

Effect of spraying concentrations of Chitosan and the green alga *Cladophora crispata* on the growth characteristics of wheat (*Triticum aestivum* L.) grown in salinity-affected soils.

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I. Abstract

A field experiment was conducted at the Research Station of the College of Agriculture, University of Basrah, during the 2024–2025 growing season, aiming to study the effect of different concentrations of the bio-stimulant chitosan and the green algae extract *Cladophora crispata* on the growth of wheat (*Triticum aestivum* L.) cultivated in salt-affected soil. The experiment included four levels of chitosan (0, 150, 250, 500 mg L⁻¹) and three levels of algae (0, 250, 500 mg L⁻¹) and was carried out according to a randomized complete block design (R.C.B.D) with three replicates. The land was prepared and sown with wheat seeds of the “Ebaa 99” variety at a seeding rate of 120 kg ha⁻¹, while foliar spraying of the treatments was performed in two stages: the first at the tillering stage and the second before flowering. The results showed that the concentrations of chitosan and algae, as well as their interaction, had significant effects on most growth traits of the crop. The chitosan concentration of 500 mg L⁻¹ showed a significant superiority, recording the highest plant height (100.36 cm), flag leaf area (40.88 cm²), chlorophyll content (32.95%), number of tillers (566.3 tillers m⁻²), and protein percentage (9.52%), compared with the untreated control. Similarly, the green algae concentration of 500 mg L⁻¹ exhibited a significant superiority in most studied traits, achieving the highest plant height, flag leaf area, chlorophyll content, number of tillers, and protein percentage, with means of 95.95 cm, 39.12 cm², 31.99%, 563.8 tillers m⁻², and 10.44%, respectively, compared with the control, which recorded the lowest trait averages. Moreover, the interaction between the two factors had a significant effect on most growth traits, where treatment A₃C₄ achieved a significant superiority in plant height, flag leaf area, chlorophyll content, number of tillers, and protein percentage, with means of 101.50 cm, 43.35 cm², 36.35%, 606.3 tillers m⁻², and 12.42%, respectively, while the untreated control A₁C₁ recorded the lowest means for the mentioned traits. The results confirm that bio-stimulants represent a promising alternative to chemical fertilizers, as they improve nutrient uptake, enhance wheat growth, and increase its ability to adapt to stressful conditions such as salinity. Therefore, these stimulants constitute an effective approach to achieving more sustainable and high-quality agriculture, ensuring healthier food, a cleaner environment, and a more stable future.

Keywords: Chitosan – *Cladophora crispata* – Wheat – Growth – Salinity

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important strategic crops in the world, ranking second after corn in grain production and first in terms of global cultivated area. Its importance lies in its high nutritional value, as its grains contain proteins, fats, vitamins, minerals, and essential amino acids necessary for human health. Recent years have witnessed a remarkable expansion in the global cultivated area, with the cultivated area reaching approximately 222.46 million hectares, with a total production of 769.85 million tons, and an average yield of 3.58 tons/ha¹ (USDA, 2025). Domestically, Iraq has achieved a qualitative leap in wheat production, with the cultivated area reaching approximately 8.177 million dunums, with a production of 5.234 million tons (Directorate of Agricultural Statistics,



2024). The Iraqi Ministry of Agriculture also announced that wheat production in 2025 exceeded 6 million tons, meeting 100% of local demand for the second consecutive year. This is accompanied by a strategic reserve of 1.3 million tons, the highest in years (Iraqi Ministry of Agriculture, 2025).

Despite this progress, wheat cultivation still faces multiple challenges, most notably the high salinity of soil and irrigation water, in addition to the negative effects of excessive use of chemical fertilizers, which negatively impact soil fertility, groundwater quality, and human health. Hence, the importance of adopting sustainable agricultural strategies that rely on reducing the use of chemical inputs and replacing them with environmentally friendly alternatives, such as biostimulants and natural organic materials, contributes to enhancing productivity and maintaining ecological balance.

