ALLELOPATHY EFFECT OF ALGAE CULTURE SUPERNATANT OF *COSMARIUM* AND *SCENEDESMUS* ON GERMINATION AND GROWTH OF THE JIHAN WHEAT VARIETY

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Abstract. This study assessed the impact of culture supernatants of two species, *Cosmarium* sp. and *Scenedesmus* sp., and a combination of the two supernatants on wheat (*Triticum aestivum* L. var. Jihan) germination and early growth. A randomized complete block design was used to achieve a pot experiment in the agricultural season 2024-2025, with three replicates. The treatments were distilled water (control), *Cosmarium* sp. supernatant, *Scenedesmus* sp. supernatant, and a 1:1 (v/v) blend of the two supernatants used at 20, 40, and 60% concentrations. The following parameters were measured: germination percent, shoot and root length, shoot and root dry weight, chlorophyll a and b levels, and leaf area. The findings revealed that there was a significant improvement in wheat, even with all the algal supernatant treatment as opposed to the control. The maximum percentage of germination (96.66%) was achieved in *Scenedesmus* sp. with 20 and 60 percent, and *Cosmarium* sp. with 40 and 20 percent of the mixed treatment. The maximum length of the shoot (29.16 cm) and chlorophyll a content (8.03 mg/g) were obtained with the combined supernatant of 40%. At 20%, the *Cosmarium* sp. had the highest root length (28.21 cm), whereas at 40%, the *Cosmarium* sp. had the highest level of chlorophyll b content (15.20 mg/g) and maximum leaf area (36.19 cm^2). All in all, *Cosmarium* sp. at 40% was the most effective treatment for improving overall traits in traits. These results affirm the fact that microalgal culture supernatants can be used as sustainable biofertilizer-like inputs to wheat to ensure environmentally sustainable agricultural practices.
Keywords: *biofertilizer, microalgae, Cosmarium, Scenedesmus, wheat, chlorophyll*

Introduction

Background and rationale

Recent research has increasingly focused on seeking alternatives to chemical fertilizers to address nutrient deficiencies, reduce their harmful effects, and enhance the soil fertility (Patil et al., 2021; García-Sánchez et al., 2022; Solomon et al., 2023). This shift aligns with the global need to have sustainable methods of agricultural production, which can remain productive and minimize the risks to the environment caused by excessive fertilization of soil with mineral products (Ibrahim et al., 2024). Biofertilizers have been discussed as a key ingredient in sustainable farming since they are cost-effective and environmentally benign, in addition to acting as a viable alternative to chemical fertilizers, since they release nitrogen gradually, which can be useful to farmers (Kanishka et al., 2025). Besides, biofertilizers may help in enhancing the biological activity and

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nutrient recycling of the soil, which leads to long-term soil quality and yield (Zhang et al., 2025).

Consequently, the development of eco-friendly biofertilizers and biofertilizers has been done using algae (Kumar et al., 2022). The macroalgae are part of a larger group of photosynthetic organisms, which may be unicellular organisms (that are in the range of one millimeter to several centimeters long), or multicellular organisms (that are up to 60 m in length) (Pereira, 2021). Macroalgae are commonly classified by their pigmentation into because they are categorized into brown, red, and green (Phaeophyceae, Rhodophyceae, and Chlorophyceae) (Biris-Dorhoi et al., 2020; da Rosa et al., 2023). They are also important natural sources of secondary metabolic products, and they display a high content of vitamins, amino acids, and carbohydrates (Aditya et al., 2022). This biochemical composition makes algae a promising resource in the elaboration of natural-friendly farming inputs, more so in those situations where sustainability intensification is needed.

Algae as sustainable biofertilizer

Sustainable farming is connected with the use of algae, as it improves the physical and chemical properties of soil that affect plant growth positively (Gonçalves et al., 2023). It is used in various ways, like the spray option, a mixture with it, or a soaking of seeds (Alling et al., 2023). The materials of algal origin are also the subject of increasing research as biostimulants and biofertilizers, as they include nutrients, organic acids, vitamins, and substances mimicking hormones that may stimulate plant metabolism and development (Braun and Colla, 2023). Application at low concentrations is preferable because its high concentrations lead to the inhibition of growth plants (Yaqub et al., 2022). This concentration-dependent reaction highlights the need to optimize application rates to maximize benefits while avoiding inhibitory effects to ensure the beneficial effects of applying a particular allelopathic are realized to their highest potential, without the occurrence of inhibitory allelopathic effects.

The extraction of such extracts can provide vegetables, herbs, and other plants with the required phytochemicals, as many of the compounds can have a positive impact on crop productivity and quality as they are capable of altering metabolism, photosynthesis, respiration, and nutrient uptake (Khaleel et al., 2024). Recent studies have demonstrated because the microalgae can enhance the vegetative growth, nutrient uptake and utilization, biomass, productivity, biotic and abiotic stress tolerance, and water utilization of most crops (Dawas and Ali, 2021; Rahman et al., 2025). Thus, extracts and culture supernatants of algae are a viable approach to ensuring that reliance on synthetic fertilizers is minimized and sustainable crop production systems are sustained (Nguyen et al., 2025).

