

Response of some metabolic activities of wheat plants to different treatments of marine macroalgae from the Red Sea, Egypt

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Abstract:

In the present study, some experiments were conducted using wheat plants (*Triticum aestivum L.*) to study the effect of different seaweed treatments on some biochemical changes in the plants. Five different species of seaweed treatments were applied, namely; *Halimeda opuntia*, *Caulerpa racemosa*, *Cystoseira myrica*, *Saragasum aquifolium*, and *Actinotrichia fragilis*. The results showed that the wheat plant treated with different seaweeds increased the activity of peroxidase enzymes at the vegetative stage of plant growth. The highest peroxidase activity was observed in the leaf plant treated with *Halimeda opuntia*. Whereas, catalase activity was increased in the strain leaves with all applied treatments, with a maximum increase with *Halimeda opuntia*, *Saragasum aquifolium*, and *Actinotrichia fragilis*. Further results showed that the level of total carbohydrates and other metabolic activities such as proline and malondialdehyde (MAD) content were raised after all treatments, in particular after the application of the species; *Sargassum aquifolium*, *Actinotrichia fragilis*, and *Caulerpa racemosa*. However, the highest increase in MAD content was recorded in the leaves treated with *Halimeda opuntia*. Thus; the application of some macroalgae on wheat plants influenced nutrient transformation and soil properties, leading to improved wheat growth, yield, and development.

Keywords Marine macroalgae, antioxidant enzymes, carbohydrates, prolines, wheat plants.

Introduction

Marine macroalgae, or seaweed, are aquatic plants that belong to the plant kingdom of Thallophyta. Brown algae (Phaeophyceae), red algae (Rhodophyceae), and green algae (Chlorophyceae) may all be classified according to their pigmentation. Among these marine macroalgae, brown seaweed is used as a bio-fertilizer in agriculture (**Bakrudeen et al., 2017**). Interestingly, bio-fertilizers play a vital role in maintaining soil fertility and essential components

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that are needed for organic farming. These organic fertilizers provide several minerals and nutrients in the soil required to improve plant growth and crop yield production (**Rouphael *et al.*, 2018**). Recently, seaweed has been replaced in agriculture to enhance plant growth because it acts as a bio-stimulant and bio-fertilizer (**Hashem *et al.*, 2019**). Treatment with seaweed biomass increases nutrient uptake of soil and makes it resistant to environmental stress. In many countries, including France, Spain, Vietnam, Thailand, Indonesia, Bangladesh, Malaysia, Philippines, Japan, China, and Korea, mass cultivation of seaweed is common (**García-Poza *et al.*, 2020**).

Moreover, seaweed and seaweed-derived compounds have been widely used as amendments in crop production due to the presence of a number of plant growth-regulating compounds, carbohydrates, vitamins, and minerals that help to maintain soil fertility, while other macronutrients and micronutrients promote faster seed germination and an increase in yield (**Mirparsa *et al.*, 2016**). Many recent researches have revealed that these marine macroalgae may be used as bio-fertilizers to increase plant growth and production (**Hashem *et al.*, 2019**). However, **Khan *et al.* (2009)** reported that the metabolic activity of many seaweed products has not been fully exploited due to the lack of scientific information on the growth factors present in seaweed and their mode of action in affecting plant growth. Although seaweed fertilizer in liquid form has gained importance in enhancing crop yield (**Salah El Din *et al.*, 2008**), there is little data on soil conditioning and crop productivity through seaweed application in granular/powder form. Algae extract foliar application was indicated in several researches to improve the growth characteristics of various crop species (**Latique *et al.*, 2013**). It was reported that the application of different successive extracts of *Asparogopsis taxiformis* thallus powder to the soil, as a biofertilizer, gave a significant increase in the growth of *Vicia faba* (**El-Barody *et al.*, 2007**). Previously, the application of an extract from algae to soil or foliage increased the ash, protein, and carbohydrate content of potatoes. However, some of the red algae species, i.e. *Laurencia obtusa*, *Corallina elongate*, and *Jania rubens*, are effective fertilizers for maize growth and sugar content (**Safinaz and Ragaa, 2013**).

The wheat plant (*Triticum aestivum* L.) is one of the important cereal crops in Egypt. It plays an essential role and is used as the basic food requirement

of the human population. This is the major crop that is being utilized for food and is cultivated in the larger areas of Egypt. According to **Muhammad *et al.* (2013)**, adding various seaweeds to the soil improved all of the soil's physico-chemical and biological characteristics, as well as the crop production. They also found that the wheat crop morphological and agronomical parameters increased positively when plants were treated with different seaweeds. Thus, seaweeds play an important role in enhancing plant defense against pests and diseases that cause problems for some plant species (**Cluz *et al.*, 2004**), reducing salt stress (**Mancuso *et al.*, 2006**), drought stress (**Zhang and Ervin, 2004**), and heat stress (**Zhang and Ervin, 2008**). In addition, enriching pea plants with 10 to 15% algae extract increased production and improved seed quality when compared to the negative control (**Nawar and Ibrahim, 2014**). There is not enough information available about the application of seaweed to the growth and yield of wheat plants. **Safinaz and Ragaa (2013)** stated that the application of an algae mixture to the soil increased the dry weight, plant phosphorus, and nitrogen content of maize (*Zea mays* L.) plants.

