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ASSESSING THE SYNERGISTIC EFFECT OF *CHLORELLA VULGARIS*, SEAWEED AND INORGANIC FERTILIZER FOR ENHANCING WHEAT GROWTH

AAMNA KIYANI[1], SALEHA JAMIL[1], MARIA HAMAYUN[2], RAMEESHA ABID[1], ASIF JAMAL[1] AND MUHAMMAD ISHTIAQ ALI[1*]

[1]*Quaid-i-Azam University, Department of Microbiology, 45320, Islamabad, Pakistan*
[2]*Oxford college, Beaves H 27-38 Hythe Bridge St. Oxford OX1 2 EP*
**Corresponding author's: ishimrl@qau.edu.pk*

Abstract

Modern agricultural strategies are evolving worldwide as sustainable, organic, and chemical-free practices. Modern agriculture aims to increase the yield with less chemical input and maintain soil health. This is possible via bio-stimulants and biofertilizers. Microalgae and seaweed fertilizers are considered as potential candidates for achieving the aforementioned objectives. In the current study, the efficacy of commercially available seaweed fertilizer and *Chlorella vulgaris* powder was evaluated by growing wheat plants in greenhouses. The results were compared with control and commercially available inorganic fertilizer. Several growth parameters were recorded. We found *C. vulgaris* powder has prominent impacts on plant growth and soil health. In the current study MB2 treatment (10g *C. vulgaris* powder/kg of soil) shows the most promising results in growth and yield parameters. It has been found that all organic treatments we used in the current study have more yield compared to commercially available inorganic fertilizers.

Key words: Microalgae; *Chlorella vulgaris;* Seaweed fertilizer; Inorganic fertilizer.

Introduction

Ending hunger, achieving food security, improving nutrition, and promoting sustainable agriculture are some of the important objectives of the United Nations Sustainable Development Goals (UNSDGs) (Biermann *et al*., 2017). The global population explosion tremendously increases pressure on major stakeholders to meet the food requirement (Abid *et al*., 2022; Abid *et al*., 2022). Food of all living beings originates from the soil. In this regard, soil health and fertility is the utmost responsible factor for increasing agricultural productivity. During the industrial revolution when chemical fertilizer was developed and implemented an enormous increase in yield was reported. However, the long-term and continuous use of chemical fertilizers poses deleterious effects on human health, aquatic life, biodiversity, and soil health (Savci, 2012). To overcome these issues sustainable and green alternative technologies to increase agricultural productivity need to be focussed.

Microalgae and seaweed fertilizers are considered as major sources of bio-stimulants and biofertilizers, alternative to chemical fertilizers (Ronga *et al*., 2019). Bio-stimulants also known as metabolism inducers, are a variety of compounds that enhance plant growth parameters when used in a minute quantity either in soil or by foliar application (Babushkina *et al*., 2017; Deepika & Mubarak Ali, 2020). Common components of these metabolism inducers are vitamins, amino acids, chitin, humic acid, and polysaccharides (Michalak *et al*., 2017; Chiaiese *et al*., 2018). Compounds reported in algal extracts and seaweed fertilizers are similar to plant growth hormones like auxin, and cytokinins which have influential effects on plant physiology when applied in small quantities. Being photosynthetic either autotrophic or mixotrophic, microalgae, have the potential to produce a vast variety of biologically active compounds like phytohormones, antimicrobial compounds, and pigments, that can be used as a source of nitrogen, phosphorus, and potassium (NPK) for plants as they enhance plant growth and soil fertility (Gonçalves, 2021). The stimulatory effect of microalgae and its extracts has been reported by various researchers. Additionally, microalgae contribute to CO_2 sequestration and add organic matter to soil. A significant amount of seaweeds are used as nutraceuticals and bio-stimulants in agriculture. Seaweed extracts have been used in agriculture as soil conditioners or as plant stimulators. Application of seaweed extracts as foliar sprays are promising plant growth promoters as they enhance photosynthetic activity, increase the resistance of plants against phytopathogens, and enhance the productivity of many crops (Bulgari *et al*., 2015; Supraja *et al*., 2020; Ahmed *et al*., 2023).

In recent years several studies have been carried out to exploit the bio-stimulant potential of microalgae and its extracts on a variety of plants (Odgerel & Tserendulam, 2016). For instance, the synergistic effect of MaB-flocs and *Nannochloropsis* biomass shows a positive effect on tomato plants. Increased growth parameters were also reported by the application of living cells of (Figueiredo *et al*., 2022) microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* on tomato plants (La Bella *et al*., 2021).

The purpose of this work was to evaluate the bio-stimulant effect of commercially available *C. vulgaris* powder on wheat crops. Various plant growth parameters were recorded and compared with control, seaweed fertilizer, and commercially available inorganic fertilizer. Results from this study will further increase understanding, importance, and bio-stimulant role of microalgae.

Material and Methods

Acquisition and analysis of *C. vulgaris* biomass: Chlorella powder was ordered from Golden Greens Organic Ltd, Company number 06799051, UK under the product name of Organic Chlorella Powder. The cell wall of *C. vulgaris*, used in this product, was already disrupted and the product fulfills the Soil Association's organic standard, and 100% purity was reported according to the company's description.

Soil collection and characterization: Fertile soil (loam texture), suitable for plantations, was obtained from a nursery and its physicochemical analysis was performed before and after the greenhouse experiment to check the effect of organic and inorganic fertilizers on soil health. The soil was sieved by using a 2mm strainer to remove any coarse particles and clumps.

Seed collection and pre-treatment: Seeds of wheat cultivar Borlaug 2016 were obtained from the National Agriculture Research Centre (NARC), Islamabad, Pakistan. Seeds were washed with 95% ethanol and soaked overnight in sterilized distilled water.

Selection of organic and inorganic fertilizers: Commercially available organic seaweed fertilizer produced by Greenbelt Organics, Manufacturer Part Number GBO00562P1LBSCA, UK, which is available in Pakistan was ordered. The most commonly used inorganic NPK fertilizer (urea) was obtained from local suppliers.