Research gap

Generally, the study has included two strains of microalgae, *Cosmarium* sp. and *Scenedesmus* sp., as microalgae supernatants that enhanced wheat *Triticum aestivum* L. growth, thereby showing the significance of microalgae as biofertilizer to reduce the use of chemical fertilizer and lead to sustainable agricultural production. Nevertheless, even though more and more groups of evidence emerge in favor of algae-based biostimulants, there are still some gaps in the literature. To begin with, most of past-researches were concentrated on macroalgae extracts or a small diversity of microalgae genera, whereas the allelopathic and biostimulatory action of culture supernatants of *Cosmarium* sp. and

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Scenedesmus sp. are not well studied. Second, there is very little similar research that compares single-species supernatants to combined supernatant treatments of different concentrations. Third, little experimental data exists that connects these treatments with combined germination, morphology, and physiological responses (chlorophyll a, chlorophyll b, and leaf area) in wheat in controlled conditions in pots.

These gaps should be addressed in order to know whether the culture supernatants of the algae can be used as acceptable and scalable biofertilizers for the cereal crops, especially wheat, as it is one of the most economically and nutritionally significant crops in the world.

Aim and hypothesis

Hence, this research paper was intended to examine the impact of the leachate (floating material) of the growth medium of *Cosmarium* sp and *Scenedesmus* sp, and a combination of both on the germination and growth of (*Triticum aestivum* L. var. Jihan). Precisely, this paper has evaluated the percentage of seed germination, shoot and root length, shoot and root dry weight, chlorophyll a and chlorophyll b concentration, and leaf area at various levels of algal supernatant levels.

The general hypothesis of the study was that the culture supernatant of algae would be more effective in promoting wheat germination, algae growth, and physiological performance in relation to the control, and that the degree of improvement would be affected by the algae genus and the algae concentration. The secondary hypothesis was that the combination of the treatment (*Cosmarium* sp. + *Scenedesmus* sp.) would exhibit greater effects as a growth promoter compared to single treatments because of the complementary bioactive compounds in the culture medium.

Materials and procedures

Study design and experimental location

The experimental station of the Mosul University, Education College of Pure Sciences, Biology Sciences Department, carried out a pot experiment in the growing season, 2024/2025, to determine the impact of the supernatant of the algae *Cosmarium* sp. and *Scenedesmus* sp. on wheat plants. The study was developed into a randomized complete block experiment (R.C.B) that can be replicated thrice. Two microalgal supernatants (*Cosmarium* sp. and *Scenedesmus* sp.), their mixture, and a control treatment were tested. Each algal treatment was applied at three concentrations (20, 40, and 60%), thus forming a factorial structure.

The test was carried out in the plastic pots (17 cm in diameter) with the mass of the soil being 3 kg. The temperature was kept at 20-23 ^{0}C during the day and 14-19 ^{0}C at night with a 16 h light/ 8 h dark photoperiod and a light intensity of approximately 200-300 µmol/m²/s. The watering was done frequently to keep the soil moist (65% of field capacity).

Algae source and identification

The algae *Cosmarium* sp. *and Scenedesmus* sp. under pure culture *were obtained in* the algae laboratories. Department of Life Sciences/College of Education, University of Mosul, and identified by using standard algal taxonomic keys to confirm the genus of the algae using an optical microscope (Prescott, 1962). One of the genera studied was the

Cosmarium sp, which is a green, one-celled alga, made up of two semi-spherical halves. The length of the cells is 30-40 microns, and the width is 26-33 microns. The other genus is *Scenedesmus* sp., which are floating on the surface of the freshwater, are single-celled, non-motile, generally mucous, and have a waist that causes it to be divided into two. Two halves: The depth of the waist is dependent on species (*Fig. 1*).

Scenedesmus sp. *Cosmarium* sp.

Figure 1. *Microscopic image(40X) of algae*

Culture medium and growth conditions

Both algae were cultured in Chu10 growth medium (Praba et al., 2016), the contents of which are presented in *Table 1*, and put in a growth incubator with the temperature set at 25 °C and the intensity of light at 60 mol photons m 2s 1fluorescent source. The algae were grown under 16 hours of light with 8 hours of darkness. The agitation of the cultures was maintained, and after 21 days of culture, the growth density of both algae was measured at a wavelength of 436 nm by the use of a spectrophotometer, and absorbance values of 2.70 and 0.425 for *Scenedesmus* sp and *Cosmarium* sp respectively.

