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INDUCES OF ANTIOXIDANT COMPOUNDS AND SALT TOLERANCE IN WHEAT PLANT, IRRIGATED WITH SEAWATER AS RESPONSE TO APPLICATION OF MICROALGAE SPRAY

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ABSTRACT

Wheat plants (*Triticum aestivum* L., cv. Giza 168) irrigated with either 10 or 20% of Seawater (SW) were treated with aqueous extracts of green microalgae *Scenedesmus obliquus* and blue green algae *Spirulina platensis* (AESO and AESP 20 g (dry weight)/L) in order to increase wheat salt tolerance. Treated plants showed higher ability to tolerate salt stress (10 or 20% SW) by significant ($p>0.5\%$) increasing of photosynthetic pigments (chlorophyll: Chlorophyll total, chlorophyll a and b types) and antioxidant low-molecular compounds (glutathione and carotenoids) contents. The increase of these contents was associated with increasing activities of antioxidant enzyme systems Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Catalase (CAT) and total Peroxidase (POD). In addition, this observation was significantly correlated with decreasing of lipid peroxide products (TBARs) and sodium ions concentrations. However, wheat plant exposed to salt stress showed significant changes in all growth parameter and antioxidant low-molecular compounds and antioxidant enzyme activities compared with that in plants irrigated with regular water (tap water). In addition, plants treated with oxalic acid as bioregulator agent implied a moderate changes on growth parameters, antioxidant capacity includes non-enzyme and enzymatic systems compared with that in wheat plants treated with algae extracts. This study indicates that the algae extracts could be used as a promising plant growth enhancer for treating wheat plants irrigated with brackish water.

Keywords: Microalgae *Scenedesmus Obliquus*, *Spirulina Platensis*, Antioxidants, Oxalic Acid, Salt Tolerance, Antioxidant Enzymes System, Irrigation with Seawater and Wheat Plants

1. INTRODUCTION

The Reactive Oxygen Species (ROS) comprises both free radical (O^{-2}, superoxide radicals; OH^{-}, hydroxyl radical; HO^{2-}, perhydroxy radical ROO^{2}-and alkoxy radicals RO^{-}) and non-radical forms (H_2O_2, hydrogen peroxide and $_1O^2$, singlet oxygen) are produced in plants under normal growth conditions and their concentration were low, while under stressful environments it is considerably high (Polle, 2001). However, exposure of plants to unfavorable environmental conditions such as temperature extremes, heavy metals, drought, nutrient deficiency, or salt stress, can induce increase the production of ROS (Karpinski *et al*., 2003; Laloi *et al*., 2004; Gill and Tuteja, 2010). In stomata closure, osmotic stress leads to leak in CO_2 availability for photosynthetic carbon assimilation, thereby causing high accumulation of superoxide in chloroplast which can cause photo-inhibition and photo-oxidation damages (Ashraf *et al*., 2008). Under pathogens, drought and salinity stress

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condition; ROS are generated through pathways such as photorespiration, mitochondrial respiration and from the photosynthetic apparatus (Pei *et al*., 2000). However, under these conditions, the cellular electron transport within the different subcellular compartments is impairs and lead to generation of ROS compounds (Ali and Alqurainy, 2006). Therefore, ROS could be considered as cellular biomarker for stresses and as secondary messenger in the stress response signaling pathways. However, plants have the high ability to scavenge ROS radicals by producing two types of antioxidants action includes: Enzymatic and non-enzymatic systems (Abd El Baky *et al*., 2010; Gupta *et al*., 1999). Enzymatic antioxidants include Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Peroxidase (POD) and Glutathione Reductase (GR). Whereas, non-enzymatic antioxidant includes (low molecular weight): Glutathione (GSH), Ascorbate (AsA), carotenoids and phenolic compounds (Asada, 1999; Kiddle *et al*., 2003).