Experimental site and greenhouse set up: The experiments were conducted in the greenhouse facility of the faculty of biological sciences, Quaid-i-Azam University Islamabad from November to April, in climatic conditions favorable for wheat growth i.e., (Temperature: 24°C, pH: 6.5-7, Humidity: an average of 0.19 inches (0.5 cm) per day, Natural day light cycle).

Plantation pots of 2kg soil holding capacity were used in this experiment. In each pot, 1kg of soil was used. Wheat seeds were treated with three different treatments i.e., seaweed fertilizer, *C. vulgaris* powder, inorganic fertilizer, and control treatment. The efficacy of different concentrations of these treatments was analyzed. Table 1 describes the treatments and concentrations of fertilizer. The efficiency of these biofertilizers and inorganic fertilizers was evaluated by comparing the data obtained from 10 different parameters of the greenhouse experiment.

Plant's physiological analysis

Chlorophyll estimation: Plant chlorophyll was extracted from leaves by using 80% acetone. Total chlorophyll of leaves was measured at 30,60 and 90 days after sowing. For this one gram of leaves was grinded in 0.5g magnesium carbonate and 80% acetone using a pestle and mortar. The resulting plant mixture was incubated at 4°C for three hours. Then it is centrifuged at 2500rpm for 5 minutes and 100ml acetone is added to the supernatant and used for chlorophyll estimation through a spectrophotometer. The absorbance was recorded at 645nm and 663nm using 80% acetone as blank (Kamble *et al.*, 2015).

The relative water content of shoots: Relative water content of shoots was found by the following formula (Turner, 1981).

$$\text{Relative water content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Plant's morphological analysis: Morphological analysis of plants is divided into two categories, pre and post-harvest analysis.

Pre-harvest analysis includes the estimation of the plant's length (shoot length) from week 1 to week 18th for all combinations.

While post-harvest analysis includes dry and fresh weight of roots and shoots, root length, shoot length, number of spikes/pot, number of grains/spike, weight of grains/pot, and weight of grains/spike.

Plant's antioxidant enzyme assay

Preparation of enzyme extract: Enzyme extract was prepared by grinding 0.5g leaves in polyvinylpyrrolidone (PVP), ethylenediaminetetraacetate (Na_2EDTA), and 10ml sodium phosphate buffer. It was subjected to centrifugation at 4°C and supernatant was collected (Alici & Arabaci, 2016).

Superoxide dismutase assay: The assay is based on the principle that superoxide dismutase inhibits the photoreduction of dye, Nitro blue Tetrazolium (NBT). The final reaction mixture (3ml) consisted of methionine, NBT, EDTA, riboflavin, enzyme extract, and sodium phosphate buffer. The reaction mixture was kept under white light for 15 minutes and absorbance was recorded at 560nm.

Table 1. Treatments with evaluated concentrations in greenhouse experiment.

Treatments	Concentrations
Control	No treatment
Microalgal biomass (MB1, MB2, MB3, MB4)	5, 10, 50, 100g/kg soil
SWF: Commercial seaweed fertilizer SWF(s): Soil application SWF(f): Foliar application	<u>Soil application</u> 12g/kg soil <u>Foliar spray</u> 2g/l
Urea fertilizer (IF)	0.03g/kg soil
Microalgae (MB1, MB2, MB3, MB4) and seaweed fertilizer	<u>Soil application</u> 12g seaweed + 50g microalgae <u>Foliar spray</u> 5g microalgae (MB1) + 2g/l seaweed 10g microalgae (MB2) + 2g/l seaweed 50g microalgae (MB3) + 2g/l seaweed 100g microalgae (MB4) + 2g/l seaweed
Microalgae + Urea fertilizer	50g microalgae + 0.03g/kg urea
Microalgae + Urea+ Seaweed fertilizer	50g microalge+0.03g urea +2g/l seaweed

Peroxidase assay: In this assay, pyrogallol was used as a substrate and an increase in absorbance was noted after 3 minutes at 430nm. The total reaction mixture was 3ml and it consisted of pyrogallol, sodium phosphate buffer, H_2O_2, and enzyme extract.

Catalase assay: The enzyme activity was measured with a spectrophotometer at 240nm by a decrease in absorbance caused by the degradation of hydrogen peroxide. The final reaction mixture (3ml) consisted of sodium phosphate buffer, hydrogen peroxide, and enzyme extract.

The final concentration of these antioxidant assays was calculated in units/mg of protein using the following equations:

$$\text{Enzyme activity} = \frac{\text{Units}}{\text{L}} = \frac{\text{Change in absorbance} * \text{Total assay volume}}{\text{Change in time} * e * I * \text{enzyme sample volume}} \times 100$$

e = Extinction co-efficient of substrate
I = Path length of the cuvette

$$\frac{Units}{mg} solid = \frac{\frac{units}{ml}\ enzyme}{\frac{mg\ solid}{ml}\ enzyme}$$

$$\frac{Units}{mg} protien = \frac{\frac{units}{ml}\ enzyme}{\frac{mg\ protien}{ml}\ enzyme}$$

Table 2. Physicochemical analysis results of soil selected for the study/experiment.

Characteristics	Results
pH	7.25 ± 0.4
E.C (dS/m)	4.25 ± 0.2
Available phosphorus (mg/kg)	4.1 ± 1.3
Available potassium (mg/kg)	98.4 ± 7.4
Available nitrogen (mg/kg)	400 ± 0.1
Saturation (%)	26 ± 3.2
Texture	Loam

Statistical analysis

The difference between the treatments/combinations was evaluated by one-way ANOVA analysis of variance. And Tukey Honest significance difference test (HSD) was used to figure out further differences among them on the basis of P values (where[`p adj`<=1, sign:= ''] [`p adj`<0.05, sign:= '*'] [`p adj`<0.01, sign:= '**'] [`p adj`<0.001, sign:= '**

Results

Soil physicochemical analysis: The physicochemical analysis of soil selected for the study has been depicted in (Table 2) and the results of physicochemical analysis of soil after harvesting are in (Table 3).