Table 1. *Components of algae growth medium*

gm/L	Components of medium
0.4	Ca $(NO_3)_2$
0.1	K_2HPO_4
0.2	Na_2CO_3
0.25	$MgSO_4.7H_2O$
0.25	Na_2SiO_3
0.005	Ferric Ammonium Citrate
0.4	Ca $(NO_3)_2$

At the age of 21 days, the culture medium was centrifuged at 10,000 rpm and 5 °C to separate the biomass of the algae and obtain an algae cell-free filtrate with the extracellular metabolites (Ibrahim et al., 2021).

Preparation of algal supernatant

Algal supernatant of the *Cosmarium* sp. and *Scenedesmus* sp. were prepared at three volumes (20, 40, and 60 ml) was adjusted to a total volume of 100 mL used distilled water. supernatant was prepared to assess potential combined effects.

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The treatments included:

1.Distilled water (control)

2.*Cosmarium* sp. supernatant at 20 %, 40 %, and 60 %.

3.*Scenedesmus* sp. supernatant at 20 %, 40 %, and 60 %.

4.Mixture of *Cosmarium* sp. and *Scenedesmus* sp. Supernatant (consisted of a mixture of the two algal materials, prepared at a 1:1 ratio (v/v), with the total application rate kept the same as that used for the individual algal treatments to ensure comparability among treatments).

For all treatments, the total applied volume per pot was fixed at 100 mL to ensure consistency across concentrations and treatment types.

Wheat grain source

The wheat cultivar sample, which was picked as Jihan, was acquired at the Agricultural Research Centre, Mosul University, Agriculture College, Iraq.

This cultivar was used due to its adjustment to the local growing conditions and its ability to be used in controlled pot experiments.

Soil sampling and analysis

The physical and chemical characteristics of the soil employed in the experiment were analyzed (*Table 2*), and some of them relied on the approach mentioned in Ryan et al. (2001).

Table 2. *Physical and chemical properties of the soil*

Soil particle size distribution (%)			**Texture**	**pH**	**EC (dS/m)**	**Organic matter (%)**	**N (ppm)**	**K (ppm)**	**P (ppm)**
Sand	Silt	Clay	Silt loam						
30	52	18		7.22	0.56	0.76	10.64	5.81	39.3

Pot experiment procedure

The experiment was carried out in plastic pots (17 cm in diameter) having 3 kg of soil, it had been initially planted (10 grains of wheat at a depth of 1 cm of soil) then *Cosmarium* sp, *Scenedesmus* sp, and algal mixture that was prepared separately were added to the soil at 20, 40 and 60 percent concentration per pot (100 mL). Two treatments (at 1 and 15 days after the grains were planted) were done on the same amount of tap water (control). Thinning of the plants was done after germination to produce 5 regular plants in a pot.

A ruler was used to measure these five plants, and three replicas of the five plants were taken to ensure the statistics were accurate. The pots were kept in the same environmental and irrigation conditions to reduce confounding factors. Waters were also put into the plants at a regular frequency to ensure that the soil was at the right moisture content (65% of the field capacity).

Measured traits

The experiment compared the germination activity, morphological developmental characteristics, biomass accumulation, and physiological characteristics (chlorophyll a, chlorophyll b, and leaf area).

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Percentage of germination of seeds (ISTA, 2016)

$$\text{Percentage of seed germination } = \frac{\text{No.of seedlings appearing}}{\text{No.of seeds planted}} \times 100 \qquad \text{(Eq.1)}$$

Leaf area (cm²)

Leaf area of the wheat was determined according to Kemp (1960):

$$\text{leaf area } = \text{ leaf length x maximum leaf width x 0.905} \qquad \text{(Eq.2)}$$

Chlorophyll a and b content

The content of chlorophyll has been estimated using the method (Niveditha et al., 2022). In it 100 mg of the same fresh leaves that we used in the measurement of the leaf area was weighed and crushed it in 10 ml of 80 per cent acetone in a ceramic mortar and centrifuged it in centrifuge 15 minutes at 3000 rpm/minute, the filtrate was drawn into it, the absorbance was measured at wavelength of 645 and 663 nm.

$$\text{Chl.a (mg / g leaf)} = Chl.\,a\ (mg/gleaf)\,\frac{(12.7)\ (\text{Abs663}) - (2.69)\ (\text{Abs645})}{\text{leaf tissue mg}} \qquad \text{(Eq.3)}$$

$$\text{Total Chl.} = 20.2(\text{Abs } 645) + (8.02\ (\text{Abs } 663) \qquad \text{(Eq.4)}$$

Chlorophyll a and b were expressed as mg/g fresh leaf tissue.

Shoot and root morphological parameters

The shoots of the wheat plant after 60 days were collected for the measurement of shoot and root length (cm).

Shoot length and root length were recorded using a ruler, and measurements were performed consistently across all treatments.

Dry biomass measurement

The collected samples were subjected to oven-drying at 70 °C till constant weights were obtained to measure the shoot and root dry weight.