Green algae are an important source of nutrients, as they possess the ability to fix atmospheric nitrogen through heterotrophic cells. They also contain natural plant hormones that stimulate growth and regulate plant biological processes. In addition, algae contribute to enhancing the activity of antioxidant enzymes, giving plants a greater ability to resist salt stress (Obaid, 2015). On the other hand, biostimulants are defined as natural organic compounds that can be added to soil or sprayed on plants to improve nutrient availability and increase fertilizer utilization efficiency (Bulgari et al., 2019). These compounds affect many basic physiological processes such as photosynthesis, respiration, nucleic acid synthesis, and increased chlorophyll content. They also contribute to enhancing plants' ability to withstand various stress conditions, including salinity, drought, and heat (Paradikovic et al., 2015). Chitosan is one of the most important and widely used natural polymers, second only to cellulose among polysaccharides. It is characterized by its linear structure consisting of 2000–3000 units and is mainly extracted from marine waste such as shrimp, crab, and lobster shells, where it constitutes 20–30% (Ahmed and Salem, 2020). The diverse biological effects of chitosan are due to its homogeneous positive charges along its chain, giving it a broad ability to interact with various molecules within the cell matrix. When deposited in the plant cell wall, chitosan acts as an anti-water loss agent by reducing stomatal conductivity, which limits evaporation and enhances water use efficiency. This effect positively impacts carbon metabolism, leading to increased biomass accumulation. Furthermore, spraying chitosan on leaves increases solar reflectance, which reduces water loss through evaporation and improves transpiration and photosynthesis efficiency, without negatively impacting the final crop yield. Numerous studies have demonstrated that chitosan possesses physiological properties that enhance growth and productivity and increase the plant's ability to withstand environmental stress. Given the strategic importance of wheat crops and the vital role of both green algae and chitosan in improving morphological and physiological plant traits under saline conditions, this study aimed to:

1. Evaluate the effect of different concentrations of green algae extract on wheat crop growth, determining the optimal concentration for achieving the best response.
2. Study the response of wheat plants to different concentrations of bio-chitosan, with the aim of determining the most effective concentration for improving crop growth.
3. Analyze the interactive effect between green algae and chitosan concentrations and determine the optimal level of interaction between them to achieve the highest efficiency in improving wheat crop growth.

I. Research materials and methods

1.2 Experimental site and characteristics of the study soil

A field experiment was conducted during the agricultural season 2024-2025 at the Agricultural Research Station of the College of Agriculture, University of Basra, Iraq, which is located at the geographical coordinates (30,56948°) north and (47,752119°) east, where soil samples were taken at a depth of 0-30 cm from different locations of the field and mixed together homogeneously. Then the sample was air-dried and passed through a metal sieve with a diameter of 2 mm holes, after which it was taken to the laboratory and its physical and chemical properties were estimated as shown in the results of Table (1).

Table (1) Some chemical and physical properties of field soil before the study

Property		The value	Unit
pH		9.23	
EC (1:1)		7.45	dS m-1
Available Nitrogen		42	Mg kg ⁻¹ soil
Available Phosphorus		18	
Available Potassium		115	
Organic Matter		1.23	g kg ⁻¹ soi
Soil texture	Sand	633.3	g kg ⁻¹ soi
	Silt	245.4	
	clay	121.3	
Soil Texture		Sandy Loam	

1-Soil acidity (pH):

The acidity was measured in the saturated soil paste extract according to the method of (Jackson 1958).

2 -Electrical conductivity (EC):

The electrical conductivity of the soil extract was measured at a ratio of (1:1) using a WTW conductivity meter, according to the method of (Page et al., 1982).

3 -Carbonate mineral determination:

Carbonate minerals were determined using 1N hydrochloric acid and 1N sodium hydroxide in the presence of phenolphthalein indicator, according to the method of (Richards 1954).

4 -Organic carbon:

The organic carbon in the soil was determined using the method of Walkley and Black, 1934. The percentage of organic matter was calculated by multiplying the percentage of organic carbon by the conversion factor.(1.724)

5 -Soil bulk density: (pb):

The bulk density of the soil was measured using the (core) method.

6 -Particle density: (ps):

Particle density was estimated to be using Pycnometer, according to the method of (Black, 1965).

7-Total porosity (f):

Total porosity was calculated using the equation:

$f = (1 - pb/ps) \times 100f$, based on the method of (Black, 1965).

8-Soil texture:

Soil texture was determined using the pipette method, according to (Black, 1965).