Plant's antioxidant enzyme assay: An antioxidant assay was carried out to determine how different fertilizer treatments affect the antioxidant potential of plants. Correlation was observed with increasing concentrations of microalgal biomass in catalase assay. Additionally, with the application of seaweed fertilizer, a further increase in catalase concentration was observed. About 100% of increase was recorded at MB4 treatment. The maximum observed concentration of catalase was recorded in treatment (SF(s)+MB) seaweed fertilizer + Microalgae, which is higher than MB4 (Fig. 1a). In peroxidase assay, the highest concentration was detected when plants were treated with a combination of all fertilizer (i.e. seaweed fertilizer + microalgal fertilizer (50g) + inorganic fertilizer), about 4 fold increase compared to the control. Treatment with Seaweed fertilizer, foliar application (SWF) has no significant effect on catalase concentration when compared to control (Fig. 1b). Superoxide dismutase (SOD) assay revealed that all biofertilizer treatments have high concentrations of superoxide dismutase compared to control and inorganic fertilizer treatment. The highest concentration of SOD was detected in the treatment SWF (f)+ MB4 which is 4 folds higher than control and plants treated with inorganic fertilizer (Fig. 1c).

Table 3. Physicochemical analysis results of soil after harvesting.

Treatments	N(mg/kg)	P(mg/kg)	K(mg/kg)	pH	E.C(ds/m)	Saturation (%)	Texture
Control	430.3 ± 3.5	3.9 ± 0.3	105 ± 11.3	7 ± 0.6	1.28 ± 0.37	28 ± 1.4	Loam
IF	403 ± 11.4	4.2 ± 0.4	104.6 ± 8.13	7.5 ± 0.26	1.89 ± 1.64	25 ± 0.7	Loam
MB+IF	539 ± 6.6	4.7 ± 0.31	105.6 ± 3.04	7.1 ± 0.62	2.06 ± 0.6	28 ± 0.22	Loam
MB1	441 ± 7.5	4.7 ± 0.7	109.1 ± 8.2	6.5 ± 0.23	1.13 ± 0.57	27 ± 0.41	Loam
MB2	459.7 ± 12.5	5.6 ± 0.4	106.6 ± 1.6	6.68 ± 0.62	1.58 ± 0.45	28 ± 0.3	Loam
MB3	494.7 ± 14.6	5.06 ± 0.4	105.4 ± 5.2	6.9 ± 0.61	1.14 ± 0.48	28 ± 0.51	Loam
MB4	502.7 ± 15.2	4.97 ± 0.6	103.3 ± 2.8	6.8 ± 0.21	2.68 ± 1.5	27 ± 0.36	Loam
SWF	461 ± 7.5	5 ± 0.3	117.3 ± 1.51	7.08 ± 0.34	0.36 ± 0.14	27 ± 0.18	Loam
SWF(f)+MB1	483 ± 12.8	5.2 ± 0.3	118.7 ± 1.6	7 ± 0.1	1.46 ± 0.13	27 ± 0.61	Loam
SWF(f)+MB2	520 ± 4	5.3 ± 0.42	115.5 ± 1.85	6.8 ± 0.4	2.4 ± 2.46	29 ± 0.19	Loam
SWF(f)+MB3	534.7 ± 9.9	5.4 ± 0.1	116.9 ± 1.76	7.04 ± 0.12	1.14 ± 0.23	28 ± 0.8	Loam
SWF(f)+MB4	552.7 ± 14.2	5.2 ± 0.5	117.3 ± 2.3	6.83 ± 0.32	1.8 ± 0.34	30 ± 0.9	Loam
SWF(s)+MB	520.3 ± 4.5	5.7 ± 0.2	105 ± 5.6	6.9 ± 0.23	2.85 ± 1.24	27 ± 0.97	Loam
SWF+MB+IF	568.7 ± 9.6	6.1 ± 0.3	112.8 ± 2.05	7.15 ± 0.23	1.08 ± 0.2	27 ± 0.37	Loam

