The chemical analysis of by-product of yeast production is available in Table 3.

Table 1: Chemical composition and mineral of algae extract and HPLC chromatogram hormones of algae extract.

	%						Ppm			
Element	N	P	K	Mg	Na	Ca	Fe	Zn	Mn	Cu
Conc.	13.30	2.22	2.13	0.22	0.01	0.33	1936.00	68.00	21.00	18.00
HPLC chromatogram hormones of algae extract sample, mg. g^{-1}										
	Indole acetic acid			Indole butyric acid				Gibberellic acid		
13.66				3.25				1.19		

Table 2: Amino acids content of the used algae extract

Amino acid	Appreviation	Concentration	Amino acid	Appreviation	concentration
Aspartic	ASP	1.85	Threonine	THR	0.83
Serine	SER	0.70	Glutamic	GLU	2.24
Proline	PRO	0.67	Glycine	GLY	1.07
Alanine	ALA	1.55	Valine	VAL	1.11
Methionine	MET	0.33	Isoleucine	ISOL	0.71
Leucine	LEU	0.29	Tyrosine	TYR	0.53
Phenylalanine	PHE	0.87	Histidine	HIS	0.24
Lysine	LYS	0.70	Arginine	ARG	0.98
Cysteine	CYC	0.22			
Total amino acids		15.89			

Table 3: Chemical analysis of by-product of yeast production (CMS)*

Amino acid(g ⁄100 ml)		Nutrient content(%)		Growth Regulators(mg/L)	
Aspartic acid	0.58	Total Amino Acid	20.00	Cytokine	762.6
Threonine	0.14	Free Amino Acid	7.00	Gibriline	495.2
Serine	0.21	Total –N	4.62	Others	
Glutamic acid	4.80	P_2O_5	0.20	(%)	
Glycine	0.25	K_2O	9.80	Organic matter	59.75
Alanine	0.38	Ca	0.87	Organic carbon	34.66
Valine	0.17	Mg	0.16	-	
Isoleucine	0.12	S	10.04	pH	7.23
Leucinc	0.17	(mg/L)			
Phenyl alanine	0.10	B	8.5		
Hisitidine	0.04	Mo	5.3		
Lysine	0.13	Fe	71.0		
Arginine	0.04	Mn	11.3		
Proline	0.14	Zn	483.9		
Cystine	0.03	Cu	5.3		
Methionine	0.06				

Treatments were: 100% Control (recommendation from fertilizers)**,** 100% recommendation + 10 ml/liter Algae extract,100% recommendation + 15 ml/liter Algae extract, 100% recommendation + 20 ml/liter Algae extract, 100% recommendation + 10 ml/liter CMS, 100% recommendation + 15 ml/liter CMS, and100% recommendation + 20 ml/liter CMS.

2.3. Greenhouse experiment (Nursery of scion and rootstocks; grafting)

The grafting experiment was carried out to produce the seedlings in nursery located in El kottna area Dakahlia governorate during 2022 and 2023 seasons. In the first season, the watermelon seeds of section were sown in the nursery on 20th January 2022, while the rootstock seeds were sown on 1st February 2022. In second season, the watermelon seeds of section were sown in the nursery on 16th January 2023, while the rootstock seeds were sown on 28th January 2023 (The seeds of the scion were sown in nursery 10: 13 days earlier than the seeds of the rootstocks to ensure both seedlings reach the right stem size for grafting at the same time). The experimental design was completely randomized blocks with three replications.

Seeds of sections and rootstock were sown in 216-cell Styrofoam trays filled with a mixture of peat moss and perlite at the ratio of 7:3 (v: v) enriched by 1g potassium sulfate, 1 g calcium nitrate, 2 g mono potassium phosphate and 0.25 g fungicide for each 1 liter of the mixture. During the greenhouse experiment fertilization applied during irrigation and the solution content has all macro and micro elements (ml-mol /liter), its electric conductivity (EC) was 1.8: 2.0 ms and its component was as follow in Table 4.

Table 4: Chemical analysis of the fertigation solution

Elements	NH_4^+	K^+	Na^+	Ca^{++}	Mg^{++}	NO_3^-	Cl-	$S0_4^{--}$	$H_3PO_4^-$	Fe	Mn	Zn	B	Cu	Mo
Conc.	12.18	1.42	3.30	5.26	4.45	20.71	1.40	5.03	0.11	16.3	105.0	22.5	104.6	4.3	1.3

The fertilizers were put on three tanks which are diluted in water, tank (A); ammonium nitrate (2.0g/liter), calcium nitrate (2.5g/liter), Fe EDEHA (0.5g/liter). Tank (B); potassium sulfate (1.6g/liter), mono potassium phosphate (3.0g/liter) and Tank (C); magnesium nitrate (1.0g/liter), Micro elements (0.25g/liter).

The grafting was conducted when the rootstocks and scion were ready to graft (10-13 days after rootstocks seeds were sown). The grafted seedlings were located under plastic tunnel completely closed under temperature (24–26◦C) and humidity (>85%RH) for 7 days for acclimatization and hardening which were the important factors for the survival of grafted plants. The tunnel was left completely closed for 3 days. After that, the hardening process started on the fourth day by opening the tunnel for 2-3 hours followed by wetting the grafted plants with fine water before sealing the tunnel again. The tunnel was again opened for 6 hours or less on the fifth day and re-opened for half a day in the sixth day, and the tunnel plastic cover was removed entirely on the seventh day of grafting. Grafted seedlings were applied with biostimulant products with recommended levels of the fertilizers one time before delivering the seedlings to the open field

2.4. Field experiment

The field experiment was carried out on a privet farm in Dakahlia government. Grafted seedlings were transplanted on 14th March 2022 and 10th March 2023 in rows 3.0 m in width, 9 m in length and spaced 1.0 m apart in the open field. The graft union of grafted seedlings was kept above the soil surface to avoid development of adventitious roots from the scion that connect the soil that may lead to infection and death of the entire plant.