Dry biomass values were expressed in grams (g) per plant.

Statistical analysis

They were analyzed as per the SAS program, were considered as the factorial experiments, and the coefficients were differentiated with the help of Duncan multiple range test at the probability of 5%.

All experimental data were statistically analyzed using SAS software. Treatments were considered within a factorial framework under a randomized complete block design (RCBD) with three replicates. Mean comparisons were performed using Duncan's multiple range test at $P \leq 0.05$.

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Results and discussion

Germination percentage

The results of *Table 3* indicate that the rate of germination has significantly changed in response to the influence of water algal filtrate. Generally, all the treatments of the algal supernatant enhanced germination relative to the control (distilled water), showing that the culture supernatants had bioactive compounds that could induce early seed metabolic activity.

Table 3. *The effect of algal supernatant and their interaction on the germination (%)*

	Treatments			
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	83.33 e	83.33 e	83.33 e	83.33 d
20	90.00 c	96.66 a	96.66 a	94.44 a
40	96.66 a	90.00 c	93.33 b	93.33 b
60	86.66 d	96.66 a	93.33 b	92.22 c
Average effect of algae	89.16 b	91.66 a	91.66 a	
±SD	5.69	6.38	5.77	

Note: According to Dunkin's multiple range test ($P \leq 0.05$) means that taking the same letter did not differ significantly

At the concentration of 20 and 60%, *Scenedesmus* sp filtrate was found to exhibit maximum germination at an average of 96.66, followed by 40% *Cosmarium* sp mixture with *Scenedesmus* sp at 20% with an average germination of 91.63. The comparison treatment (control) had the lowest germination of 83.33 percent. Though the rate of germination was a bit lower than the expected rate of certified seeds, it was the Jihan variety that was chosen deliberately due to the fact that it is highly adaptable to the local environment and is resistant to diseases, coupled with high productivity that facilitates reproducibility in the case of the experiment.

The observed increase in germination may be explained by the fact that the algae contain certain nutritional elements that increase the nutritional value of the plant, which, in turn, can be applied to the physiological functioning of the plant. In this constituent, magnesium is its constituent because this plays an important role in carbohydrate metabolism in the activation of key enzymes such as amylase that triggers the germination activities in the seeds (Nguyen et al., 2025; Alfaro et al., 2025). Moreover, algal-produced compounds can enhance the water intake during imbibition and enzyme activation needed to induce germination (Puente-Padilla et al., 2025). This is consistent with the research by Yaqub et al. (2022), which mentions that the stimulatory effect of green extract biomasses of Cladophora and Spirogyra was perceived to be present on the process of seed germination and certain growth parameters in three cultivars of bread wheat.

Shoot length

Table 4 indicates that most treatments had a significant effect on increasing shoot length. The highest height was attained by *Cosmarium* sp. and *Scenedesmus* sp. supernatant with mean heights of 24.42 and 25.36, respectively, and the 40 percent concentration gave the highest height with a mean of 24.42 and 25.36, respectively. These

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findings suggest that the algal supernatants produced the same positive influence on the vegetative elongation, especially in the middle range of concentration.

Table 4. *Influence of algal supernatant and their interaction on shoot length (cm)*

	Treatments			
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	20.80 i	20.80 i	20.80 i	20.80 d
20	25.11 c	23.13 f	22.20 h	23.48 b
40	24.17 d	22.76 g	29.16 a	25.36 a
60	23.40 e	19.02 j	25.53 b	22.65 c
Average effect of algae	23.37 b	21.43 c	24.42 a	
±SD	1.85	1.90	3.73	

Note: According to Dunkin's multiple range test (P ≤ 0.05) means that taking the same letter did not differ significantly

The maximum shoot length (29.16 cm) and the minimum (19.02 cm) were achieved with the *Cosmarium* and *Scenedesmus* supernatant with a concentration of 40%. This indicates that the joint algal supernatant was the most effective in the growth of shoots. *Fig. 2* also shows that the *Scenedesmus* supernatant at a concentration of 60% had affected the shoot length to 27.88 cm.

Figure 2. *The effect of the growth medium supernatant of the algae Cosmarium sp. and Scenedesmus sp. on the growth of wheat*

Living organisms are *Cosmarium* sp. and *Scenedesmus* sp., interaction with which can cause the development of plants and the increase in the activity of living organisms. The algae also contain the element of iron that plays a role in the formation of the primitive constituent of the plant cell, e.g., cytochromes and ferredoxins, which are engaged in the transfer of electrons in the photosynthesis process, thus causing the plant to grow and be taller and have more leaves (Al-Bdairi and Kamal, 2021). Consequently, the recorded growth of the shoot may be a result of improved photosynthetic potential and increased availability of nutrients encouraged by the algal supernatant treatments

Root length

As the findings provided in *Table 5* show, the treatments are different. At 20%, the *cosmarium* sp. of the experiment had a higher average of root length at 28.21 cm, whereas the *Scenedesmus* algal supernatant of the experiment had a lower average at 19.01 cm.