When plants are exposed to environmental stress, reactive oxygen species (ROS) are produced. These activated oxygen damages protein, membrane lipid, and nucleic acid cellular constituents (**Xie *et al.*, 2019**). Plant cells accumulate various compounds, such as antioxidants and secondary metabolites, as an adaptive response to stress conditions (**Kosar *et al.*, 2015**). A number of enzymatic antioxidants (Catalase and peroxidase) are produced in plants due to abiotic stresses that protect plants against ROS-produced oxidative agents (**Nazi *et al.*, 2016**). The positive effects of seaweed on the plant growth criteria of various plant species may be due to the presence of plant growth-promoting substances/hormones in these marine algae (**Thorsen *et al.*, 2010**). The benefit of seaweed as a source of organic matter and fertilizer nutrients has led to its use as a soil conditioner for centuries (**Nabti *et al.*, 2017**). Moreover, the studies of **Kumar and Sahoo (2011)** and **Mohy El-Din (2015)** demonstrated that seaweed could serve as an alternative biofertilizer as it is ecofriendly, cheaper, and delivers substantial economic and environmental benefits to farmers.

Regarding the effects of marine macroalgae on the germination and early growth stages of plants, wheat genotypes is an effort to improve production potential for farmers and provide knowledge of suitable tolerant cultivars for

constrained soil. The goal of this study was to assess strategies for maximizing the benefits of applying marine macroalgae (seaweeds) to wheat genotypes.

Materials and Methods

1. Marine macroalgae (seaweeds) and cultivar conditions

Five marine macroalgae (*Halimeda opuntia*, *Caulerpa racemosa*, *Cystoseira myrica*, *Saragasum aquifolium*, and *Actinotrichia fragilis*) were seasonally collected from summer 2017 to spring 2018 from two different sites. These sites represent different hydrographic habitats along the north western coast of the Red Sea. The sample collection was performed using snorkeling at the two sites. The first site was the shore in front of the National Institute of Oceanography and Fisheries, Hurghada Branch, while the second site was a mangrove forest 15 km south of Safaga City. Seaweeds are dried and ground to a fine powder. About 30 g of the dried powder was mixed with the experimental soil before plants were cultivated. The investigated marine algae were identified by **Aleem (1978)**, **Coppejans and Beeckman (1990)**. After that, wheat grains were surface sterilized for 3 minutes with 1% sodium hypochlorite and then rinsed three times with sterile distilled water. The first group was grown under the above seaweed and the second group was untreated, which was used as a control (plants without algal treatment).

2. Growth conditions and plant cultivation:

The experimental plant used in the present investigation was *Triticum aestivum* L (Gemiza 11, Gemiza 171 and Misr 2, which are represented by S1, S2 and S3, respectively). The grains of the plant were obtained from the center of Agriculture, Assiut University. The plant was grown in the normal clay soil of 4000 g per plastic pot (30 cm in diameter) in a greenhouse under natural conditions at the Faculty of Science, Al Azhar University, Assiut, Egypt. All pots were seeded with ten grains per pot and three pots were used for each treatment. The pots were irrigated with tap water every 6 days until the fourth leaf appeared. The pots were irrigated with tap water to 60-70% of their water-holding capacity (WHC) just after growing. The experiment was set up in a fully random manner,

with pots being randomized every two days and three replications. The experiment was carried out in the open air under natural conditions with 26 ± 2 °C at day and 18 ± 2 °C at night. After plants were harvested, for dry weight measurements, the plant samples were cleaned with distilled water, divided into shoots and roots, weighed, and oven-dried at 80°C for 24 hours. The plant's leaf length, fresh weight, and dry weight were all measured.

3. Determination of photosynthetic pigments

At harvesting, the photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) were measured using the spectrophotometric method as recommended by **Lichtenthaler, 1987**.

4. Determination of total carbohydrates

The Anthrone-sulphuric acid method (**Fales, 1951 and Schlegel, 1956**) was used for the determination of carbohydrates. A calibration curve using pure glucose was made and the data expressed as mg glucose/g DW.

5. Determination of proline and lipid peroxidation contents

According to **Bates et al. (1973)**, proline content was measured. The proline concentration was determined using a calibration curve for proline and expressed as mg/gm DW. Malondialdehyde (MDA) formation was measured using the thiobarbituric acid reaction as described by **Madhava and Sresty (2000)** to determine lipid peroxidation (MDA). The concentration of MDA was calculated by using an extinction coefficient (155 mM) and the results were expressed as µg MDA/gm FW.

6. Determination of plant antioxidant enzyme activities

a. Catalase activity measurement:

The activity of catalase was determined by **Aebi (1984)**. Add 3 ml of the reaction solution (50 mM, of phosphate buffer, pH 7.0, and 30% W/V H₂O₂) to 0.5 ml of enzyme extract. The activity of catalase was estimated by the decrease

in absorbance at 240 nm as a consequence of H₂O₂ consumption (**Havir and Mellate, 1987**).

b. Determination of peroxidase activity:

The peroxidase activity was determined following Anjum *et al.* (2011) with slight modifications. The assay medium consisted of 2.5 mL of 100 mM K-phosphate buffer (pH 5.5), 100 µL of 1 mM guaiacol, and 0.1mL of enzyme extract. The reaction was started by the addition of 300 µL of 1.3 mM H₂O₂ to oxidize guaiacol and produce a dark brown product. The increase in absorbance at 470 nm was recorded using a spectrophotometer.

7. Statistical analysis:

Using the SPSS 20.0 software program, the data was subjected to one-way ANOVA. Three replicate values were used to calculate the mean. Duncan's multiple range tests were used to compare means, and statistical significance was determined at the 5% level.