The experimental design was completely randomized blocks with 7 treatments (6 biostimulants products) + control (recommendation) with 3 replicates and the plot area was 81 m^2 included 27 plants. In both seasons, all field practices (irrigation, fertilization, weeding, and pest control) were performed according to the recommendations of the Egyptian Ministry of Agriculture for watermelon production. Mean physical and chemical characteristics of soil of the experimental site were determined according to Chapman & Pratt [34] and are shown as follows: Texture: clay, pH 7.93, EC (ds/m) 2.42, N 30.3, P 19.21, K 670, Fe 21.1, Mn 7.5, Zn 2.24, Cu 24ppm.

Applying the treatments during growth stages: vegetative growth, flowering and fruit Set, fruit development; the treatments of the biosimulate products were applied by foliar application one time during each stage.

2.5. Data recorded

2.5.1. Seedlings growth characters

Seedlings growth characters were recorded after 50 days from planting date in nursery. Samples of four Seedlings were chosen randomly from each treatment to evaluate Chlorophyll as SPAD readings by Minolta chlorophyll meter, Seedling length (cm) and Seedling diameter (mm).

2.5.2. Vegetative growth characters

Vegetative growth characters were recorded after 50 days in field on samples of four plants were chosen randomly from each plot to record the following parameters,[35]Number of leaves per plant, Number of branches per plant, Plant length (m)

2.5.3. Yield and fruit characters

Total yield after harvest all fruit (early yield and final yield) according to Islam et al., and Petropoulos et al.[36, 37]The fruits were harvested two harvest times, after 85 and 95 days from transplanting, respectively. Three ripen fruits were randomly collected from each experimental plot's subsamples for fruit quality measurements, thickness peel, fruit weight, fruit length and fruit diameter.

2.5.4. Fruit quality

Three fruits were taken randomly from each plot and subjected to measure content of total soluble solids (TSS) in fruit juice by using a hand refract meter. This was estimated according to the methods of AOAC international [38]. Total sugar (TS %) was determined using the sulfuric acid/phenol technique described by Dubois et al.[39]

2.5.5.Nutrient content of leaves

Leaf samples from the fourth upper leaf of 6 plants were taken at 50 days from transplanting was used to determine nutrient content according to Cottenie et al. [40]

2.6. Statistical analysis

The data will be triplicate and analyzed by analysis of variance (ANOVA) using static 8 computer program. The significance of differences will determine according to DMRT. P values ≤0.05 were considered to be significant. [40]

3. Results

3.1. Greenhouse experiment

3.1.1. Effect of biostimulants application on Seedling growth characters:

Seedling growth characters (chlorophyll, Seedling length and stem diameter) are presented in (Table 5 and Pic 2). The highest chlorophyll index was recorded in plants treated with CMS at 20 ml/L, with values of 51.30 and 52.17 in the 2022 and 2023 seasons, respectively, resulting in an overall mean of 51.73. This treatment was statistically superior to all others. The next highest chlorophyll index was recorded for the algae extract at 20 ml/L, with a mean of 50.60 for 2022 and 2023. The control treatment consistently recorded the lowest chlorophyll values, (46.00 and 45.96 in 2022 and 2023, respectively). Both CMS and algae extract treatments significantly enhanced chlorophyll index compared to the control. It also shows that the application of CMS at 20 ml/L resulted in the longest seedlings, with a mean length of 17.37 cm across seasons. This was followed closely by the algae extract at 20 ml/L, which achieved a mean seedling length of 16.50 cm across seasons. There was no statistically significant difference between these two treatments, indicating comparable effectiveness. The control treatment recorded the shortest seedling length; with a mean of 12.27 cm across seasons. The algae extract also showed consistent performance across different concentrations, with mean seedling lengths of 12.98 cm and 14.05 cm at 10 and 15 ml/L, respectively.

As shown in Table 5 **and Figure 2**, the highest stem diameter was recorded with the application of CMS at 20 ml/L, reaching a mean value of 0.32 mm. This was followed closely by algae extract at 20 ml/L, which produced a mean stem diameter of 0.31 mm. The lowest stem thickness was observed in the control treatment, with a mean of 0.27 mm. These findings indicate that the application of both CMS and algae extract at higher concentrations positively affected stem development, contributing to greater seedling vigor and structural robustness. Memorable, CMS at 20 ml/L consistently performed best across all measured seedling parameters during both growing seasons.

Figure 2: Effect of biostimulants application on grafted watermelon seedlings.

Table 5: Effect of biostimulants application on chlorophyll index, seedling length and diameter of watermelon during 2022 and 2023 seasons

Biostimulants Treatments		Chlorophyll index				Seedling length(cm)				Seedling diameter (mm)			
		2022		2023		2022		2023		2022		2023	
CMS	10	48.43	ab	47.03	bc	14.03	c	13.17	d	0.291	e	0.293	b
	15	48.90	ab	50.37	ab	15.57	b	13.97	c	0.297	c	0.300	b
	20	51.30	a	52.17	a	17.80	a	16.93	a	0.316	a	0.318	a
Algae extract	10	46.57	b	45.40	c	12.87	d	13.10	d	0.281	f	0.285	c
	15	48.87	ab	47.17	bc	14.17	c	13.93	c	0.295	d	0.296	b
	20	49.50	ab	51.70	a	16.97	a	16.03	b	0.314	b	0.315	a
Control		46.00	b	45.96	c	12.63	d	11.90	e	0.269	g	0.269	d

3.2. Field experiment

3.2.1. Effect of biostimulants foliar application on vegetative growth characters

Both CMS and Algae extract significantly increased the Vegetative growth characters and that shown in Table 6**.** At 20 ml/L of CMS, produced the highest total number of leaves (Mean = 245.00), reveling a significant dose- dependent response. The control number of leaves also consistently had the lowest leaf count (Mean = 132.51) representing the positive effects of the biostimulants. CMS at 20 ml/L increases leaf numbers above algae extract and control by 84.9% and 62.13, respectively.