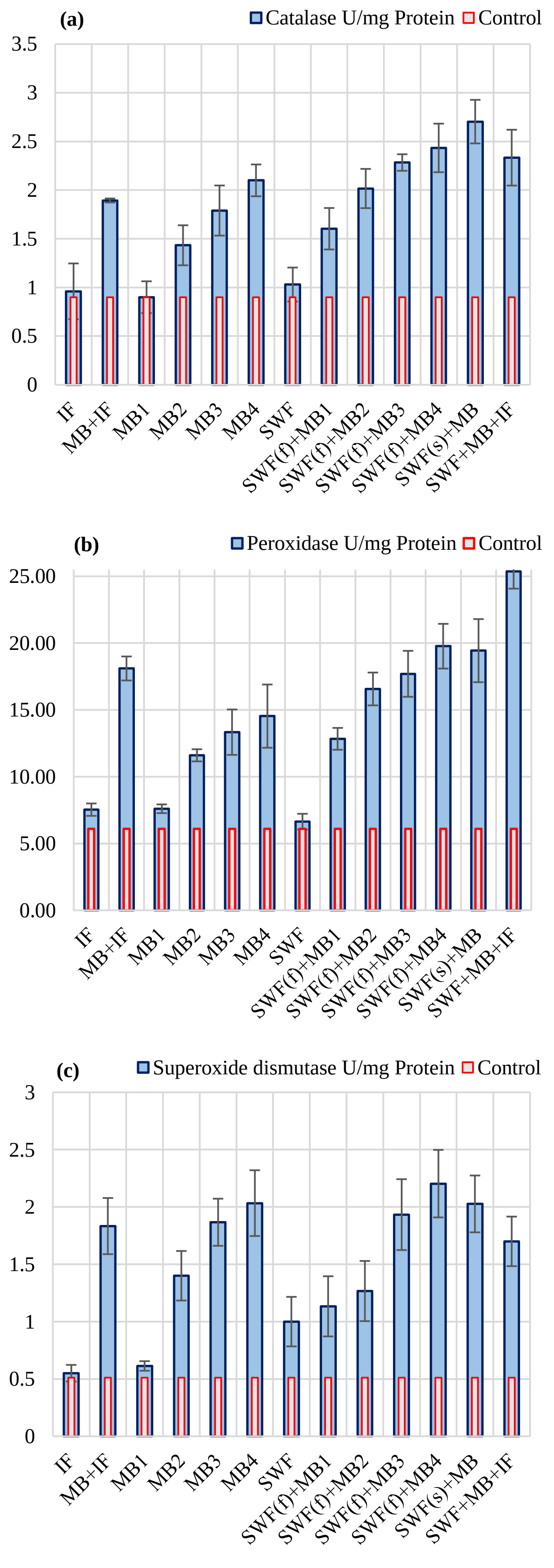


Fig. 1. Antioxidant enzymes Assay a) Catalase enzyme activity, $p<0.05$ b) Peroxidase activity, $p<0.05$ c) Superoxide dismutase activity, $p<0.05$.

Plant's physiological analysis

Real-time images of crop (Wheat) cultivation experiment can be seen in (Fig. 2).

Chlorophyll estimation: Chlorophyll was measured in (mg/g) for each treatment at days 30, 60, and 90. The mean of the total chlorophyll for each treatment was calculated and compared with the control. A significant correlation was observed at different treatments (Fig. 3).

The relative water content of shoots: In this study, we found MB2-treated plants have the highest water content compared to control which corresponds healthy physiological state of the plants (Fig. 4). A decrease in relative water content was observed as we increased the concentration of microalgal biomass. We also observed plants treated with seaweed fertilizer as foliar application and microalgal biomass (SWF(f)+MB2) had no significant difference compared to the plants treated with only microalgal biomass (MB2) which corresponds that seaweed fertilizer as foliar spray does not affect relative water content.

Plant morphological analysis

Shoot parameters

Shoot length measurements: The shoot length of the plants was measured from week 1st to week 18th regularly (Fig. 5a and 5b), and then in week 22nd (harvesting week) (Fig. 5c). From week 19th till 22nd no change in height was recorded. These results show that four treatments (MB1, MB3, SWF(f)+MB2, SWF(f)+MB4) have a significant difference from the control. All four contain microalgal biomass alone or in combination with seaweed fertilizer, which proves the beneficial effects of organic biomass on plant health as compared to its inorganic counterpart. All other combinations with microalgal biomass also show notable differences from control and inorganic fertilizer (urea). The lowest shoot length was observed in the plants treated with the combination of microalgal biomass and seaweed fertilizer (directly in the soil). The treatment was applied as a soil drench technique and then seeds were added which consequently slowed down the germination and growth of the plant. This could happen because of nitrogen toxicity as both seaweed and microalgal biomass are rich in nitrogen and other macro-elements. A significant difference in the shoot length was observed in the plants treated with MB2 after harvesting. A description of the shoot length measurements during the greenhouse experiment from week 1 to week 18 is given in (Fig. 5a and 5b) and the final shoot length at the time of harvesting is in (Fig. 5c). Similar studies are performed by (Renuka *et al*., 2016; Refaay *et al*., 2021; Wang *et al*., 2024).

Fresh and dry weight of shoots: Compared with the control and relatively with other parameters, the fresh and dry weight of shoots was significantly high in the plants treated with MB2 (Fig. 6a and 6b). A significant decrease weight of shoots was observed in the plants treated with SWF(s)+MB. The differences in root weight observed are correlated to all other parameters we checked in the greenhouse experiment. (Fig. 6a and 6b) shows the observed differences among treatments.

(a+b) (c) (d)

Fig. 2. Images from crop cultivation experiment a+b) Growth of wheat plants from week 1st to week 13th c) Riped crop ready for harvesting d) Replicates of MB2 before harvesting.

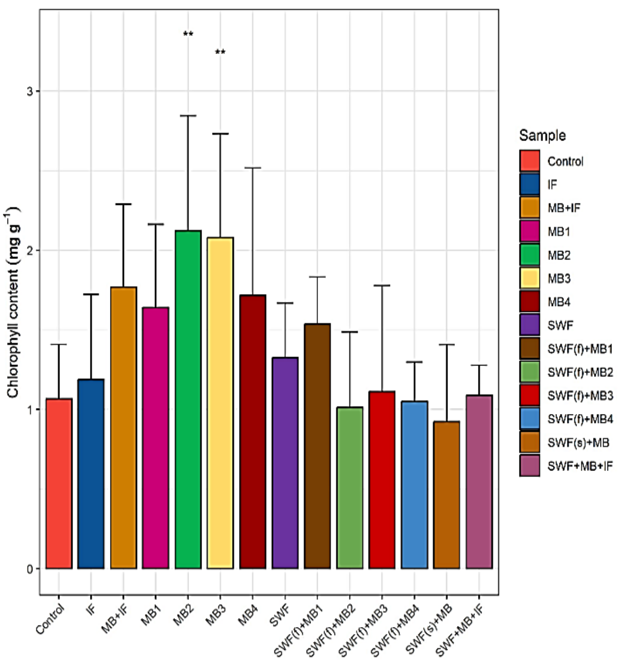


Fig. 3. Total chlorophyll content for all treatments (mg/g). [`p adj`<0.01, sign := '**']

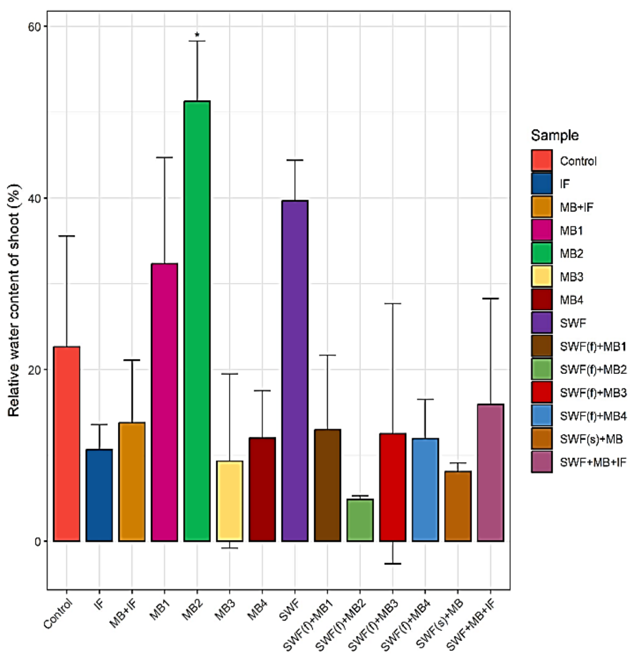


Fig. 4. Relative water content of shoots for all treatments (%). [`p adj`<0.05, sign := '*']