Results

1. Effect of seaweed application on the growth rate of a wheat plant

As a general trend; the seaweed application influenced wheat (*Triticum aestivum* L.) genotypes to varied degrees, while the control plant showed normal shoot growth. Under *Halimeda opuntia*, *Saragasum aquifolium*, and *Actinotrichia fragilis*; the plants treated with these seaweeds showed a reasonably high improvement in growth rate (**Fig. 1**). When the wheat plant Misr 2 was treated with all marine algae, the leaf length was the longest, as seen in **Fig 1**. While *Halimeda opuntia* improved the shoot length of the wheat genotypes Gemiza 11 and Gemiza 171 by about 51.3 cm plant⁻¹, Gemiza 11 and Gemiza 171 had the same effect on the leaf length as *Actinotrichia fragilis*, *Saragasum aquifolium*, and *Caulerpa racemosa*, with 44.00 and 48.60 cm plant⁻¹, respectively (**Fig. 1**) as the control.

Changes in the fresh and dry weights of *Triticum aestivum* shoots as a result of different treatments with marine algae are presented in Tables 1 and 2. In wheat genotypes Gemiza 11, Gemiza 171, and Misr 2, the highest shoot fresh weight was found in the plants irrigated with *H. opuntia* by about 54.41, 56.84, and 58.74 g pot⁻¹, respectively. The shoot's fresh weight, on the other hand, was lowest in all wheat genotypes (Gemiza 11, Gemiza 171, and Misr 2) with only 37.63, 23.81, and 36.70 g pot⁻¹, respectively, when treated by *C. racemosa* application as shown in **Table 1**. In regards to shoot dry weight, a significant difference ($P \leq 0.5$) among the treatments was found. The highest shoot dry weight was found in the plant treated with *H. opuntia* and *S. aquifolium*, followed by the plant treated with *A. fragilis*, whilst, the lowest value was recorded in the plant irrigated with *C. racemosa* as compared to the control sample as shown in **Table 2**.

2. Carbohydrate content in the wheat genotypes under algal application:

When seaweed was applied to the plant growth, it caused significantly increased in carbohydrate content in all tested wheat genotypes (**Fig. 2**). All selected species showed an increase in the content of total carbohydrates in the genotype Misr 2 as compared to other wheat plants Gemiza 11 and Gemiza 171.

The maximum increase in total carbohydrate content was observed in the genotype Misr 2 when the plant is grown under *S. aquifolium* (350 mg.g⁻¹DW) compared to untreated. Compared to control, *A. fragilis* caused a significant increase in total carbohydrate content in all wheat genotypes Gemiza 11, Gemiza 171, and Misr 2 with 340, 310, and 250 mg g⁻¹ DW, respectively. When the plant was used to grow *H. opuntia* and *S. aquifolium*, however, genotype Gemiza 11 had lower carbohydrate content, ranging from 160 mg g⁻¹DW to 230 mg g⁻¹DW.

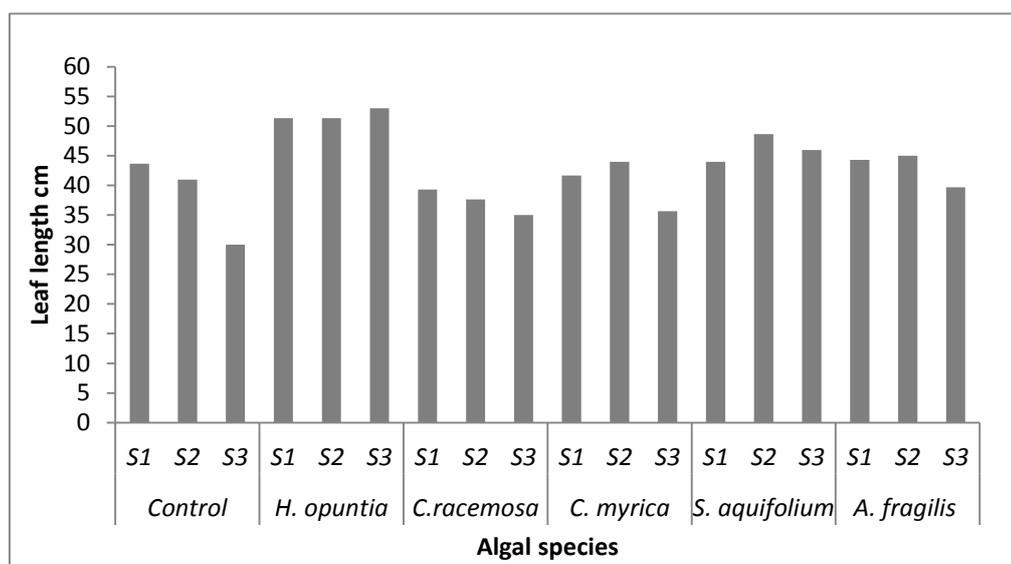


Fig. 1: Leaf length of various wheat genotypes that grown with some marine algae which are collected from some coasts of the Red Sea. Data are means of three replicates \pm SE (S1= Gemiza 11, S2= Gemiza 171 and S3 = Misr 2).

Table 1: Shoot fresh weight of wheat plants cultivated with some marine algae which were collected from some coasts of the Red Sea.

Treatment	Shoot fresh weight of wheat plants (g pot ⁻¹)					
	Gemiza 11	%	Gemiza 171	%	Misr 2	%
Control	49.38 \pm 1.80	100	46.11 \pm 1.38	100.00	40.71 \pm 0.81	100.00
<i>H. opuntia</i>	54.41 \pm 1.54	110.19	56.84 \pm 1.70	123.28	58.74 \pm 1.03	144.26
<i>C. racemosa</i>	37.63 \pm 4.67	67.20	23.81 \pm 2.61	51.65	36.70 \pm 1.45	90.13
<i>C. myrica</i>	41.74 \pm 3.09	84.53	44.93 \pm 1.34	97.45	44.73 \pm 0.82	109.86
<i>S. aquifolium</i>	51.49 \pm 2.09	104.27	42.64 \pm 0.73	92.47	52.67 \pm 1.77	129.36
<i>A. fragilis</i>	50.38 \pm 3.54	102.02	51.52 \pm 5.47	111.74	44.92 \pm 5.79	110.32
LSD=0.05	=12.9734870268		= 9.61257195634		= 10.9458695024	

Table 2: Shoot dry weight of wheat plants cultivated with some marine macroalgae which were collected from some coasts of the Red Sea.