Table 6andFigure 3 show that the highest number of branches was recorded with the application of CMS at 20 ml/L, which produced a mean value of 6.41 branches per plant. This was followed by algae extract at the same concentration, with a mean value of 5.62 branches per plant. The control treatment resulted in the lowest number of branches, with a mean of 3.53. These data indicate a notable increase in branching due to the application of biostimulants, particularly CMS at 20 ml/L, which outperformed both algae extract and the untreated control. The increase in branch number per plant for the CMS treatment was 81.86 % compared to the control.

As presented in Table 6 and Figure 3, the tallest plants were recorded in the CMS with 20 ml/L treatment, with a mean length of 3.29 m, followed closely by the Algae extract 20 ml/L treatment (Mean = 3.14 m). Lower concentrations of both treatments resulted in intermediate plant length, suggesting a possible dose-response effect. The control treatment produced the shortest plants, with a mean length of 2.48 m. In terms of relative improvement over the control, Algae extract at 20 ml/L resulted in a 26.6% relative increase in plant length, whereas CMS at the same concentration increased by 32.66%. These results indicate that both biostimulants significantly enhanced plant length compared to untreated controls.

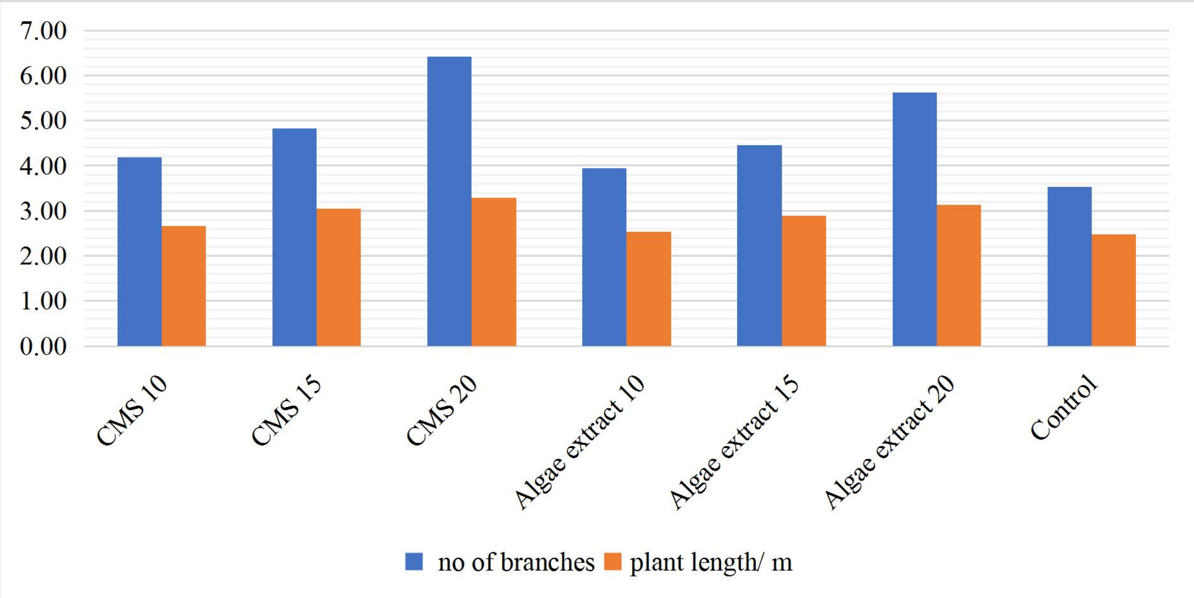

Figure 3: Effect of biostimulants foliar application on number of branches/plant and plant length of watermelon during 2022 and 2023 seasons

Table 6: Effect of biostimulants foliar application on number of leaves, number of branches and plant length of watermelon during 2022 and 2023 seasons.

Biostimulants Treatments		number of leaves/plants				number of branches/plants				Plant length (m)			
		2022		2023		2022		2023		2022		2023	
CMS	**10**	160.67	d	163.44	c	4.03	d	4.34	d	2.76	c	2.57	c
	15	187.67	c	218.33	b	4.73	c	4.93	c	2.90	bc	3.18	ab
	20	247.67	a	242.33	a	6.17	a	6.66	a	3.21	a	3.37	a
Algae extract	**10**	156.67	d	158.00	c	3.80	d	4.08	e	2.54	d	2.52	c
	15	178.00	c	165.67	c	4.47	c	4.45	d	2.76	c	3.00	b
	20	204.67	b	225.00	b	5.50	b	5.73	b	3.09	ab	3.19	ab
Control		122.33	e	142.69	d	3.40	e	3.66	f	2.47	d	2.48	c

3.2.2. Effect of biostimulants foliar application on yield and fruits characters

As shown in Table 7, thickness peel increased significantly with higher doses of CMS. In 2023, the thickest peel (1.62 cm) was recorded for the 20 ml/L CMS treatment. Across both seasons, this treatment also achieved the highest mean peel thickness (1.57 cm), significantly higher than the control (1.02 cm). Algae extract exhibited a similar trend, though with slightly lower values; the 20 ml/L algae extract treatment resulted in a mean peel thickness of 1.40 cm. In both years of the study, the control treatment consistently recorded the lowest peel thickness values. Particularly, the 20 ml/L CMS treatment yielded the largest increase in peel thickness, representing a 53.92% improvement compared to the control. The most pronounced effects for both biostimulants were observed at the highest concentration (20 ml/L), demonstrating a clear dose-dependent relationship in peel thickness enhancement.

CMS treatments significantly increased fruit weight (Table 7), with the highest mean (11.20 kg) recorded at the 20 ml/L concentration. Algae extract also led to improved fruit weight, though to a lesser extent, with the highest mean value (10.34 kg) observed at the 15 ml/L concentration. The control treatment had the lowest mean fruit weight (8.81 kg) across both seasons. Among all treatments, CMS at 20 ml/L achieved the greatest enhancement in fruit weight, showing a 27.13% relative increase over the control. Algae extract treatments in the 15–20 ml/L range also resulted in notable increases in fruit weight, although slightly below the levels achieved with CMS.