Root parameters: In the current study, we observed a significant decrease in fresh and dry weight of roots together with root length, in plants treated with SWF(s)+MB. This might be because of excessive availability of nutrients especially nitrogen which can halt the overall growth of plants. On the other hand, plants treated with only microalgal biomass (MB2) significantly enhance all the aforementioned root parameters. After increasing the concentration of microalgal biomass, no prominent improvement was observed. Details of the observed root parameters i.e. root length, dry weight of roots and fresh weight of roots are described in (Fig. 7a, 7b and 7c) respectively. Similar studies are performed by (Sial *et al*., 2019; Jungk, 2001; Wang *et al*., 2024).

Yield parameters: The spike's length, number, and weight are the essential parameters to measure the yield of wheat. In the current study several yield parameters i.e. number of spikes per pot, no of grains per spike, weight of spikes per pot, weight of grains per pot, and weight of grains per spike are evaluated for each treatment. In all parameters, substantial differences were observed in plants treated with microalgal biomass at the concentration of 10gm/ kg. All these yield parameters are correlated with growth parameters. Plants with enhanced growth and chlorophyll content are eventually outperformers in yield. Interestingly yield parameters of all organic treatments were higher than inorganic commercial fertilizer treatment. Description of yield parameters i.e. number of spikes per pot, no of grains per spike, weight of spikes per pot, the weight of grains per pot, and weight of grains per spike are given in (Fig. 8a, 8b, 8c, 8d, 8e) respectively.

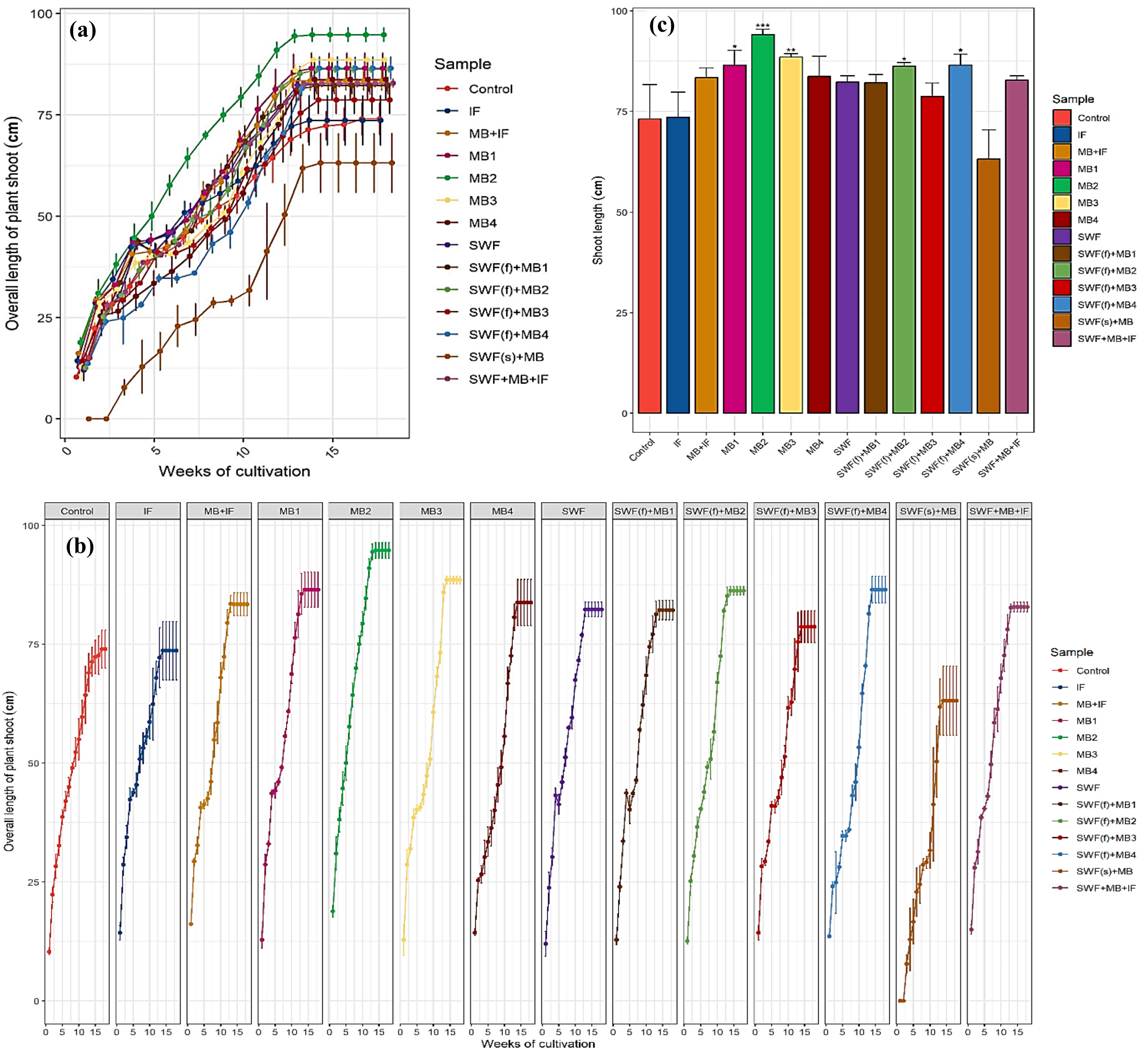


Fig. 5. a and b) Shoot length measurement for all treatments (cm) from week 1st to week 18th. c) Final shoot length at the time of harvesting. [`p adj`<0.05, sign := '*'] [`p adj`<0.01, sign := '**'] [`p adj`<0.001, sign := '***']