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This shows that the type and concentration of algae had a significant effect on root growth, and the low concentration of *Cosmarium* sp. had a more pronounced effect on root growth compared to other algal types.

Table 5. *Effect of algal supernatant and their interaction on root length (cm)*

	Treatments			
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	25.16 c	25.16 c	25.16 c	25.16 a
20	28.21 a	22.80 e	23.06 c	24.69 b
40	26.77 b	19.83 f	25.25 c	23.95 c
60	24.28 d	19.01 g	25.00 c	22.65 c
Average effect of algae	26.11 a	21.70 c	24.62 b	
±SD	1.74	2.82	1.04	

Note: According to Dunkin's multiple range test ($P \leq 0.05$) means that taking the same letter did not differ significantly

Growth of some of the parameters of growth can be attributed to the fact that as the organic matter gets increased and the pH level decreases, then the solubility of the nutrients increases and thus the rhizosphere becomes more acidic, thus leading to the growth of the root system of the plant and, consequently, the increase in the absorption of the nutrients which translates to the increased height of the plant and therefore the increased shoot and root dry weight and leaf area among other growth parameters (Skifa et al., 2024). First, it possesses growth regulators, cytokinins that lead to the division of the meristematic cells and then increase the number and size of the cells (Han et al., 2018).

Shoot dry weight

According to *Table 6,* the tests are not significantly different between the treatments except for *Cosmarium* supernatant in 40% concentration and the interaction of the two algae in 60%. The weight increase of wheat shoots recorded values of (0.11 and 0.10) g, at 40% and 60% respectively, as compared to others.

Table 6. *Effect of algal supernatant and their interaction on shoot dry weight (g)*

	Treatments			
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	0.07 cd	0.07 cd	0.07 cd	0.07 b
20	0.08 bc	0.05 d	0.05 d	0.06 b
40	0.11 a	0.05 d	0.09 abc	0.08 a
60	0.07 cd	0.05 d	0.10 ab	0.07 ab
Average effect of algae	0.08 a	0.05 b	0.08 a	
±SD	0.02	0.01	0.02	

Note: According to Dunkin's multiple range test ($P \leq 0.05$) means that taking the same letter did not differ significantly

These findings indicate that the biomass in the shoot was not as sensitive as the shoot length, although they responded positively to particular algal treatments. This may be explained by the fact that the algae have zinc, which plays the role of raising the

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chlorophyll content that completes an important task in the photosynthesis process leading to greater nutrient accumulation and, by extension, the growth of the weight of living plant mass, which is reflected in the increase of the shoot and root dry weight (Umair et al., 2020). These results are in line with Al-Khafaji and Al-Jubouri (2023) that Spirulina algal extract at concentrations of 5 and 10% stimulated lettuce growth indicators.

Root dry weight

Table 7 indicates impressive values in which the root system has greatly gained weight when subjected to *Cosmarium* sp. supernatant at 40 and 60 percent, with an average value of (0.083 and 0.084) g, respectively. It proves that *Cosmarium* sp. had the best ability to enhance the biomass of roots, especially at the moderate-to-high levels.

Table 7. *Effect of algal supernatant and their interaction on root dry weight (g)*

Treatments				
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	0.062 c	0.062 c	0.062 c	0.06 a
20	0.036 e	0.034 e	0.038 e	0.04 c
40	0.083 a	0.037 e	0.056 d	0.06 b
60	0.070 b	0.034 e	0.084 a	0.06 a
Average effect of algae	0.06 a	0.04 c	0.06 b	
±SD	0.02	0.01	0.02	

Note: According to Dunkin's multiple range test ($P \leq 0.05$) means that taking the same letter did not differ significantly

The findings were in line with those of another investigation (Gitau et al., 2021) in which the presence of the chlorella algae extract level 0 05 g/L led to the impartation of an increment in the plant, its wet weight, and the chlorophyll subsequent increase in the plant's. According to another study, the inclusion of the *Trichoderma* fungus, combined with the *Chara* sp., resulted in the greatest increase in the number of seeds and the weight of 1, 000 g (Al-Ziadi and Al-Jubouri, 2023).

Chlorophyll a content

The results of *Table 8* indicated that the addition of the supernatant algal and the interference between the two samples at various concentrations led to a significant increase in the values of chlorophyll a content of wheat leaves under the influence of the majority of treatments. The greatest amount of chlorophyll a was in the effect of *Cosmarium* sp. and *Scendesmus* at 40%, having an average of 8.03 mg/g.