Table 7: Effect of biostimulants foliar application on thickness peel and fruit weight of watermelon during 2022 and 2023 seasons.

Biostimulants Treatments		thickness peel (mm) 2022		thickness peel (mm) 2023		fruit weight (kg) 2022		fruit weight (kg) 2023	
CMS	10	1.17	bc	1.12	cd	9.79	c	10.27	c
	15	1.40	a	1.28	b	10.33	b	10.65	bc
	20	1.53	a	1.62	a	10.97	a	11.43	a
Algae extract	10	1.10	cd	1.12	cd	8.83	d	9.06	d
	15	1.24	b	1.14	c	10.06	bc	10.62	bc
	20	1.44	a	1.37	b	10.90	a	11.03	ab
Control		1.00	d	1.04	d	8.92	d	8.70	d

Table 8 and Figure 4 present data of the effect of biostimulants foliar application on fruit length, fruit diameter and yield / plant of watermelon during 2022 and 2023 seasons. The highest fruit length was recorded with the application of CMS at 20 ml/L, reaching 27.95 cm, corresponding to a 13.72% relative increase over the control. Algae extract at 20 ml/L produced a slightly lower but still considerable fruit length of 27.66 cm. The control treatment yielded the shortest fruits, with an average length of 24.62 cm. In the 2023 season, algae extract at 20 ml/L again produced the longest fruits of 27.87 cm, while the control recorded the lowest mean fruit length of 24.03 cm.

The highest fruit diameter (Table 8 and Figure 4) was recorded with the application of algae extract at 20 ml/L (mean of 25.67 cm), while the control treatment exhibited the lowest fruit diameter (21.60 cm). In terms of fruit diameter, CMS at 20 ml/L recorded the highest values. Both CMS and algae extract treatments at 20 ml/L significantly increased fruit length and diameter compared to the control, with the most substantial improvements observed at the highest concentration applied.

Table 8 and Figure 4present the impact of two biostimulants—algae and CMS—applied at three different doses (10, 15, and 20 ml/L) on the yield per watermelon plant during the 2022 and 2023 seasons, compared to a control. The data reveals a clear trend where increased doses of biostimulants positively influenced watermelon yield across both seasons, with CMS at 20 ml/L, yielding the highest production. The use of biostimulants such as algae and CMS significantly enhanced the yield per plant in both years compared to the control. particularly: CMS at 20 ml/L was the most effective treatment in both seasons, showing 24.16% increase in 2022 and 31.36% increase in 2023 compared with control. Algae at 20 ml/L also provided high yield improvement, nearly matching CMS, with 23.42% (2022) and 22.07% (2023) increases compared with control.

Table 8: Effect of biostimulants foliar application on fruit length, fruit diameter and yield / plant of watermelon during 2022 and 2023 seasons.

Biostimulants Treatments		fruit length (cm) 2022		fruit length (cm) 2023		fruit diameter (cm) 2022		fruit diameter (cm) 2023		Yield / plant (kg) 2022		Yield/plant (kg) 2023	
CMS	10	26.00	b	25.87	bc	24.12	c	24.56	cd	34.26	c	35.95	c
	15	27.60	a	27.37	a	25.16	b	25.27	bc	36.16	b	37.82	bc
	20	27.95	a	27.87	a	26.70	a	26.65	a	38.38	a	40.00	a
Algae extract	10	25.31	bc	25.23	c	23.30	d	23.90	d	31.23	d	31.71	d
	15	26.17	b	26.31	b	25.15	b	25.27	bc	35.21	bc	37.17	bc
	20	27.66	a	27.87	a	25.20	b	25.67	b	38.15	a	38.61	ab
Control		24.62	c	24.03	d	22.27	e	21.60	e	30.91	d	30.45	d

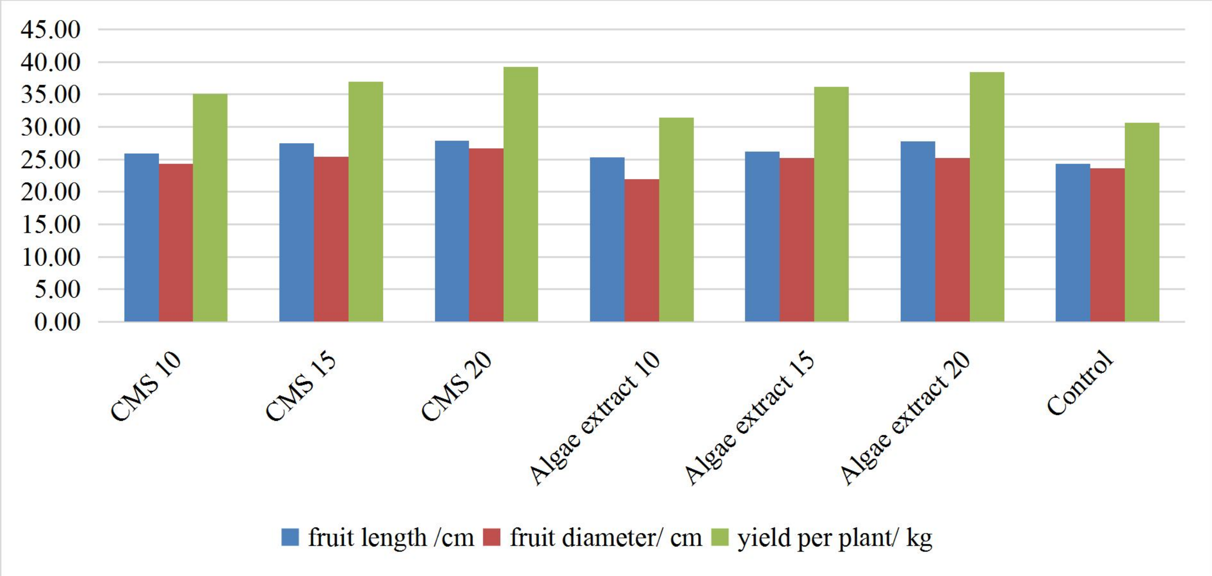

Figure 4: Effect of biostimulants foliar application on fruit length, fruit diameter and yield/ plant of watermelon during 2022 and 2023 seasons

3.2.3. Effect of biostimulants foliar application on fruits quality characters:

In 2022, as presented in Table 9 and **Figure 5**, the highest total soluble solids (TSS) value was observed in the CMS treatment at 20 ml/L, recording 12.83%, while the control recorded the lowest value of 9.78%. In 2023, the same trend was noted, with CMS at 20 ml/L achieving the highest TSS value of 12.59% compared to the control of 9.18%. Algae extract at 20 ml/L also demonstrated an improvement in TSS across both years, with a noted increase of 19.41% over the control. Overall, CMS at 20 ml/L achieved the most substantial increase in TSS across both years, with a mean enhancement of 34.07%.