Microalgae are one of the potential organisms and useful to mankind in various ways: Marine culture, food, feed, fuel, medicine, industry and in combating pollution (Thajuddin and Subramanin, 2005; Abd El-Baky and El-Baroty, 2012). Also, it is conceder as a rich source of several fine chemicals of economic value such as vitamins, carotenoids, phycobiliprotein, polysaccharides, fatty acids which possess varied biological properties and showed antibacterial, antifungal, antioxidant, anticancer and immune modulator agents (Abd El Baky *et al*., 2012; Abd El Baky and El-Baroty, 2013a). In the agriculture and horticulture field, microalgae exhibited stimulate action to growth of plants and can enhanced the plant to tolerate the a biotic stress, due to the presence of auxine, cytokinins, gibberellins, other related growth regulators and antioxidant compounds (Ordog *et al*., 2004; Molnar and Ordog, 2005; Abd El Baky *et al*., 2010). However, many studies indicated that some natural compounds might play an important role in enhancing plant tolerance to some abiotic stresses such as salt, drought and extreme temperatures (Ashraf, 2010; Abd El Baky *et al*., 2010). However, a positive association between the high accumulation of antioxidants and degree of salt tolerance has been drawn in different plant species. As examples, guaiacol Peroxidase (POX), SOD, POX and CAT enzyme activities play a significant role in the protection and recovery of several plants against oxidative stress induced by salt stress (Ashraf, 2010; Turhan *et al*., 2008). Moreover, plants contain a variety of non-enzymatic molecules (ascorbic acid, tocopherols, carotenoids, flavonoids, glutathione) which play a substantial role in counteracting oxidative stress caused by stresses (Tausz *et al*., 2000; Schafer *et al*., 2002). Abd El Baky *et al*. (2010), reported that microalgae could be enhanced plant salt tolerance through increasing the production of non-antioxidant compounds and elevate the activities of antioxidant enzyme system. Since, evaluation of functioning of all natural components in salt tolerance including that of oxidative stress tolerance is necessary. Therefore, this study was aimed to understand the impact of treated wheat plants irrigated with 10 and 20% seawater with algal extracts as bioregulator to enhance salt tolerance.

2. MATERIALS AND METHODS

2.1. Algal Strains and Growth Conditions

Scenedesmus obliquus and *Spirulina platensis* algae were grown in 30 L medium containing 10 ppm (NSI media) and 51 ppm (Zarriuk media), respectively as stress N_2 concentration in order to induction antioxidant compounds. All culture flasks were illuminated by continuous cool white fluorescent lamps (Philips at 200 W m^{-2} as optimum illumination conditions) as reported by Abd El Baky *et al*. (2012) and Abd El-Baky and El-Baroty (2012).

2.2. Plant Materials

Wheat cultivar named Giza 168 was obtained from Wheat Department, Agriculture Research Center, Giza, Egypt.

2.3. Cultivation of Wheat

A pot experiment was conducted in the greenhouse of National Research Centre Dokki, Cairo, Egypt during 2012/2013 winter season in order to evaluate the effect of spraying foliar of two algal strains (*Scenedesmus obliquus* and *S. platensis*) extracts (20 g L^{-1} dry weight in 0.1% Tween solution) as biofertillizer and sources of growth promoter to enhance salt tolerance of wheat plant irrigated with 10 and 20% sea water in tap water. Also, wheat plants irrigated with tap water were used as control plants. Organic acid (Oxalic acid 200 mg L^{-1}) was used as positive control plants. The experimental design was split plot (each 6 replicates). Salt stress treatments were started at 57 days from cultivation where plants irrigated by saline water and in next irrigation followed by tap water alternatively until the end of experiment. Samples from treated plants were taken at 70 days from sowing for chemical analysis. At the same time the plant received the optimum fertilizer (PO^-_4, K^+ and N as recommended by Agriculture of Ministry in Egypt).

2.4. Extraction and Determination of Antioxidant in Algal cells Wheat Leaves

2.4.1. Extraction of Carotenoids and Tocopherols

Carotenoid and tocopherol compounds were extracted from freshly plant material with 1: 10 (w/v) Tetrahydrofuran (THF) in present 30 mg^{-1} of BHT (2,6-ditertra-butyl-p-cresol) and magnesium carbonate (1 g 10 g^{-1} sample). After 24 h, an aliquot of the clear extracted pigments was filtered and evaporated to 5 mL using a stream of N_2 gas. The extracted pigments were saponified with 25 mL of ethanolic KOH (10%), for 2 h at room temperature and then carotenoids and tocopherols were extracted with dichloromethane. The solvent layer was then separated, washed several times with distilled water, dried over Na_2SO_4 and complete dryness was attained by a stream of nitrogen (Abd El Baky and El-Baroty, 2013b).