On the contrary, the control group showed a significantly lower chlorophyll a content (4.41 mg/g), which validated the fact that supernatants of algal resuscitated cultures enhanced pigment accretion of photosynthesis. The lowest chlorophyll a content, which was 3.38 mg/g, was measured in *Cosmarium* sp at 60 percent concentration. This implies that the efficiency of the physiology could be impaired by increased concentrations, and this is consistent with concentration-dependent inhibition reported in earlier studies on algae-based biostimulants.

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Table 8. *Effect of algal supernatant and their interaction on chlorophyll a content (mg/g)*

	Treatments			
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	4.41 h*	4.41 h	4.4 h	4.41 d
20	5.43 g	6.71 c	5.69 f	5.94 b
40	4.32 i	6.69 d	8.03 a	6.35 a
60	3.38 j	5.97 e	7.17 b	5.51 c
Average effect of algae	4.39 c	5.95 b	6.33 a	
±SD	0.84	1.08	1.61	

Note: According to Dunkin's multiple range test (P ≤ 0.05) means that taking the same letter did not differ significantly

Chlorophyll b content

Table 9 results demonstrated that algal treatments had a significant effect on the chlorophyll b content. The highest average of chlorophyll b was 15.20 mg/g with the treatment of *Cosmarium* sp. at 40 percent, and the lowest average of chlorophyll b was in the control, which had an average of 6.5.

Table 9. *Effect of supernatant algal and their interaction on chlorophyll b content mg/g*

	Treatments			
Concentration (%)	***Cosmarium***	***Scenedesmus***	**Interaction**	**Average con.**
0	3.54 j	3.54 j	3.54 j	3.54 d
20	3.76 h	7.91 c	3.73 i	5.13 c
40	15.20 a	6.71 d	5.30 f	9.07 a
60	10.15 b	5.93 e	3.95 g	6.68 b
Average effect of algae	8.16 a	6.02 b	4.13 c	
±SD	5.60	1.84	0.80	

Note: According to Dunkin's multiple range test (P ≤ 0.05) means that taking the same letter did not differ significantly

This means that *Cosmarium* sp. supernatant specifically was found to be effective in improving the accumulation of chlorophyll b that is of paramount importance in increasing the light-harvesting capacity in wheat leaves. This is because of the high chlorophyll content attributed to the presence of nutrients, vitamins in the algae, and the hormone-like materials (auxins and gibberellins) which spur the growth of plants.

Leaf area

The application of *Cosmarium* sp. supernatant resulted in a significant increase in leaf area (*Table 10*). In area of the leaves, and the greatest area was approximately 21.95 cm^2, and 40 percent provided the maximum area of 22.63 cm^2. The largest area measure was acquired in the *Cosmarium* filtrate treatment, with the average of 36.19 cm^2, because the concentration was 40%, and the least was taken in the control treatment, with the average of 9.75 cm^2.

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Table 10. *Effect of algal supernatant and their interaction on leaf area (cm²)*

	Treatments			
Concentration (%)	**Cosmarium**	**Scenedesmus**	**Interaction**	**Average con.**
0	9.75 i	9.75 i	9.75 i	9.75 d
20	10.80 g	26.42 c	10.39 h	15.87 c
40	36.19 a	19.20 d	12.50 f	22.63 a
60	31.04 b	16.44 e	10.66 g	19.38 b
Average effect of algae	21.95 a	17.95 b	10.82 c	
±SD	13.65	6.90	1.18	

Note: According to Dunkin's multiple range test ($P \leq 0.05$) means that taking the same letter did not differ significantly

This finding greatly supports the contention that algal supernatant treatments increased canopy growth and leaf expansion in wheat, which are important factors that define the extent of the photosynthetic surface area and biomass accumulation.

Discussion

Summary of key findings

The current experiment indicated that wheat (*Triticum aestivum* L. var. Jihan) was responsive to the culture supernatant treatment using *Cosmarium* sp. and *Scenedesmus* sp., as well as their mixture. In most of the measured characteristics, the algal supernatant treatments showed a significant improvement in seed germination, vegetative growth, biomass accumulation, and photosynthetic pigment content over the control. The best germination percentage (96.66%) was obtained with a combination of various treatments: *Scenedesmus* sp. at 20 and 60, *Cosmarium* sp. at 40, and the mixture of the supernatants (water) at 20, which proves that not only the supernatant of the individual algae can enhance the initial stages of developing performance, but also the mixture of supernatants.

The strongest response was observed with the combined treatment of 40%, reaching 29.16 cm, and this means that the interaction between the two algal supernatants gave the best stimulation of the shoot elongation. Conversely, the algal genus and concentration were more significant in root growth responses with *Cosmarium* sp. at 20 percent, giving the highest root length (28.21 cm). A more selective response pattern was observed in the attainment of biomass accumulation, with the shoot dry weight showing a more significant improvement at 40 and 60 percent, mainly under *Cosmarium* sp. and the combination of *Cosmarium* sp., respectively. While root dry weight showed the highest improvement at 40 percent and 60 percent at *Cosmarium* sp. and *Cosmarium* sp., respectively.