As shown in Table 9 and **Figure 5** and **Figure 6**, the highest total sugar percentage was observed with the application of CMS at 20 ml/L, which recorded a mean value of 10.44%. Algae extract at 20 ml/L also led to an important increase in sugar content, reaching a mean value of 10.34%. In contrast, the control treatment showed the lowest mean sugar content of 9.31%. The CMS treatment at 20 ml/L demonstrated the most pronounced increase in total sugars, with a percentage gain of 12.14% over the control. Algae extract at 20 ml/L followed closely, contributing to a 11.06% increase compared to the control.

Table 9: Effect of biostimulants foliar application on TSS and Total sugar of watermelon during 2022 and 2023 seasons.

Biostimulants Treatments		TSS %				Total sugar %			
		2022		2023		2022		2023	
CMS	**10**	10.03	d	10.00	d	9.50	cd	9.82	ab
	15	10.83	c	10.49	c	9.97	abc	10.18	ab
	20	12.83	a	12.59	a	10.34	a	10.53	a
Algae extract	**10**	10.00	d	9.83	d	9.25	d	9.53	b
	15	10.18	d	10.16	cd	9.70	bcd	10.13	ab
	20	11.30	b	11.34	b	10.25	ab	10.43	a
Control		9.78	d	9.18	e	9.15	d	9.47	b

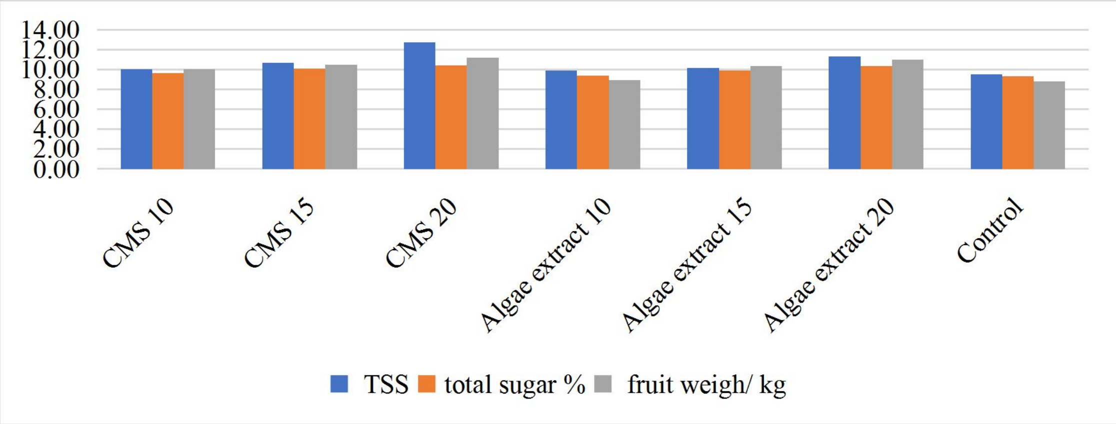

Figure 5:Effect of biostimulants foliar application on TSS, Total sugar and fruit wight of watermelon during 2022 and 2023 seasons