Shoot dry weight of Wheat plants (g pot ⁻¹)						
Treatment	Gemiza 11	%	Gemiza 171	%	Misr 2	%
Control	10.56±0.35	100	9.62±0.25	100	7.18±0.32	100
<i>H. opuntia</i>	12.31±0.32	116.60	12.65±0.44	131.49	12.38±0.63	172.34
<i>C. racemosa</i>	10.85±1.12	102.74	7.27±0.67	75.64	8.62±1.17	120
<i>C. myrica</i>	12.39±0.64	117.93	13.89±0.42	144.45	13.60±0.36	189.37
<i>S. aquifolium</i>	12.24±0.64	115.97	10.86±0.43	112.88	11.50±0.60	160.18
<i>A. fragilis</i>	12.15±1.14	109.31	12.95±1.47	88.46	12.44±1.44	89.46
LSD= 0.05	= 1.26982642007		=2.2196976478		1.42433869314	

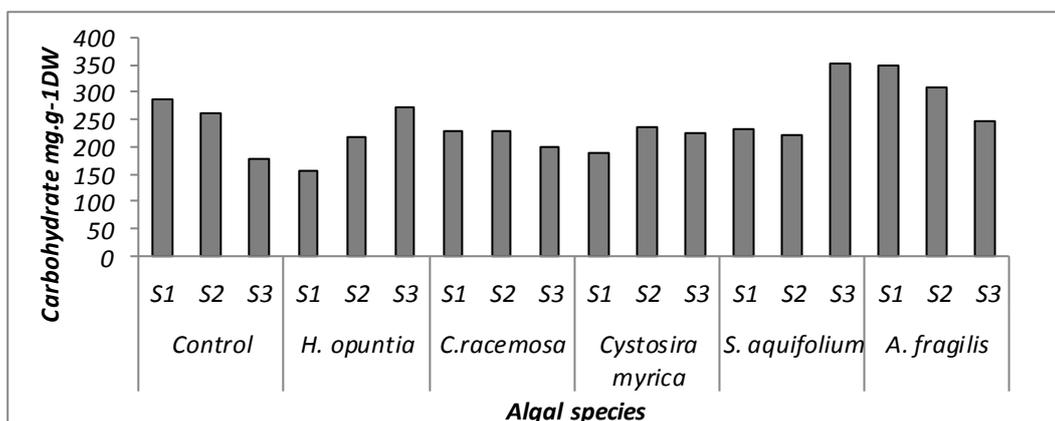


Fig. 2: Carbohydrate contents of various wheat genotypes that treated with some seaweeds. Data are means of three replicates ± SE. Significant differences (P ≤ 5%) between treatments and genotypes are indicated by different letters (S1= Gemiza 11, S2= Gemiza 171 and S3 = Misr 2).

Similar to control treatments, there was no discernible difference in the content of total carbohydrates in the genotype Gemiza 171 when the plant was treated with seaweed (macro marine algae). Based on these results, the application of selected seaweeds increased the content of total carbohydrates in the leaf cells of wheat genotypes, which led to the improvement of plant growth and yield production (**Fig. 2**).

3. Proline and MDA content

It was obvious from **Fig. 3a** that the addition of *C. racemosa* to the wheat plant led to a great increase in the shoot proline content of all wheat plant genotypes (11, 7 and 5 mg/g fresh wt. in Gemiza171, Misr 2 and Gemiza 11, respectively), followed by the plant watered and treated by *A. fragilis* (6 mg/g fresh wt. in Gemiza171 and Misr 2) with respect to the control (4.5 mg/g fresh wt.). While, the lowest proline content was found in the shoots of all wheat plants treated with *H. opuntia*, followed by Gemiza 11 and Misr 2 when plants were treated with *A. fragilis* and *S. aquifolium*, respectively; as shown in **Fig.3a**.

In comparison to other macroalgae; only *C. myrica* showed no significant effect on the shoot proline content as compared to the control in all plant species. The changes in lipid peroxidase are represented as MDA accumulation in plant cells when plants grow under environmental stress. Similarly, MDA content in the wheat genotypes Gemiza171 and Gemiza 11 was increased when the plant was treated with *S. aquifolium* followed by the wheat genotype Gemiza171 under the macro marine algae *C. racemosa* as compared to the control sample.

As mentioned in the results of proline content, application of marine algae also decreased MDA content in all tested wheat genotypes. However, *H. opuntia* showed the lowest MDA content of all wheat plants compared to other ones. Also, in the same trend as proline content, the seaweeds *A. fragilis* and *C. myrica* showed no significant effect on the MDA accumulation in the wheat genotypes Gemiza 171, Misr 2, as shown in **Fig. 3b**. Based on these results, the decrease in proline content in the leaf plant of wheat genotype under seaweed helps in the increase in plasma membrane stability of plant cells by making osmo-protectant.