These trends were supported by the physiological traits. The highest content of chlorophyll a became 40 percent (8.03 mg/g) under the combined treatment, whereas chlorophyll b became highest in *Cosmarium* sp. at 40 percent (15.20 mg/g). There was also a great improvement in leaf area, especially with *Cosmarium* sp. at 40% (36.19 cm^2), indicating that treatment with algal supernatant improved photosynthetic surface development. Taken together, the above findings point to a clear conclusion that algal culture supernatants may be used effectively as biofertilizer-like inputs in wheat when used under controlled pot conditions.

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Mechanisms: the way algal supernatant enhances wheat

The positive results of algal supernatants, which were reported in the current study, can be attributed to a number of complementary mechanisms. To begin with, it is known that microalgae are sources of nutrients, vitamins, and secondary metabolites, which can be discharged into the culture medium during the growth process and could be retained in the supernatant following the separation of the biomass. These compounds can either serve as direct nutrients available to plants or they can mediate their indirect effects as biostimulants that mediate their metabolic and physiological activity. One important reason why germination improves is that algal supernatants can increase the enzyme activation during the initial metabolism of seeds. Specifically, magnesium and other micronutrients aid in carbohydrate breakdown and enzyme activation that aid in the germination process. This is in accordance with the observation that germination was consistently higher in the various algal treatments than in the control. Moreover, algae have the potential to enhance the physical properties of soils and water supply, which facilitates the imbibition of seeds and even germination.

The growth in the length of the shoot and leaf area indicates that algal supernatants led to improved photosynthesis and nutrient acquisition. The availability of iron, e.g., is critical to cytochromes and ferredoxins, which are vital in the transport of electrons during photosynthesis (Al-Bdairi and Kamal, 2021). Thus, micronutrients of algal origin were probably added to a better chlorophyll synthesis and photosynthesis that was converted into more vegetative growth. The responses of root length and root dry weight could be explained by nutrient availability and hormone-like substances. Microalgae are also reported to have or induce growth regulators, such as cytokinins, to increase cell division of meristematic tissues and the growth of roots (Han et al., 2018). The root development is especially sensitive to the variations in the nutrient solubility, organic matter, and rhizosphere chemistry, and the algal supernatants could be of assistance with the provision of organic compounds that enhance the nutrient mobilization (Skifa et al., 2024).

Further evidence that algal supernatants enhanced physiological activity is the chlorophyll a and b enhancements. High chlorophyll content has commonly been associated with greater nitrogen uptake, availability of micronutrients, and stability of photosynthetic machinery. Nevertheless, the lower chlorophyll a content in *Cosmarium* sp. at the 60% concentration indicates that more concentrations might have an inhibitory effect, and some earlier works have indicated that algal extracts may become suppressive at high concentrations (Yaqub, 2024). Such a pattern of concentration dependence shows that it is important to optimize application rates to ensure that fields are viable and crops are safe.

Comparison with the previous studies

This research has found itself in line with emerging findings that algae-based materials can also act as sustainable biostimulants and biofertilizers. Other past studies have revealed that algae extract and biomass can enhance germination and growth parameters in wheat and other crops. As an example, Yaqub et al. (2022) found that green extract biomasses of Cladophora and Spirogyra were able to promote the seed germination and growth parameters in bread wheat cultivars, cultivar-specifically. Alling et al. (2023) also found that Nordic microalgae exerted biostimulant effects on germination in tomato and

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barley seeds and indicated the overall ability of algae-derived products to stimulate early germination.

Biomass and chlorophyll content improvements in the given study are also consistent with the previous results. Gitau et al. (2021) showed that green microalgae like *Chlorella* and *Chlamydomonas* may have a strain-specific biostimulant effect on plant biomass and chlorophyll growth. Moreover, shoot and root dry weights were higher in this study and consistent with the physiological functions of micronutrients like zinc in facilitating chlorophyll synthesis and tolerance of plants to stress (Umair et al., 2020).

The results on the changes in the leaf area and chlorophyll content also confirm the findings of Prisa (2022), who found that the application of algae had the potential to increase plant height, leaf numbers, shoot weight, root weight, and soil balance. Moreover, the results are consistent with those of Mal Allah and Al-Katib (2024), which have shown that aqueous algal extracts could improve nutritional and biochemical levels in wheat plants. Therefore, the current research contributes to the evidence base by giving comparative outcomes of two genera of microalgae and a combination of their supernatant at three concentration levels.

Practical implications for sustainable wheat production

The results of the present research are significant to sustainable wheat production. The continuous increase in the percentage of germination indicates that algal supernatants may be used as an amendment treatment to soak the seeds or as an early-stage amendment soil treatment to encourage homogeneous emergence and initial vigor. The importance of early vigor is especially relevant in a wheat production system due to the enhanced competitiveness against the weeds, as well as enhanced stability in yield, during times of variable conditions.