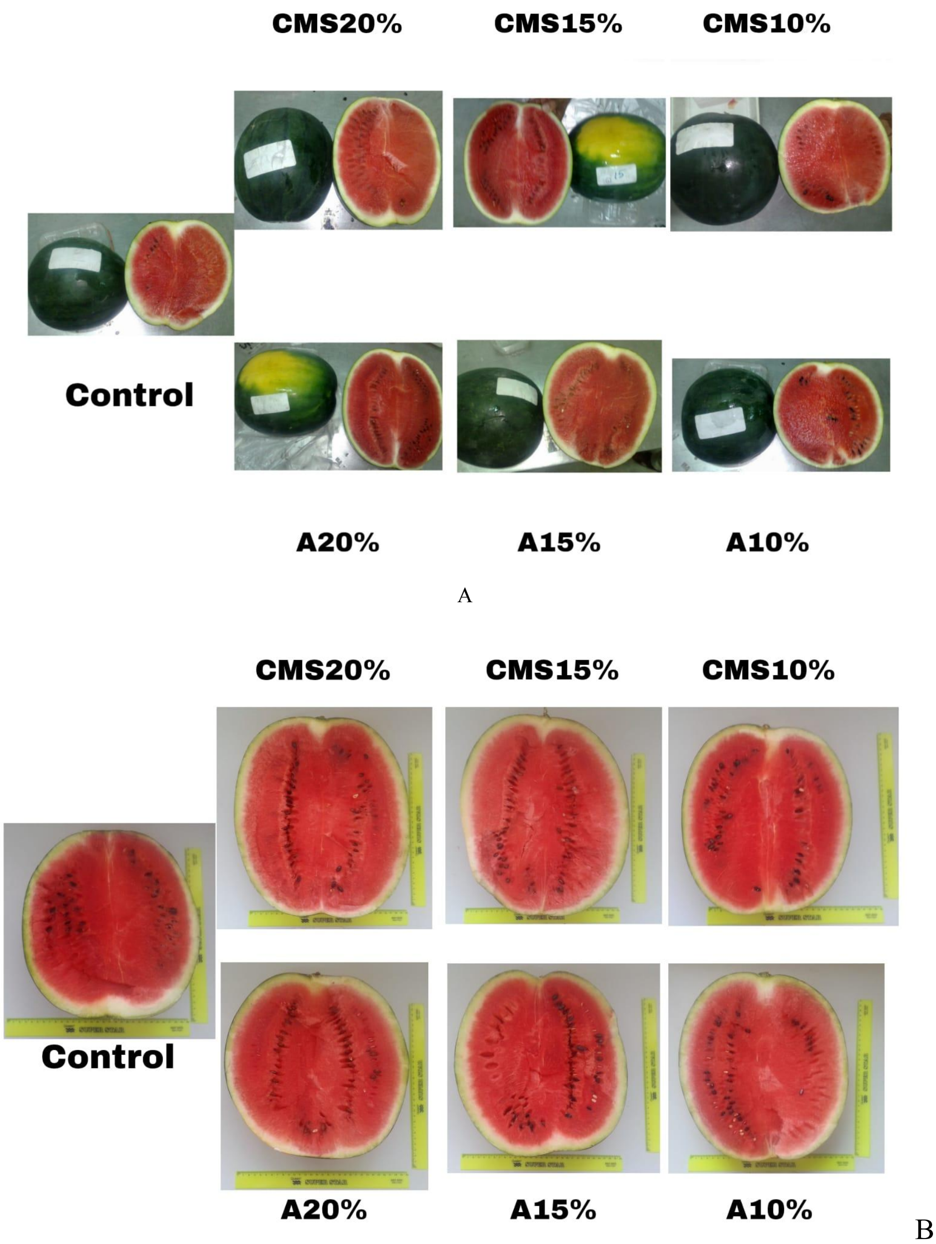

Figure 6: Effect of biostimulants application on yield quality of watermelon

3.2.4. Effect of biostimulants foliar application on Nutrient content of leaves:

As shown in Table 10, the highest nitrogen (N %) concentration was recorded with the CMS treatment at 20 ml/L, reaching a mean value of 3.43%. The algae extract at the same concentration also resulted in a high N% value of 3.40%. In contrast, the control treatment had the lowest N% of 2.65%. The greatest percentage increase in nitrogen content (29.43%) was observed with the CMS 20 ml/L treatment compared to the control.

The data presented in Table 10show that the highest phosphorus content (P%) was observed in the CMS treatment at 20 ml/L, with a value of 0.79%, followed closely by the algae extract at 20 ml/L (0.78%). The control treatment recorded the lowest P% of 0.58%. The greatest improvement in phosphorus content was observed with the CMS 20 ml/L treatment, representing a +36.21% increase compared to the control.

The potassium content (K %) **(**Table 10**)** was the highest in the CMS treatment at 20 ml/L, with a recorded value of 5.32%. The algae extract at 20 ml/L also resulted in a high K% value of 5.26%. The lowest K% was recorded in the control treatment of 4.97%. The CMS at 20 ml/L demonstrated the greatest improvement over the control in K%, representing a 7.04% increase compared to the control.

Table 10: Effect of biostimulants foliar application on N%, P% and K% of watermelon leaves during 2022 and 2023 seasons.

Biostimulants Treatments		N%				P%				K%			
		2022		2023		2022		2023		2022		2023	
CMS	**10**	3.10	b	3.22	b	0.67	b	0.69	d	5.09	d	5.12	e
	15	3.22	ab	3.48	a	0.74	a	0.76	b	5.17	c	5.22	c
	20	3.33	a	3.53	a	0.78	a	0.80	a	5.29	a	5.34	a
Algae extract	**10**	2.92	c	3.16	b	0.60	c	0.62	e	5.07	d	5.12	e
	15	3.19	ab	3.25	b	0.72	ab	0.73	c	5.16	c	5.18	b
	20	3.29	a	3.52	a	0.78	a	0.79	ab	5.24	b	5.28	b
Control		2.61	d	2.69	c	0.57	c	0.59	f	4.94	e	5.00	f

Table 11 presents the effects of two biostimulants—CMS and algae extract—applied at three concentrations on magnesium (Mg) content in watermelon leaves. The highest magnesium content was recorded in the treatment with CMS at 20 ml/L (0.42%), followed closely by the algae extract at 20 ml/L (0.40%) and shows that calcium (Ca) content in watermelon leaves was the highest with the application of CMS at 20 ml/L. The algae extract at 20 ml/L also resulted in a notable increase in calcium content, though slightly less than that observed with CMS.

Table 11 indicates that the Iron content was significantly increased by CMS 20 ml/L (53.03 ppm) - possibly due to the soil being more acidic and Fe in greater availability. Algae extract 20 ml/L increased Iron content by also allowing access to Fe by siderophores and/or phytohormone-like effects. Control had Iron content of 44.10 ppm, the high Fe level for the CMS 20 ml/L treatment (53.03 ppm) increased from the peak Fe concentration by 20%, Algae extract at 20 ml/L increased Fe (49.47 ppm), however the lower doses, especially 10 ml/L did show less increase.

Table 11: Effect of biostimulants foliar application on Mg%, Ca% and Fe ppm of watermelon leaves during 2022 and 2023 seasons.

Biostimulants Treatments		Mg%				Ca%				Fe (ppm)			
		2022		2023		2022		2023		2022		2023	
CMS	**10**	0.31	d	0.33	e	3.85	d	3.88	d	44.77	c	45.33	cd
	15	0.35	c	0.37	d	3.91	c	3.93	c	45.67	c	48.00	bc
	20	0.41	a	0.43	a	4.12	a	4.18	a	52.07	a	54.00	a
Algae extract	**10**	0.30	de	0.33	e	3.74	d	3.78	d	44.00	c	44.83	d
	15	0.35	c	0.34	c	3.84	b	3.83	b	45.00	c	47.00	bcd
	20	0.39	b	0.40	b	4.04	b	3.93	b	49.50	b	49.43	b
Control		0.29	e	0.31	f	3.72	d	3.80	d	43.93	c	44.27	d

The data copper (Cu) content represented **in** Table 12 shows that the copper (Cu) content in watermelon leaves varies significantly among treatments. The highest Cu content was recorded with the CMS

20 ml/L treatment, reaching 33.80 ppm, which represented a 17.36% increase compared to the control. The Algae extract at 20 ml/L also showed a strong response, with a Cu content of 33.05 ppm, corresponding to a 14.76% increase. The control treatment exhibited the lowest Cu content of 28.80 ppm. All biostimulant treatments led to increased Cu content compared to the control.