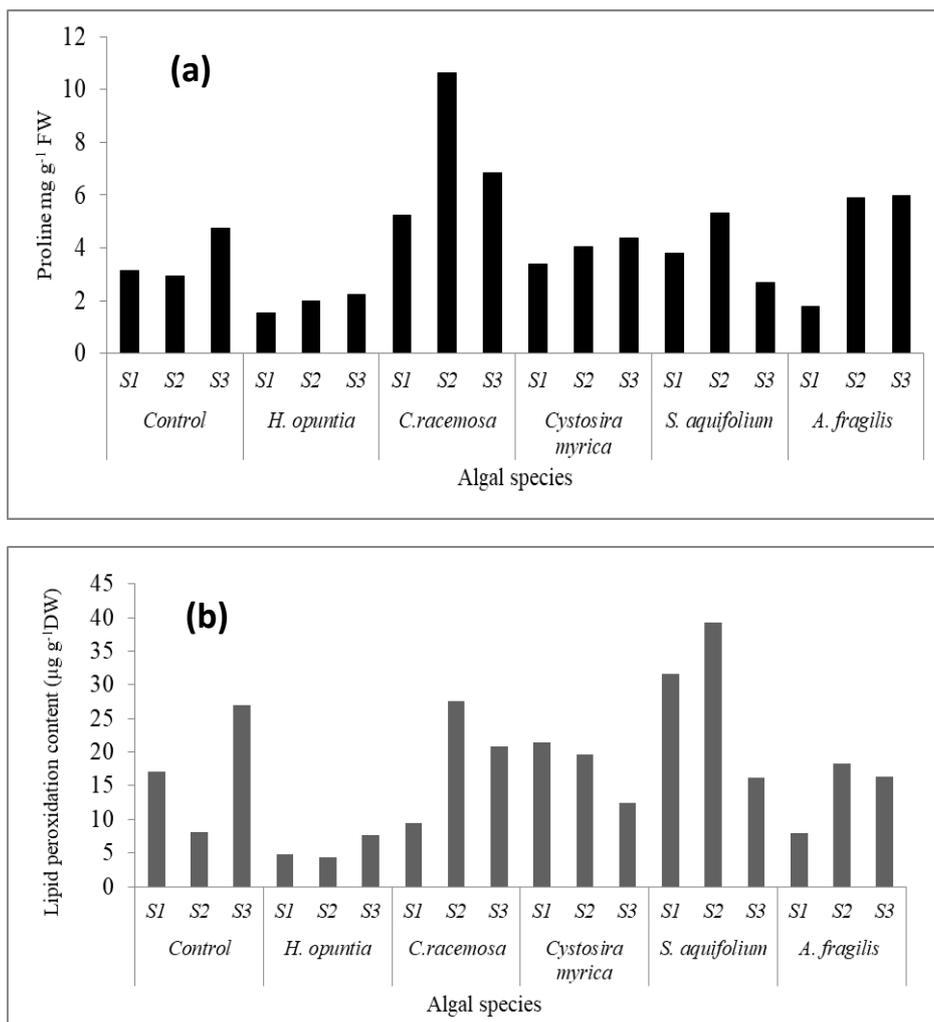


Fig. 3a, b: Proline contents (3a) and lipid peroxidation contents (MAD) (3b) of various wheat genotypes that treated with some seaweeds. Data are means of three replicates ± SE. Significant differences ($P \leq 5\%$) between treatments and genotypes are indicated by different letters (S1= Gemiza 11, S2= Gemiza 171 and S3 = Misr 2).

4. Catalase and peroxidase antioxidant enzymes

The activity of catalase (CAT) and peroxidase (POD) of wheat genotypes is presented in **Fig. 4a, b**. The results showed that catalase activity was significantly enhanced by the application of seaweed as a bio-fertilizer on wheat plant species. However, the genotypes Gemiza 11 and Misr 2 showed lower catalase activity when plant-treated with all seaweeds compared to the genotype Gemiza171; it was significantly greater significant increase in the activity of the catalase antioxidant enzyme of the wheat plant under all seaweed application. As shown in **Fig. 4a**, the marine algae *C. myrica* and *C. racemosa* increased the catalase activity in Gemiza171 (0.9 unit mg⁻¹ FW) in comparison to Misr 2, which showed the lowest catalase antioxidant activity (0.4 unit mg⁻¹FW) that was grown under *S. aquifolium*. Among the genotypes, Gemiza171 doesn't differ in the activity of the antioxidant catalase enzyme when plants are treated with *H. opuntia* and *S. aquifolium* as compared to control. However, treatment of wheat genotypes with seaweed increased POD activity slightly, as shown in **Fig.4b**. In contrast to the control plants, the application of plants with seaweed resulted in a significant decrease in peroxidase activity. However, the lowest reduction was reported in the genotypes Gemiza171 and Misr 2 in comparison to Gemiza11 which had higher POD activity under *C. myrica*, *S. aquifolium*, and *A. fragilis* as compared to control (**Fig. 4b**).

Discussion

The aim of this study was to compare the relative changes in shoot growth of three wheat genotypes under-treated and untreated irrigated-plants and to access the possible efficiency of seaweeds for use as a biofertilizer.

The seaweed fertilizers could help the soil to create an environment suitable for root growth by increasing microbial diversity, resulting in improved soil content and water holding capacity (**Abbott *et al.*, 2018**). Also, the application of marine algae improves biological activities like respiration and nitrogen mobilization, and mineralization of mineral nutrients (**DePascale *et al.*, 2017**).