2.2 Experimental Factors

The experiment included the study of two factors:

The first factor: four levels of the biostimulant chitosan: 0, 125, 250, and 500 mg L⁻¹, designated C₁, C₂, C₃, and C₄, respectively.

The second factor: three levels of green algae: 0, 250, and 500 mg L⁻¹, designated A₁, A₂, and A₃, respectively.

3.2 Experimental Design

The experiment was conducted according to the factorial experiment method using a randomized complete block design (R.C.B.D), with three replicates. All treatments were randomly distributed within each block, resulting in a total of 363 experimental units (3x4x3).

4.2 Field Operations

The soil was prepared for cultivation by loosening it with a subsurface plow, leveling it, and smoothing it. The land was then divided according to the approved design into experimental units measuring 2x2 meters. Each experimental unit contained 10 lines, 2 meters long, with a distance of 20 cm between lines. A distance of 60 cm was left between units to prevent overlap between treatments. Wheat seeds of the "Ibaa 99" variety, approved by the Iraqi Ministry of Agriculture, were sown on November 10, 2024, at a rate of 120 kg/ha. The field was irrigated upon planting, and hand weeding was performed when necessary. The crop was harvested on April 15 after full maturity.

5.2 Preparation and Application of Experimental Treatments

The ethanolic extract of the algae was obtained by adding 100 grams of dried and ground algae to 100 ml of a 70% ethanol solution. The extraction process was carried out using a magnetic mixer for 24 continuous hours, and the mixture was then filtered through 0.45-µm filter paper. The liquid products were then collected in Petri dishes and left exposed under normal laboratory conditions to allow the alcohol to gradually evaporate. At the end of this step, the precipitate was obtained and was recognized as the algal extract (Obaid, 2015).

The plants were treated by foliar spraying using both the algal extract and the biostimulant chitosan. Chitosan was obtained from a company specializing in marketing chemical and laboratory materials in Baghdad and was of American origin. The spraying process was carried out in two batches: the first during the branching stage, and the second just before the plants entered the flowering stage, at predetermined concentrations. Spraying was carried out using a 20-liter backpack sprayer, with care taken to spray in the early morning hours to avoid the negative effects of high temperatures. To increase the efficiency of the spray solution and ensure even leaf coverage, a spreading agent (Enforce) was added at a concentration of 2.5 cm³/L¹ to reduce the surface tension of the water and achieve complete wetting of the leaf surfaces.

I. Results and Discussion

3.1 Plant Height

Table 2 shows that the C4 chitosan concentration recorded the highest average plant height, reaching 100.36 cm, compared to the treatment without C1, which recorded the lowest average height, reaching 85.24 cm. This may be due to chitosan's effectiveness in stimulating the plant to produce certain hormones responsible for growth and development. This occurs by enhancing the growth and branching of the root system, which contributes to increasing the efficiency of water and mineral absorption from the peri-root zone. In addition, chitosan can stimulate cells to produce gibberellins-like compounds, which stimulate cell multiplication and division, which in turn affects plant growth and development (Zeng et al., 2010). These results are consistent with the findings of Islam et al. (2016), Khati et al. (2017), and El-Wakeel et al. (2024). As shown in Table 2, the A3 algae concentration achieved the highest average plant height, reaching 95.95 cm, while the A1 control treatment achieved the lowest average for this trait, reaching 91.38 cm. The increase in plant height can be attributed to the biological activity of the natural hormones present in the seaweed extract, particularly auxins and cytokinins, which stimulate the elongation and expansion of internodal spaces and increase the rate of cell division and cell number in developing tissues. Gibberellins, in turn, contribute to enhancing cell elongation in the apical meristem, which positively affects plant height (Mohsen and Mahmood, 2025). These results are consistent with those of Salim et al. (2016), Al-Hasany et al. (2019), and Al-Qashaam and Al-Mustafa (2023). Regarding the effect of the interaction between chitosan and green algae, treatment A3C4 achieved the highest average plant height of 101.50 cm, compared to the treatment without addition, A1C1, which achieved the lowest average for this trait, at 81.77 cm. The interaction between chitosan and green algae significantly enhanced plant growth, as Chitosan stimulated plant vital processes, while the algae provided the plant with the nutrients and hormones necessary for growth. This resulted in a significant increase in plant height compared to the treatment without addition.