Table 12 reported the zinc (Zn) content in watermelon leaves under different treatments. The control treatment showed a mean Zn content of 37.78 ppm. The highest Zn content was observed with CMS at 20 ml/L, reaching 44.83 ppm, representing an 18.66% increase over the control. The Algae extract at 20 ml/L also improved Zn content to 43.65 ppm and a 15.5% relative increase compared to the control. All treatments significantly increased Zn levels compared to the control, with CMS consistently outperforming Algae extract across all concentration.

In Table 12**,** the mean manganese (Mn) content in the control treatment was 22.83 ppm. The CMS treatment at 20 ml/L increased Mn to 24.76 ppm, representing an 8.45% increase over the control. The Algae extract at 20 ml/L showed a moderate increase in Mn content to 23.95 ppm and a 4.9% improvement compared to the control.

Table 12: Effect of biostimulants foliar application on Cu, Zn and Mn ppm of watermelon leaves during 2022 and 2023 seasons.

Biostimulants Treatments		Cu ppm				Zn ppm				Mn ppm			
		2022		2023		2022		2023		2022		2023	
CMS	**10**	30.50	b	31.48	c	41.81	c	41.97	e	23.00	e	23.18	d
	15	31.33	b	32.47	b	42.57	bc	43.17	c	23.33	c	23.64	c
	20	33.50	a	34.10	a	44.50	a	45.17	a	24.61	a	24.92	a
Algae extract	**10**	29.31	c	29.57	d	40.90	d	41.93	e	22.93	f	23.15	d
	15	30.80	b	31.70	c	42.07	c	42.57	d	23.07	d	23.49	c
	20	32.83	a	33.50	a	43.20	b	44.10	b	23.71	b	24.19	b
Control		28.47	c	29.13	d	37.47	e	38.10	f	22.55	g	23.10	d

4. Discussion

4.1. Seedling growth characters

The observed increase in chlorophyll index in plants treated with 20 ml/L CMS (mean values of 51.73 in 2022, 52.10 in 2023) aligns with previous studies. Similar enhancements were reported in grafted plants [41-44] and in response to beet molasses carbon dots [45] and molasses treatments in pepper and tomato. [46, 47]These effects are attributed to cytokinin and other growth regulators in molasses and algae extracts, which enhance chlorophyll synthesis and delay senescence,[45, 47, 48] supported by nitrogen's role in chlorophyll structure**)**.[49] Seedling length (11.90–17.80 cm) was consistent with findings by Basavaraja et al.[50] and Muthurakku, O. and Sadhana, B.[51]which highlighted the role of algal extracts in enhancing growth via hormones and improved photosynthesis. [52]also noted that CMS improves early vegetative development, while Karaağaç [53]and Yang et al.[54]emphasized the influence of treatments and environment on hypocotyl growth. Mean stem diameters reached 0.32 mm (molasses) and 0.31 mm (algae), supported by reports that algal biostimulants, rich in auxins and brassinosteroids, enhance stem development. [55, 56]Overall, the improvements in chlorophyll content, seedling length, and stem thickness confirm that biostimulants promote early seedling vigor through nutritional and hormonal pathways.

4.2. Vegetative growth characters

The application of CMS and algae extract at 20 ml/L significantly enhanced the vegetative growth parameters of watermelon compared to the control (Table 6). CMS treatment resulted in an increase in number of leaves by 62.40% (2022) and 45.79% (2023), branches by 46.47% (2022) and 45.08% (2023), and plant length by 19.84% (2022) and 22.58% (2023). Algae extract produced similar trends, with respective increases of 46.96% and 28.17% in leaf number, 35.00% and 29.23% in branches, and 13.36% and 16.94% in plant length across the two seasons. These results align with findings by Goñi et al.,[57] Ali et al. [58] **Zaki et al.** and Nugroho et al., [59] who emphasized the role of leaf number as an indicator of photosynthetic potential and plant sustainability. CMS, rich in sugars, amino acids, and micronutrients, supports growth through leaf initiation and expansion. Algal extracts, containing hydroxylated algal acids and phytohormones, promote chloroplast development and cell division, thereby enhancing vegetative growth—a mechanism also supported by Rouphael et al.[60]The observed improvements in branching further highlight the biostimulants' role in promoting stem and shoot development, with CMS showing superior efficacy. Previous studies [24, 57, 61]have attributed such effects to enhanced nutrient uptake and hormonal balance restoration. Algal extracts also stimulate the expression of expansin genes, facilitating cell wall loosening and elongation, contributing to increased shoot and branch formation. Contrasting views were reported by Khalid et al.,[62] who favored grafting as a more sustainable solution for increasing plant vigor and mitigating climate-related stress. Rootstocks such as *Cucurbita moschata* have been shown[63, 64]to enhance early growth and disease resistance in grafted plants. Similarly, Mohamed and El-Tawashy[65]reported improved strength and length in grafted plants. Further supporting the role of algae-based products, several studies [66, 67] indicated that algal extracts, rich in macro- and micronutrients, improve plant vigor, water absorption, and nutrient uptake while enhancing resistance to pathogens. Likewise, sugar beet molasses has been shown to improve soil microbial activity and nutrient efficiency, [46, 68]with economic benefits such as reduced fertilizer use and increased cost efficiency.