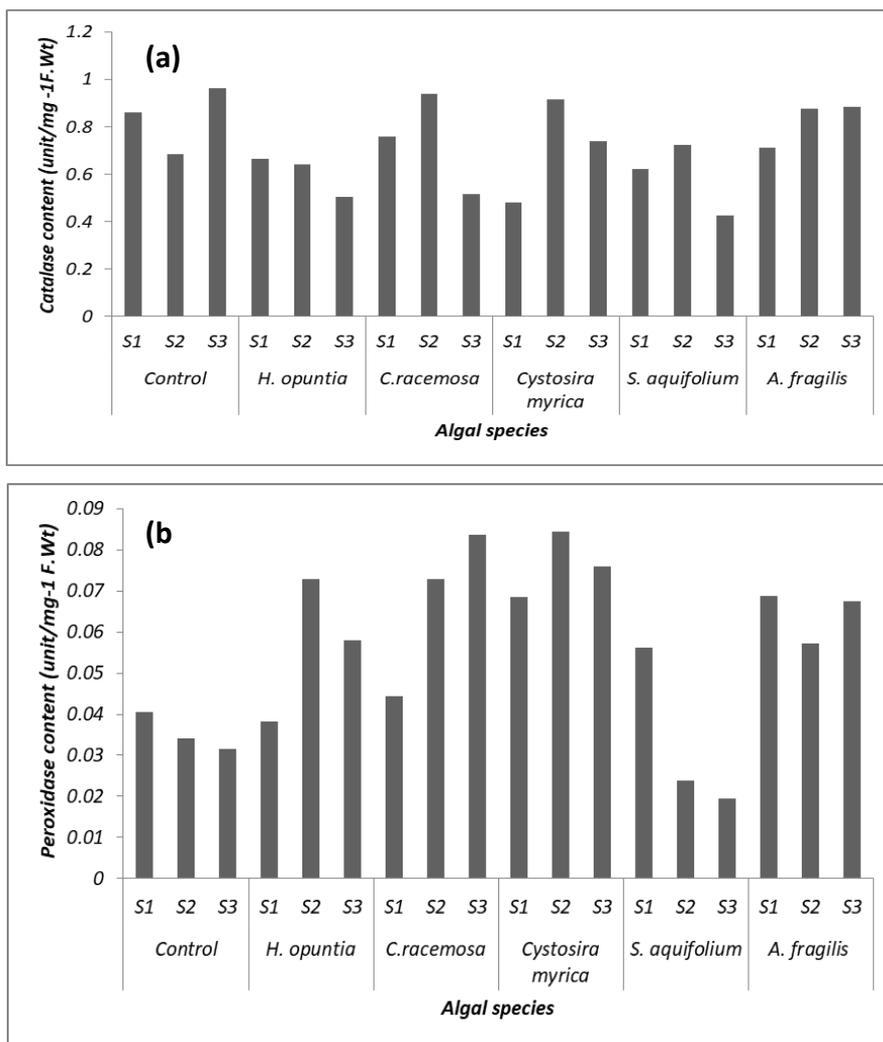


Fig. 4a, b: Catalase enzymes (CAT) (4a) and peroxidase enzymes (POD) (4b) of various wheat genotypes that treated with some marine algae. Data are means of three replicates \pm SE. Significant differences ($P \leq 5\%$) between treatments and genotypes are indicated by different letters (S1= Gemiza 11, S2= Gemiza 171 and S3 = Misr 2).

Even so; *H. opuntia* can be used to improve the physicochemical properties of soil, which in turn increases plant growth and productivity. Seaweeds are a rich source of polysaccharides that may affect soil aggregation directly or indirectly after their decomposition by soil microorganisms (**Heltan *et al.*, 2015**). Also, **Davari *et al.* (2012)** reported that organic matter contained in seaweed biofertilizer is known to stimulate the growth rate of some plant species due to its nutrient content. The application of seaweed as a biofertilizer in sufficient quantities increased the level of soil nutrients, minerals necessary for plant growth and stimulated the growth rate of field crops (**Mirparsa *et al.*, 2016**). The presence of some growth regulators, macro and micronutrients, may be responsible for the positive increase in plant growth and division (**Tarakhovskaya *et al.*, 2007**).

The most effective seaweed treatment in this study was *Halimeda opuntia*, which caused the greatest stimulation in wheat growth under normal conditions. The presence of a higher level of total carbohydrates and proline content in *H. opuntia* could explain the observed benefit over the other used seaweeds. The distinctive effect of *S. aquifolium*, on the other hand, could be attributed to its significantly higher carbohydrate content than the other seaweeds studied. Because of their high content of polysaccharides and plant growth regulators with a wide range of biological content, most marine microalgae are considered valuable resources for plant improvement (**Hamed *et al.*, 2018 and Ganesan *et al.*, 2019**). Our findings were similar to the recent investigation by **Hashem *et al.* (2019)**. They hypothesized that the stimulatory effect of seaweed on growth and productivity was due to its constituents, which included a wide range of growth-promoting hormones, total carbohydrates, and phenolic compounds.

Leaf growth can possibly be induced due to the high uptake of water and minerals from the soil after being treated with marine macro-algae. In the present study, leaf length was significantly increased by the treatment with seaweeds; *H. opuntia*, *S. aquifolium*, and *A. fragilis* with wheat varieties compared to *C. racemosa* and *C. myrica*. In comparison to the Gmazia 11 genotype, wheat plants such as Misr 2 and Gmizia171 had a significant increase in leaf length. This result could be attributed to the presence of some hormonal compounds in marine macro-algal granular, such as auxins and gibberellins, which mitigate the negative

effects of environmental conditions on wheat growth (Nassar *et al.*, 2016) and help recover plants after damage (El Shoubaky and Salem, 2016). The current result was in agreement with those of Tuhy *et al.* (2015). They suggested that the application of different seaweeds led to an increase in plant biomass, which is due to the presence of a high amount of mineral fertilizer.