Table (2) Effect of concentrations of chitosan and the green alga *Cladophora crispate* and the interaction between them on the plant height trait (cm)

treatment	C ₁	C ₂	C ₃	C ₄	mean
A ₁	81.77	90.27	93.97	99.53	91.38
A ₂	84.83	92.43	94.67	100.03	92.99
A ₃	89.13	94.83	98.33	101.50	95.95
mean	85.24	92.51	95.66	100.36	
LSD (0.05)	Cladophora crispate		Chitosan		interfere
	1.028		1.188		2.057

3.2 Number of days from planting to 50% flowering

The results of Table 3 showed that chitosan concentrations had no significant effect on the number of days from planting to 50% flowering. These results are consistent with those obtained by Al-Tawaha et al., 2013. The results of Table 3 indicated that green algae concentrations played a role in reducing the number of days from planting to 50% flowering. Concentration A3 recorded the lowest average for the trait, at 96.58 days, while concentration A1 recorded the highest average number of days, at 101.25 days. The reason for the reduced number of days required for the plant to reach the flowering stage is attributed to the role of the algae extract in promoting early growth and improving vegetative growth. The extract contains nutrients and plant hormones such as auxins and cytokinins, which accelerate cell division and expansion, leading to accelerated growth stages (Sethi and Adhikary, 2008).

Table (3) The effect of concentrations of chitosan and the green algae *Cladophora crispate* and the interaction between them on the trait of the number of days from planting to 50% flowering.

treatment	C ₁	C ₂	C ₃	C ₄	mean
A ₁	103.00	100.00	101.00	101.00	101.25
A ₂	98.00	98.00	97.00	98.00	97.75
A ₃	96.33	96.00	96.00	98.00	96.58
mean	99.11	98.00	98.00	99.00	
LSD (0.05)	Cladophora crispate		Chitosan		interfere
	1.150		N. S		N. S

3.3 Number of days from 50% flowering to full maturity

Table 4 shows that chitosan concentrations increased the number of days from 50% flowering to full maturity. The C4 concentration recorded the highest average for this trait, at 41.00 days, while the treatment without C1 application recorded the lowest average, at 39.33 days. The reason for the increased number of days can be explained by chitosan increasing the concentration of simple organic molecules, such as sugars, free amino acids, and soluble phenols, which play a role in regulating osmosis in plants, thus improving growth and production. It also increased osmoprotective compounds, such as TSS, FAA, and soluble phenols, which may improve plant tolerance to environmental stress conditions, which in turn extends the grain filling period (Li et al., 2017). These results were inconsistent with the findings of Islam et al. (2016), who showed that chitosan concentrations had no effect on the number of days from planting to full maturity. Table 4 also shows that the green algae *Cladophora SP.* concentrations increased the number of days from 50% flowering to full maturity. Concentration A3 recorded the highest average number of days, reaching 43.92 days, while concentration A1 recorded the lowest average, reaching 35.50 days. This is attributed to the algae extract contributing to enhancing gene expression related to nutrient uptake from the soil and increasing the efficiency of photosynthesis. This delayed the onset of senescence symptoms in the last two leaves, which helped prolong the grain filling period and positively impacted the yield (Shah et al, 2013).

Table (4) The effect of concentrations of chitosan and the green algae *Cladophora crispate* and the interaction between them on the number of days from 50% flowering to full maturity.

treatment	C ₁	C ₂	C ₃	C ₄	mean
A ₁	34.00	36.00	36.00	36.00	35.50

A ₂	42.00	41.00	42.00	41.00	41.50
A ₃	42.00	44.00	45.00	44.67	43.92
mean	39.33	40.33	41.00	40.56	
LSD (0.05)	Cladophora crispate		Chitosan		interfere
	0.855		0.987		N. S