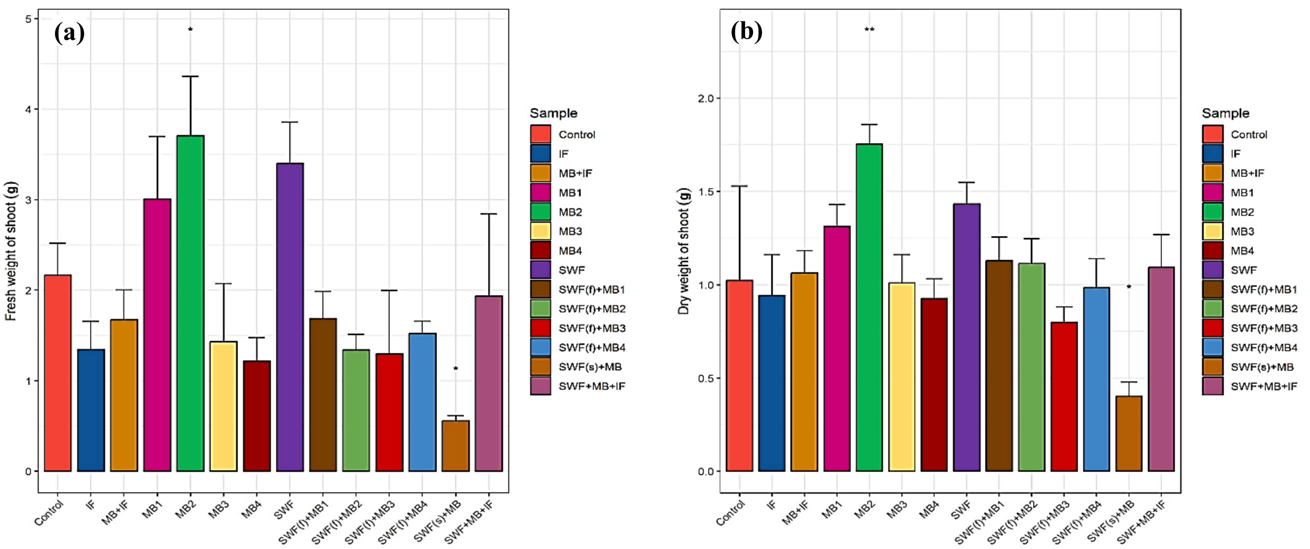


Fig. 6. Weight of shoots for all treatments (g) a) Fresh weight of shoots b) dry weight of shoots. [`p adj`<0.05, sign := '*'] [`p adj`<0.01, sign := '**'].

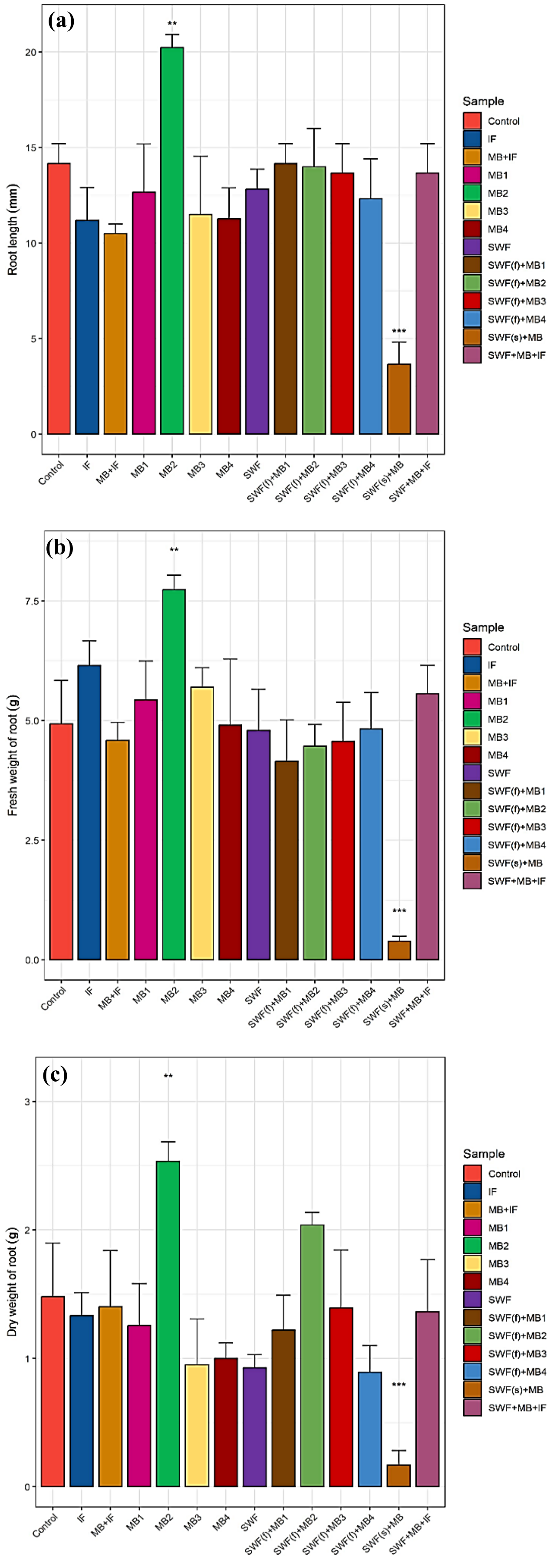
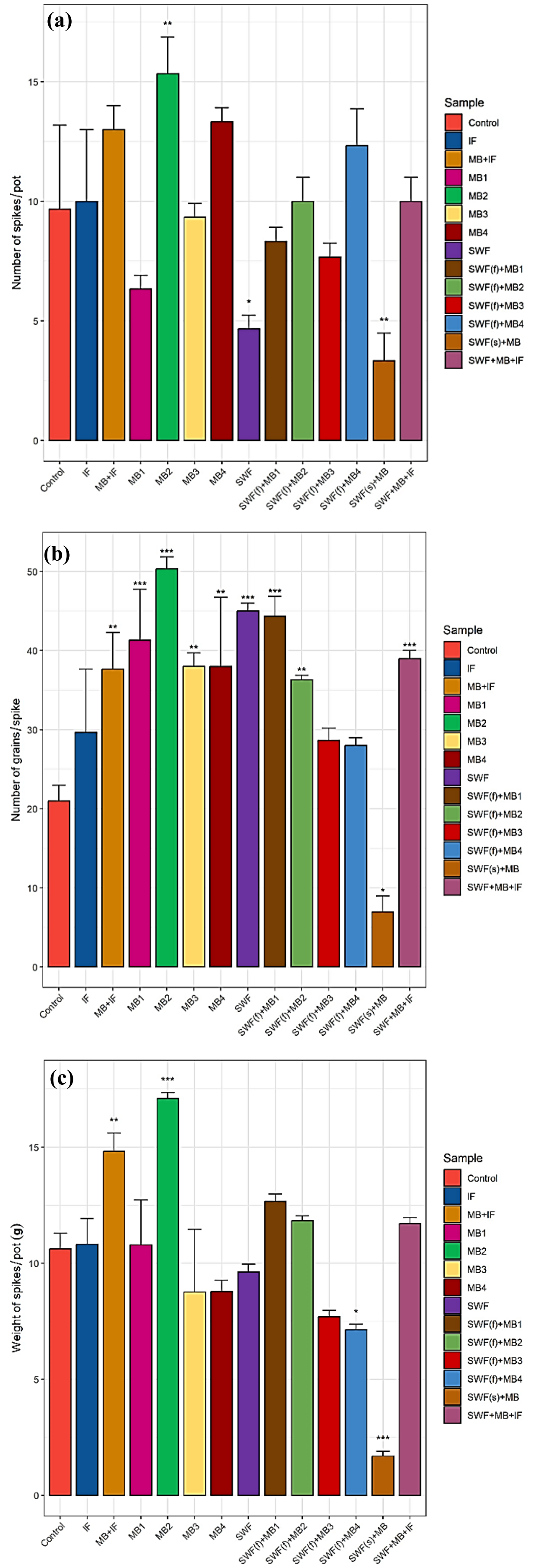