Similarly, wheat plants treated with seaweed exhibited an increase in fresh and dry weights, which indicates that the components in seaweed had a considerable effect on shoot development. The highest values were found for plants treated with *H. opuntia*, *S. aquifolium*, and *A. fragilis*. While the lower values were observed when the plants were applied by *C. racemosa* and *C. myrica*. The presence of some growth-promoting substances could explain the increase in growth parameters (MohyEl-Din, 2015) or due to nutrient content (Kalaivanan *et al.*, 2012). Our results coincided with those of earlier studies on *Vigna sinensis* (Sivasankari *et al.*, 2006), *Vigna mungo* (Kalaivanan *et al.*, 2012), *Zea mays* (Al-Shakankery *et al.*, 2014), and wheat plants (Kumar and Sahoo, 2011, Kumar *et al.* 2012, and MohyEl-Din, 2015). They concluded that the application of seaweed to pea plants significantly increased the vegetative growth characteristics such as leaf length and total fresh and dry weight. Our findings revealed that *H. opuntia*, *S. aquifolium*, and *A. fragilis* had the highest maximum values in this regard, with no statistically significant difference between them. The phytohormone content of the algal application may be responsible for the enhanced effect on wheat plant growth characteristics (Yakhin *et al.*, 2017). Algal granules, which play an important role in cell division and expansion. As a result, shoot growth and plant fresh and dry weight increased, as suggested by (Chiaiese *et al.*, 2018).

Carbohydrate contents

The results of the present study indicated an increase in the total carbohydrates of the plant irrigated with untreated and treated algae. This is due to the absence of some minerals in untreated algae that alter carbohydrate accumulation and distribution in wheat plants. The results were in agreement with Ganesan *et al.* (2019), who reported increased carbohydrate content in some crop species irrigated with seaweed than in untreated ones. In contrast, the decreases in the total content of carbohydrates of the tomato shoot and root of the irrigated

plant with marine algae were detected in the work done by **EL-Sheikh *et al.* (2020)**.

It was reported that the application of seaweed has a significant positive effect on wheat yield and its carbohydrate content. Our results confirm the data of **Hashem *et al.* (2019)**, who claimed that the effect of seaweed on plants is a favorable condition that promotes plant growth (in all growth parameters) and total carbohydrate content, resulting in higher crop yields. Macroalgae treatments such as *H. opuntia* and *S. aquifolium* tended to stimulate a positive effect on wheat yield. An increase in carbohydrate levels in treated seaweed plants is a common indicator of antioxidant parameters; this has been linked to plant growth stimulation and a high level of the antioxidant enzyme (**Pise & Sabale, 2010**). Wheat plants are protected from the negative effects of declining carbohydrate content by adding *H. opuntia* and *S. aquifolium* to the soil. Under normal conditions, the observed enhancement effect of algal treatments on carbohydrates indicated that these treatments maintained a higher carbohydrate content than the control. Similar results were recorded by **Rathore *et al.* (2009)**, who discovered that SLF-treated soybean plants had higher carbohydrate levels than untreated controls. **Korzen *et al.* (2015)** discovered that the plant growth substances in SLF boost the carbohydrate content of plant leaves. Total carbohydrate accumulation was found to be slightly higher in all wheat genotypes used in this study than in the control. Meanwhile, when compared to untreated wheat plants (Misr 2), seaweed-treated wheat plants (Misr 2) had significantly higher total carbohydrates.

This finding is consistent with the observed increase in proline content in response to seaweed treatments, which occurred in the same order. This means that seaweed increased total carbohydrates by stimulating proline biosynthesis, which helps plants grow and produce more carbohydrates. The same outcomes were obtained by **Korzen *et al.* (2015)** for *Ulva rigida* and **Pramanick *et al.* (2017)** for Potato tubers. Increased amounts of carbohydrates, which are known to play an important role in osmoregulation and also improve plant growth in a way similar to hormones, may be attributed to the growth-enhancing potential of seaweeds like *H. opuntia* and *S. aquifolium*.

Proline content, lipid peroxidase level (MAD)

Decreased levels of some metal ions are known to affect the permeability of membranes, which may lead to water stress conditions inducing the production of proline (**Basak et al., 2001**). Proline content is proved to be essential for stress tolerance because of its active role in osmotic adjustment, protection of the enzyme structure, stabilization of membranes, and defense against hydroxyl radicals (**Silva-Ortega et al., 2008**). According to the obtained data, proline content and lipid peroxidase level (MAD) reached their maximum effect when plants were treated with seaweed. In comparison to the control, proline levels were higher in seaweed-treated plant wheat leaves. Furthermore, when plants were treated with marine algae, higher levels of proline were accumulated. Proline may protect plant cells from harmful oxidative stresses by reducing the harm caused by reactive oxygen species (ROS) as a result of various defense mechanisms, according to (**El Rabey et al., 2016**). As a result, the increased proline content of wheat plants treated with *C. racemosa* compared to control and other treated plants suggests that proline is one of the wheat plant's defense mechanisms against oxidative problems caused by environmental conditions. Proline content can use osmotic adjustment to stabilize proteins and cellular structures, as well as redox metabolism to scavenge excess ROS and restore cellular redox balance (**You and Chan, 2015**). Plants grown in soil supplemented with seaweed accumulate higher levels of proline. It is well known that high proline accumulation is linked to a higher tolerance to stress in the environment (**Mansori et al., 2016**).

Similarly, an increase in lipid peroxidation under stress conditions is due to the generation of free radicals that distort the membrane architecture, causing oxidative damage (**Nada et al., 2007**). Due to the relatively higher availability of ion metals in plants irrigated by treated algae, the requirement for antioxidants was low and hence the induction of antioxidants was also lower compared to plants irrigated by untreated algae. The study suggests that plants growing in treated algae irrigated areas have potentially developed a defense strategy to combat environmental stress due to plant hormones, which help to support plant growth under nutrient-stressed conditions and to recover plants after damage (**El Shoubaky and Salem, 2016**).