4.3. Yield and fruits characters

The application of CMS and algae extract at 20 ml/L significantly improved various fruit quality and yield parameters in watermelon. CMS at 20 ml/L produced the greatest increases in peel thickness (by 54.90% over the control), fruit weight, length, and diameter, as well as yield per plant across both 2022 and 2023. These enhancements reflect the biostimulants' ability to promote both vegetative and reproductive development. In terms of fruit quality, CMS 20 ml/L application led to the highest increase in total soluble solids (TSS), with an improvement of 34.07%, and recorded the highest total sugar content at 12.14%. Peel thickness—a critical trait for postharvest durability and shelf life—was important enhanced by both biostimulants, likely due to improved uptake of calcium and boron, facilitated by increased microbial activity in the rhizosphere. [69]Algal extracts, through activation of secondary metabolic pathways and pectin biosynthesis, also contributed to cell wall reinforcement and peel firmness. [58, 70]

Improvements in fruit weight and morphology (length and diameter) were associated with enhanced vegetative growth (e.g., increased leaf and branch number), leading to better photosynthetic efficiency and carbohydrate allocation. Similar effects have been reported in various crops with foliar applications of biostimulants.[71, 72] These effects are attributed to the presence of trace elements, plant growth hormones, and organic matter in CMS and algae extracts, which stimulate nutrient uptake and metabolic activity.[72, 73]

The observed enhancement in TSS and total sugars likely stems from the rich content of carbohydrates, vitamins, and micronutrients in molasses, which promote sugar biosynthesis and enzymatic activity. Algae extract's phytohormones (e.g., cytokinin and auxins) further enhance photosynthesis and nutrient translocation. [57]Supporting studies have reported increased TSS and sugar accumulation in various crops following biostimulant applications.[74-76] Moreover, biostimulant treatments may up regulate sugar metabolism genes (e.g., sucrose synthase, invertase, sugar transporter genes), leading to better sugar partitioning into the fruit. [58] These findings suggest that biostimulants not only improve fruit yield but also enhance overall fruit quality, contributing to greater marketability and postharvest performance.

4.4. Nutrient content of leaves

Data reveals that CMS at 20 ml/L resulted in the highest concentrations of N (3.43%), P (0.79%), and K (5.32%), slightly surpassing algae extract at the same concentration (N: 3.40%, P: 0.78%, K: 5.26%). Compared to the control, molasses achieved a 7.05% increase in K%. The enhanced nitrogen levels with CMS are likely due to its rich organic carbon content, which promotes microbial activity and nitrogen mineralization, improving N availability. [69]Algal extracts, rich in amino acids and growth regulators, further support nitrogen metabolism and uptake. [57] The increase in phosphorus content is attributed to organic acids in CMS and chelating compounds in algae extract, which solubilize bound phosphorus and facilitate its uptake. These effects align with findings by El-Nwehy et al. and zaki et al. [24, 77, 78] highlighting enhanced nutrient accumulation under biostimulant treatments. Potassium accumulation improved due to biostimulant-induced enhancement of root function, microbial mineralization, and the presence of cytokinin that stimulate photosynthesis and biomass production.[79, 80] Both biostimulants thus enhance macro-nutrient acquisition through synergistic effects on soil biology and plant physiology.

Data in Table 11 shows that CMS at 20 ml/L resulted in the highest uptake of Mg (0.42%), Ca (4.15 %) Fe (53.03 ppm), and Cu. With algae extract at 20 ml/L closely following for all elements (Mg: 0.40%, Fe: 49.47 ppm). Compared to the control, CMS improved Fe content by ~20%, while lower concentrations showed reduced efficacy. The enhanced Mg uptake is linked to improved chlorophyll synthesis, cation exchange capacity, and microbial activity induced by organic acids and polysaccharides in both biostimulants.[79, 81]Calcium accumulation increased due to improved root development and chelation effects from CMS and algae extracts. Phytohormones like auxins and cytokinin in algae stimulated root proliferation, enhancing Ca uptake critical for cell wall integrity and fruit quality. [57] These effects support micronutrient solubility, transporter activation, and overall nutrient assimilation.[82]

Data in Table 12shows that CMS at 20 ml/L achieved the highest uptake of Cu (33.80 ppm), Zn (44.83 ppm), and Mn (24.76 ppm). With algae extract at 20 ml/L closely following (Cu: 33.05 ppm, Zn: 43.65 ppm, Mn: 23.95 ppm). Compared to the control, CMS improved Cu and Zn by ~17–19% and Mn by ~8%; algae extract also showed consistent increases but with slightly lower efficacy. The enhanced Cu and Zn uptake is linked to improved chelation, microbial stimulation, and cation exchange capacity induced by CMS. These mechanisms increase solubility and transport of trace elements and activate nutrient transporter pathways. Algae extract improved micronutrient uptake through hormone-driven root proliferation and acidification of the rhizosphere, enhancing metal bioavailability. Zn uptake benefited from auxin-mediated root expansion and biochemical interactions in the rhizosphere. Mn uptake, while generally lower in response magnitude, increased due to chelation and root zone acidification, with algae extract potentially favoring Mn solubility via phytohormone-containing exudates and pH modulation.[83]

5. Conclusion

CMS showing superior efficacy enhance chlorophyll content, seedling growth, and stem thickness by supplying growth regulators and nutrients, thereby improving early seedling vigor. The application of CMS (20 ml/L) significantly boost watermelon growth by enhancing leaf development, branching, and plant elongation, also enhances watermelon yield, fruit quality, and postharvest durability by improving nutrient uptake, photosynthetic efficiency, and sugar metabolism. These biostimulants offer a sustainable strategy to boost both productivity and marketability in watermelon cultivation. CMS as biostimulants improve nutrient availability through microbial activation, chelation, and hormonal regulation. Biostimulants, especially microalgae-based ones, are emerging as sustainable tools in modern agriculture to enhance plant growth, nutrient uptake, and stress resilience. Their diverse application methods influence key metabolic pathways, offering promising alternatives to synthetic inputs and supporting organic and ornamental plant cultivation.

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7. Institutional Review Board Statement

Not applicable.

8. Data Availability Statement

The data presented in this study are available on request from the corresponding author.

9. Acknowledgments

Not applicable

10. Conflicts of Interest

No conflict of interest was reported by all authors.

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