Fig. 7. Fresh and dry weight (g) and length of roots (mm) for all treatments a) Root length b) Fresh weight of roots c) Dry weight of roots. [`p adj`<0.01, sign := '**'] [`p adj`<0.001, sign := '***'].



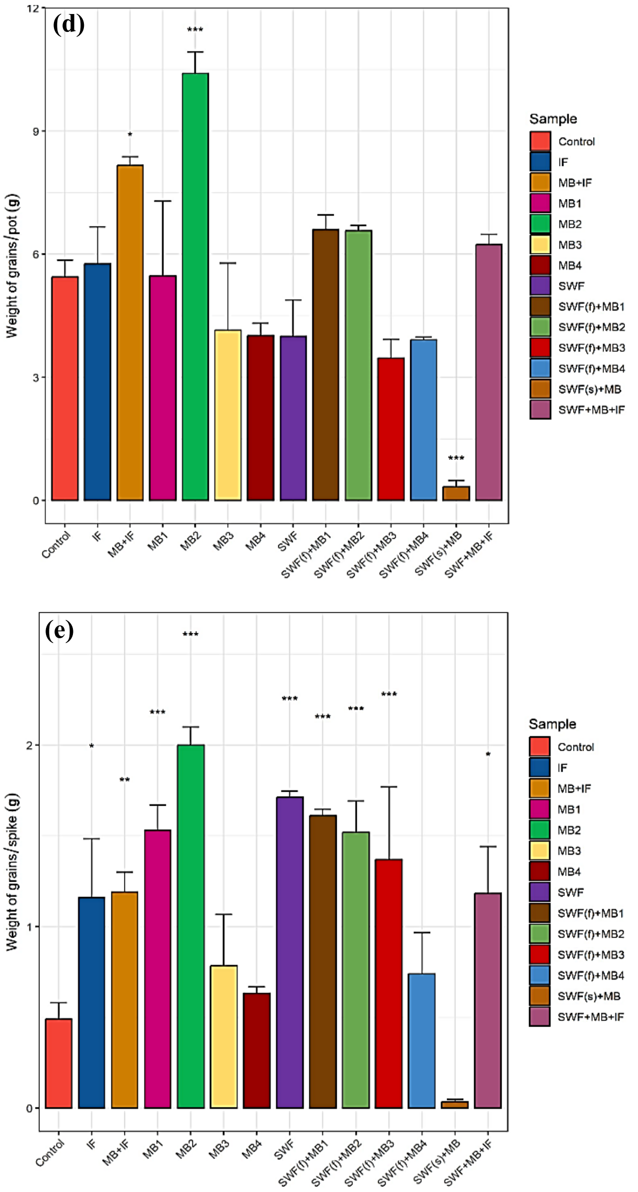


Fig. 8. Yield parameters for all treatments a) Number of spikes/pot b) Number of grains/spike c) Weight of spikes/pot (d) D) Weight of grains/pot (e) E)Weight of grains/spike. [`p adj`<0.05, sign := '*'] [`p adj`<0.01, sign := '**'] [`p adj`<0.001, sign := '***']

Discussion

Post-harvest soil analysis shows that soil treated with organic treatments has a comparatively high number of N, P, and K as compared to initial soil analysis results (Tables 2 and 3). We can conclude that the application of organic treatments improves soil fertility, maintains nutrients, and has long-term beneficial effects on the environment as suggested by Jadhav *et al*., 2022. Moreover, the burden of chemicals can be reduced and it will also support the soil's microbial community (Hussain & Hasnain, 2011; Gougoulias *et al*., 2018).