Antioxidant activity

Antioxidant enzymes are the first protection mechanism against environmental stress conditions. Plant cells have several response mechanisms against ROS production (Saeed *et al.*, 2020). The present results revealed a significant decrease in the activities of CAT and SOD in the untreated plants. At the same time, treatment of wheat plants by the application of marine algae, especially *H. opuntia*, increases the activity of antioxidant enzymes which are involved in the scavenging of active oxygen species (Mohy El-Din 2015). The enzyme activities could be attributed to the presence of antioxidant and osmo-protectant components, such as proline, in the investigated marine algal compounds.

Seaweeds have recently been extensively studied for their potential as natural antioxidants (Ozyigit *et al.*, 2016). Similarly, when compared to the control, wheat plants treated with seaweed had significantly higher levels of antioxidant activity. In response to *Caulerpa racemosa* and *Sargassum aquifolium*, especially genotype Gemiza171, the highest antioxidant activity was detected. Antioxidants protect plants by maintaining the balance between the synthesis of free radicals and their detoxification, where the accumulation of some ion metals induces oxidative stress because they are involved in several different types of ROS-generating mechanisms. Singh and Agrawal (2010) reported that the antioxidative enzyme; peroxidase showed an increment in its activity in plants grown under the application of seaweed as compared to control ones. Peroxidases play a significant role in defense against oxidative stress (Ozyigit *et al.*, 2016) and are suggested to be indicators of metal toxicity accumulation (Ghori *et al.*, 2019). An increase in peroxidase activity under environmental stress has also been reported in palak spinach grown at different application rates of seaweed (Xu and Leskovar, 2015).

Conclusion

In conclusion, the application of some macroalgae, namely *Halimeda opuntia*, *Sargassum aquifolium*, *Actinotrichia fragilis*, *Caulerpa racemosa*, and *Cystoseira myrica* on wheat plants influenced nutrient transformation and soil properties, leading to improved wheat growth, yield, and development. The

improved soil conditions supported greater plant mineral uptake and utilization. The enhanced capacity of treated leaves to scavenge free radicals by elevated antioxidants like peroxidase and catalase and rapid mobilization of stored carbohydrates, proline, and malondialdehyde (MAD) contents during vegetative stages could at least partially explain the beneficial effects of seaweed application on different wheat plants.

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إستجابة بعض الأنشطة الأيضية لنباتات القمح للمعاملات المختلفة للطحالب البحرية من البحر الأحمر، مصر

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في هذه الدراسة تم تجميع خمسة أنواع من الطحالب البحرية الكبيره لمدة أربعة مواسم خلال عامي 2017 – 2018 من موقعين مختلفين بساحل البحر الأحمر المصري. تم تجميع العينات من منطقه المد والجزر على عمق من 1-2 مترا بإستخدام طريقه الغطس الحر (السنوركل).

تم إجراء بعض التجارب على ثلاثة سلالات من نبات القمح (*Triticum aestivum* L.) بعد معاملاتها ببعض الطحالب البحرية لمعرفة مدى تأثير إضافة الأعشاب البحرية المختلفة على بعض التغيرات البيوكيميائية في هذه النباتات. تمت معاملة سلالات القمح بخمسة أنواع مختلفة من معالجات الأعشاب البحرية وهى: *Halimeda opuntia* و *Caulerpa racemosa* و *Cystoseira myrica* و *Saragassum aquifolium* و *Actinotrichia fragilis*.

أظهرت النتائج أن نباتات القمح المعالجة بالأعشاب البحرية إزداد فيها نشاط إنزيمات peroxidase في المرحلة الخضرية لنمو النبات؛ وقد لوحظ أن أعلى نشاط peroxidase كان في أوراق النباتات المعالجة بطحلب *Halimeda opuntia*؛ بينما زاد نشاط أنزيم ال Catalase نسبيا في أوراق كل السلالات مع معظم الأنواع الطحلبية المختلفة؛ وقد سجلت الزيادة القصوى لهذا الإنزيم عند المعالجة بأنواع *Halimeda opuntia* و *Saragassum aquifolium* و *Actinotrichia fragilis*.

أوضحت النتائج أيضا أن مستوى الكربوهيدرات الكلية والأنشطة الأيضية الأخرى مثل محتوى البرولين والمالونديالدهيد (MAD) قد إزداد في سلالات القمح مع كل المعالجات الطحلبية خاصة مع بعض الأنواع مثل *Sargassum aquifolium* و *Actinotrichia fragilis* و *Caulerpa racemosa*؛ وقد لوحظت أعلى زيادة في محتوى المالونالدهيد (MAD) في الأوراق المعالجة بطحلب *Halimeda opuntia*.

في الختام؛ أثر تطبيق بعض الطحالب الكبيرة على بعض سلالات نباتات القمح على تحول المغذيات وخصائص التربة مما قد يؤدي إلى تحسين نمو القمح وإنتاجيته وتطوره؛ وقد ساعدت ظروف التربة المحسنة على زيادة إمتصاص النبات للمعادن وإستخدامها وقد ساعدت القدرة المعززة للأوراق المعالجة على التخلص من الجذور الحرة عن طريق مضادات الأكسدة المرتفعة مثل peroxidase و catalase والتعبئة السريعة للكربوهيدرات المخزنة والبرولين ومحتوى المالونالدهيد (MAD) خلال المراحل الخضرية يمكن أن تفسر جزئيا على الأقل الآثار المفيدة لتطبيق الأعشاب البحرية على نباتات القمح المختلفة.