4.3 Number of branches (branch/m²)

Table 5 shows that the C₄ chitosan concentration produced the highest number of steppes per square meter, with an average of 566.3 branch/m², compared to the C₁ treatment, which produced the lowest average number of steppes, with 479.2 branch/m². This is because chitosan helps convert organic nutrients into inorganic forms, which are easily absorbed by plant roots. Therefore, chitosan acts as a non-toxic and biodegradable plant growth promoter by enhancing crop production performance. Chitosan also has the ability to stimulate signals for plant hormone synthesis and pathways associated with auxin synthesis to promote plant growth and development (Uthairatanaki et al., 2007). Furthermore, chitosan may contribute to stimulating stress responses in plants, prompting them to produce more steppes (supports) as an adaptive mechanism to enhance survival under stressful conditions (Abubata, 2023). The results are consistent with those of El-Rahman et al. (2025). Table 5 also shows that the algal extract concentrations led to an increase in the number of seedlings. Concentration A₃ achieved the highest average seedling count, reaching 563.8 branch m², while the control treatment A₁ achieved the lowest average for this trait, reaching 468.6 branch m². The results are consistent with the findings of Aldulemy and Abdul-Razak (2021), who attributed the increase in seedling count to the algal extract containing high concentrations of cytokinins, auxins, amino acids, and a number of major and minor minerals, which contribute to stimulating cell division and expansion. It also works to achieve a balance in physiological and biological processes, positively impacting root growth and increasing their ability to absorb water and dissolved nutrients. This directly impacts the improved growth of the vegetative system and better nutrition of the lateral branches, which increases the number of shoots and reduces their mortality due to competition for food resources. Regarding the interaction between chitosan and green algae concentrations, treatment A₃C₄ recorded the highest average number of shoots, reaching 606.3 branch m², while the control treatment A₁C₁ recorded the lowest average number of shoots, reaching 429.3 branch m². The reason for the increase is that chitosan enhanced nutrient availability and absorption, while the green algae provided natural growth regulators and amino acids, which led to increased cell division and stimulated the formation of new shoots, thus increasing the number of shoots compared to the control treatment.

Table (5) The effect of chitosan and green algae *Cladophora crispate* concentrations and the interaction between them on the number of branches per square meter.

treatment	C ₁	C ₂	C ₃	C ₄	Mean
A ₁	429.3	451.7	487.0	506.3	468.6
A ₂	511.7	493.3	559.7	586.3	537.8
A ₃	496.7	565.0	587.0	606.3	563.8
mean	479.2	503.3	544.6	566.3	
LSD (0.05)	Cladophora crispate		Chitosan		interfere
	13.21		15.26		26.42

3.5 Flag leaf area (cm)

Table 6 shows the effect of chitosan concentrations on flag leaf area. The C₄ concentration achieved the highest average for the trait, reaching 40.88 cm², compared to the C₁ treatment, which achieved the lowest average, reaching 30.10 cm². This is due to the vital role chitosan plays in activating enzymes responsible for nitrogen metabolism, which contributes to enhancing leaf growth and development and improving their functional efficiency, which positively impacts overall plant growth and development (Chibu et al., 2000). The results are consistent with those found by Agbodjato et al. (2016) and Behboudi et al. (2018).

It is noted from Table 6 that spraying algae extract at a concentration of A₃ recorded the highest average flag leaf area, reaching 39.12 cm², while the control treatment, A₁, recorded the lowest average for the trait, reaching 34.11 cm². This increase in flag leaf area can likely be explained by the algae extract containing plant growth regulators such as cytokinins and gibberellins, along with trace elements and vitamins, which contributed to the activation of plant physiological processes. Feeding the leaves with the algae extract enhanced nutrient transport and distribution within the plant, increased leaf area, improved dry matter production, and increased crop growth rate (Devi and Mani, 2015). These results are consistent with those obtained by Ahmed (2024) and Al-Shammery and Aziz (2024). Regarding the interaction between the two factors, the A₃C₄ treatment recorded the highest mean for this trait, at 43.35 cm², while the A₁C₁ control treatment recorded the lowest mean for the trait, at 29.14 cm². The increase in flag leaf area is attributed to the improved vegetative growth of the plant as a result of the optimal nutritional conditions provided by the A₃C₄ treatment, which led to increased nutrient absorption efficiency and balanced hormonal activity within the plant. This was reflected in an increase in the size of the flag leaf, which is the main source of photosynthesis and supplies the grains with nutrients during the grain filling stage. Therefore, the increase in its area reflects a higher physiological efficiency of the plant compared to the non-additive treatment.