Different growth and productivity parameters calculated in greenhouse experiments show that biofertilizer application has better results compared to control and organic fertilizer. This is probably due to the availability of nutrients and organic matter to plants when biofertilizer is applied (Suhag, 2016). Chlorophyll content, considered as one of the health parameters, was reported significantly high in plants treated with MB2 and MB3 with microalgal fertilizer at 10g/I and 50g/I respectively. Between both these treatments no significant differences were found which points out that the uptake of nutrients or other bioactive compounds from the soil by plants is limited to a certain amount. If less concentration is effective for the desired results, employing high concentration can lead to nutrient toxicity in plants (Iwuagwu *et al*., 2013; Alves *et al*., 2018). Weekly observation of shoot length revealed that MB1, MB2, and MB3 treatments performed better compared to inorganic fertilizer and seaweed fertilizer. Among the treatments, it was observed that MB2 has a greater length than MB3 which is probably because of Nitrogen toxicity, as microalgal fertilizers are rich in Nitrogenous compounds. The productivity of grains was compared with all the treatments and it was observed that MB2 and MB+ IF were significant. The synergistic effect of seaweed fertilizer (s) + microalgal fertilizer (50g/I) was not observed in case of grains production. This could be possible because of the nutrient toxicity and pH. In another study, Odgerel & Tserendulam, 2016 also proved the beneficial effects of *C. vulgaris* on wheat plants. Early germination, plant growth, and an increase in fresh and dry weight are also reported by Rehmat *et al*., 2021 and these results are congruent to our findings.

Plants generate ROS (reactive oxygen species) during their metabolism. These ROS are deactivated by antioxidant enzymes. Superoxide dismutase converts superoxide anion to oxygen and hydrogen peroxide (less reactive) (Kusvuran & Can, 2020). Then hydrogen peroxide is converted to water by peroxidase or catalase enzyme. Out of these three enzymes Superoxide dismutase is the major antioxidant enzyme (Alici & Arabaci, 2016; Rajput *et al*., 2021). A study on the effect of adding urea fertilizer on plants in different concentrations has shown that a high concentration of fertilizer results in greater oxidative stress. The greater the stress greater the resistance shown by plants to combat the stress (Kerchev *et al*., 2022). Similar trends can be seen in our study where the lowest concentrations of catalase, peroxidase, and superoxide dismutase were detected compared to biofertilizer treatments. It is concluded that inorganic fertilizers don't play a critical role in ROS management. The most important stakeholder in ROS management is SOD, as it has first exposure to ROS and converts it into hydrogen peroxide, a less reactive compound compared to ROS. A high concentration of SOD signifies better plant health. In the current study, we observed that all biofertilizer treatments induced the production of SOD in plants and probably this is one of the reasons for employing microalgal-based biofertilizers in modern agriculture. After the conversion of ROS to H_20_2, a further breakdown is performed by peroxidases. If initially plants are exposed to high oxidative stress and in the presence of abundant SOD, plants will experience high concentrations of H_20_2, which needs further neutralization. This neutralization is performed by peroxidases. Low levels of peroxidases, will leave the plants to stress of H_20_2. Direct correlation between increasing biofertilizer concentration and peroxidases in our study clarifies the potential of seaweed and microalgal fertilizer.

Additionally, the biostimulant effect of microalgae and seaweed couldn't be neglected. In the current study, we majorly applied biomass to soil and treated it as a soil amendment to compare its biofertilizer potential but in case of foliar application or treatment of specific compounds derived from microalgae or seaweed like phytohormones or antistress compounds, biostimulant effect should also be considered as contributing factor to yield and plant health (Refaay *et al.*, 2021; Minaoui *et al.*, 2024).

Despite the fact of sustainable nature and environmentally friendly composition, microalgal fertilizers face several limitations compared to traditional agrochemicals, particularly in terms of production costs, nutrient concentration, and slow nutrient release. Cultivating and processing microalgae can be expensive, making large-scale applications less economically feasible. Issues with standardization and nutrient consistency, along with limited long-term research and regulatory barriers, further hinder their widespread adoption. Lastly, storage and shelf-life challenges make them less practical than conventional, chemically stable agrochemical products (Muhammad *et al.*, 2020; Peter *et al.*, 2022).

Conclusion

This study concludes that *C. vulgaris* has excellent antioxidant potential which results in increased concentrations of SOD, and POD enzymes in treatments where we have higher amounts of fertilizers and lower concentrations of CAT enzyme. In the plant's physiological analysis, plants treated with MB2 and MB3 have a significantly high value of total chlorophyll. The lowest concentration of chlorophyll can be seen in plants treated with the combination of microalgal biomass and seaweed fertilizer (SWF(s)+ MB). The presence of betaines and betain-like compounds in seaweed fertilizers is responsible for enhancing chlorophyll concentrations. In our results, the higher concentration of seaweed fertilizer could be a factor in reduced chlorophyll concentration and hence the various concentrations of seaweed fertilizers to evaluate this effect will be beneficial. The morphological analysis also proves that combination MB-2 contains the best concentration of *C. vulgaris* powder, which is sufficient to provide all major nutrients for the optimal growth and yield of a wheat plant.

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List of Abbreviations.

MaB-flocs	**Microalgae-Bacteria flocs**
NARC	National Agriculture Research Canter
UNSDGs	United Nations Sustainable Development Goals
NPK	Nitrogen, Phosphorus, Potassium
MB	Microalgal Biomass
IF	Inorganic Fertilizer
SWF	Seaweed Fertilizer
SWF(s)	Seaweed Fertilizer in Soil
SWF(f)	Seaweed Fertilizer as Foliar spray
PVP	Polyvinylpyrrolidone
EDTA	Ethylenediaminetetraacetic Acid
Na_2EDTA	Sodium Phosphate Buffer
NBT	Nitro Blue Tetrazolium
H_2O_2	Hydrogen peroxide
ANOVA	Analysis of Variance
HSD	Tukey Honest Significance Difference Test
Ab	Absorbance
E.C	Electrical Conductivity
SOD	Superoxide Dismutase
POD	Peroxidase
ROS	Reactive Oxygen Species
CAT	Catalase

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