The changes in the length of shoots, leaf area, and chlorophyll content of the plants show that algal supernatants increased the photosynthetic potential of wheat plants. This implies that microalgal supernatants may help decrease reliance on chemical fertilizers as growth promoting inputs may be partly replaced with biologically-based material. The highest responses observed at 40 percent concentration in some of the traits also reveal that the intermediate concentrations can be the most effective in relation to the effects on benefits to risk ratio.

Moreover, it is also possible that the high levels of root responses noted in *Cosmarium* sp. treatments indicate that this supernatant could be used to support enhanced nutrient and water uptake by enhancing the root system. The effect of this might especially be useful in semi-arid or drought-affected conditions where the extent of wheat performance is highly dependent on root water uptake. All in all, this paper confirms the possibility of using algae culture supernatants as greener biofertilizer-like inputs, which, besides minimizing the chemical input needs of farmers, may enhance early growth performance.

Limitations of the study

Although such positive results were evident in this experiment, there are a number of limitations that ought to be mentioned. First, this was a pot experiment under controlled conditions, which is not a complete representation of the complexity of the field, such as fluctuations of the environment, soil heterogeneity, and microbial relationships. Second, the experiment also compared early vegetative growth characteristics, chlorophyll concentration, and biomass parameters, whereas final yield characteristics (spike number, grain, and thousand-grain weight) were not analyzed.

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Third, although the paper has proven that algal supernatants stimulate wheat growth, it has not determined the chemical content of the supernatants (macro- and micronutrients, phytohormones, amino acids, or organic acids). This type of analysis would enhance mechanistic interpretation and foster scalability in the future. Fourth, the researchers tested three concentrations (20, 40, and 60%), although the lower levels of 20% were not tested; in the previous studies, powerful responses were reported at very low concentrations (Yaqub et al., 2022).

Lastly, the interaction treatment was made in a 1:1 ratio, and no other ratios of the mixtures were done. The ratios can produce greater synergistic or decreased inhibitory effects at higher concentrations.

Future research directions

Since these findings have been conducted under the open-field conditions in single seasons and also on different soils, future studies should authenticate such results in warm seasons and soil types to establish consistency and agronomic feasibility. Yield-related characteristics like grain yield, thousand-grain weight, harvest index, and nutrient content in grains should also be incorporated in future studies in order to establish whether the gains associated with earlier growth actually result in significant production advantages. Moreover, chemical profiling of the supernatants of the culture is advised in detail. These must involve examination of nitrogen, phosphorus, potassium, micronutrients, and phytohormone-like compounds. The profiling would enable the association of particular biochemical constituents with the observed plant responses, and it would aid in the formulation of standardized biochemical formulations to be used in agriculture.

The other direction which should also be considered is to consider a broader concentration range and other application methods. The best mode of delivery of wheat should be compared between seed priming, foliar spraying, and combined soil + foliar. Also, the alternative microalgae strains and mixed ratios should be tested to determine the synergistic combinations that can maximize the benefits of growth and reduce the possible inhibitory effects. Lastly, the work in the future should explore the dynamics of soil microbes and rhizosphere reactions after the addition of algal supernatant. Since products of the microalgae could alter microbial vitality, nutrient circulation, and root-related consortia, the incorporation of microbial measures would give a more detailed insight into the activities of algae-based biofertilizers in sustainable agricultural frameworks.

Conclusions

This experiment has shown that wheat (*Triticum aestivum* L. var. Jihan) has a positive response to culture supernatants of *Cosmarium* sp., *Scenedesmus* sp., and a combination of both, which demonstrates the promise of microalgal supernatants as a biofertilizer-like input in the future. In the measured parameters, the algal supernatant treatments were found to have a significant effect on germination, vegetation growth, biomass and accumulation, chlorophyll a, chlorophyll b level, and the leaf area of the vegetation in comparison with the control. Consistently and substantially larger improvement was found in most measured traits with *Cosmarium* sp. at 40 percent and the combination of supernatant treatment also gave good effects, especially in the shoot length and chlorophyll a. These findings suggest that the positive impacts of the algal supernatants

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are concentration dependent and depend on the algal genus and mixture interaction hence the need to identify the most optimal application rate.

In practical terms, the results indicate that the supernatants of microalgae culture can be used as biostimulant materials in the environment, which are environmentally friendly and cheap, in order to promote sustainable wheat production and curb the use of chemical fertilizers. These increases in germination and early growth performance suggest that the biological results could be useful in the improvement of crop establishment and early vigor, which are essential in wheat productivity. Nevertheless, the experiment was done under pot conditions; thus, it is advised that more field-scale experimentation should be done to confirm the effects on actual agronomic conditions and to determine the ultimate yield and grain quality response.

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