Table (6) Effect of concentrations of chitosan and the green alga *Cladophora crispate* and the interaction between them on the area of the flag leaf

treatment	C ₁	C ₂	C ₃	C ₄	Mean
A ₁	29.14	32.74	36.62	37.92	34.11
A ₂	30.17	35.85	38.32	41.35	36.42
A ₃	30.99	40.47	41.66	43.35	39.12
mean	30.10	36.35	38.87	40.88	
LSD (0.05)	Cladophora crispate		Chitosan		interfere
	0.718		0.829		1.435

3.6 Leaf Chlorophyll Percentage (SPD%)

It is noted from Table 7 that the concentration of chitosan C₄ recorded the highest average for this trait, at 32.95%, compared to the treatment without C₁, which recorded the lowest average, at 25.54%. This is due to chitosan improving cytokinin content, which stimulates chlorophyll formation and releasing amino compounds that contribute to stimulating chlorophyll synthesis (Chibu, 2001). Additionally, chitosan may increase the content of both nitrogen and potassium in plant shoots, thereby increasing the number of chloroplasts per cell and increasing chlorophyll content (Hidangmayum et al., 2019). These results are consistent with those of Behboudi et al., (2018) and Farouk and El-Metwally, (2019). This positive effect also applies to the green algae concentrations, as shown in Table 7. Concentration A₃ recorded the highest average chlorophyll content, reaching 31.99%, compared to the treatment without addition, A₁, which recorded the lowest average, reaching 26.57%. This is attributed to the vital role of the active compounds present in the algae extract, which are characterized by their content of plant hormones and natural

growth regulators, in addition to the availability of major nutrients such as nitrogen and magnesium, which directly contribute to the formation of the chlorophyll molecule. These components work together to enhance the vital processes within the plant, particularly the process of chlorophyll formation and synthesis, which positively impacts the efficiency of photosynthesis, improves vegetative growth, and increases overall plant vitality (Embarek and Mumin, 2024). The results were similar to those obtained by Aldulemy and Abdul-Razak (2021) and Aljana et al. (2022). The results of Table 7 also demonstrated the interaction effect between the two factors. Treatment A₃C₄ outperformed, recording the highest average chlorophyll concentration of 36.35%, while the control treatment A₁C₁ recorded the lowest average for this trait, at 21.17%. This is attributed to the synergistic effect between the two factors. Chitosan enhances the absorption of mineral elements and activates the physiological processes associated with photosynthesis, while the green algae, with its micronutrients and natural growth regulators, stimulates the formation of chloroplasts and increases their efficiency. This complementarity was clearly reflected in the increased chlorophyll content compared to the other treatments.

Table (7) Effect of concentrations of chitosan and the green alga *Cladophora crispate* and the interaction between them on the percentage of chlorophyll in leaves

treatment	C ₁	C ₂	C ₃	C ₄	Mean
A ₁	21.17	26.23	28.73	30.13	26.57
A ₂	26.21	28.43	30.72	32.37	29.43
A ₃	29.23	29.27	33.12	36.35	31.99
mean	25.54	27.98	30.86	32.95	
LSD (0.05)	Cladophora crispate		Chitosan		interfere
	0.802		0.926		1.603

II. Conclusions

The study results showed that spraying wheat plants with different concentrations of the biostimulant chitosan and the green algae extract *Cladophora crispata* significantly improved most growth traits. The best responses were achieved when using a concentration of 500 mg/L¹ for both chitosan and algae. The highest values were recorded for plant height, number of branches, and flag leaf area, as well as increased chlorophyll content. This improvement is attributed to the effective role of both chitosan and algae in enhancing nutrient accumulation and improving plant physiological processes, which increases the efficiency of nutrient absorption. The extracts are also rich in natural plant hormones that contribute to stimulating growth and increasing plant resistance to salt stress. The results of the interaction between chitosan and algae showed a more pronounced effect compared to the individual treatments, with treatment A₃C₄ achieving the highest averages for all studied traits. Therefore, biostimulants can be considered a promising and effective alternative to traditional chemical fertilizers, especially under saline conditions. The study recommends further research to uncover the precise mechanisms behind the effect of these stimulants on improving the growth and productivity of agricultural crops.

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