Determination of algal total carotenoids: The total carotenoids were determined by spectrophotometric method at 450 nm. βcarotene served as a standard compound, was used for preparing the calibration curve (Semeneko and Abdullaev, 1980).

2.5. Extraction and Determination of Ascorbic Acid

Ascorbic acid (vitamin C) was extracted from the plant material with 2% metaphosphoric acid and determined by spectrophotometrically using 2, 6 di-chlorophenol indophenol dye (Augustin, 1985).

2.6. Determination of Total Phenolics

Total phenolic content was determined by the Folin-Ciocalteu method (Li *et al*., 2007). In brief, 1 mL of 1:10 diluted Folin-Ciocalteu reagent were added to 200 µL aliquot of extract, after 4 min, 800 µL of saturated sodium carbonate (75 g L^{-1}) was added. The mixture was incubated for 2 h at 25°C and then the absorbance was measured at 765 nm. Gallic acid (0-500 mg L^{-1}) was used for preparation of standard calibration curve. The results were expressed as gallic acid equivalent (GAE)/g dry weight and calculated as mean value ± SD (n = 3).

2.7. Extraction and Estimation of Chlorophyll

One gram of wheat leaves (FW) dried on filter paper, was ground in acetone (5 mL, 80%) and allowed to stand overnight in dark at 4°C followed by centrifugation at 10,000×g for 5 min. The contents of total Chlorophyll (T-Chl), Chlorophyll a (Chl-a) and Chlorophyll b (Chl-b) in the supernatant were determined according to Lichtenthaler (1987).

2.8. Preparation of Cytosolic Fraction

Five grams leaves of wheat were excised and homogenized in 5 mL of ice-cold grinding buffer (250 mM sucrose, 25 mM Tris and the pH was adjusted to 7.2). The homogenate was centrifuged at 12000×g for 15 min at 4°C. The resulting supernatant was used for analyses of antioxidant enzyme activities, GSH, lipid peroxidation and protein content.

2.9. Determination of Glutathione (GSH)

The GSH contents of algal cells and wheat extracts were measured by reaction with 5,5`Dithiobis -2-Nitrobenzoic (DTNB) reagent to give a compound that absorbed at 412 nm (Silber *et al*., 1992).

2.10. Enzymes Assays

The activity of cytosolic SOD (EC 1.15.1.1) was determined by photochemical method (Ginnopolitis and Ries, 1977). Spectrophotometrically estimated as described by Chance and Maehly (1955) was used to assay the POD activity (EC 1.11.1.7). Ascorbate Peroxidase (APX) (EC 1.11.1.11) was determined according to Nakano and Asada (1981).

2.11. Determination of Lipid Peroxidation Products

The lipid peroxidation products was estimated by the formation of Thiobarbaturic Acid Reactive Substances (TBARS) and quantified in term of Malonaldhyde (MDA) as described by Haraguchi *et al*. (1997). The lipid peroxidation was expressed as micromoles of MDA. The extinction coefficient of TBARS was taken as 1.56×10^5 at 532 nm.

2.12. Determination of Proteins

Protein concentration of cytosol was determined spectrophotometrically at 595 nm, using comassein blue G 250 as a protein binding dye (Bradford, 1976). Bovine Serum Albumin (BSA) was used as a protein standard.

2.13. Estimation of Growth and Grain Yield

Nine plants from each treatment (three plants per replicate) were collected and immediately rinsed with iso-osmotic solution, blotted on filter paper and weighed to obtain the Fresh Weight (FW). For determination of Dry Weight (DW) the plant parts from each treatment dried to a constant weight at 65°C. Leaves and stems

were separated and weighed. After harvest, the weight of 100 grains and grain yields were calculated.

2.14. Statistical Analysis

Results were statistically analyzed by Snedecor and Cochran (1989).

3. RESULTS

3.1. Total Antioxidant Content of Algal Extracts

According to our previous study, microalgae could be grown under nitrogen limitation in order to enhance biosynthesis some fine biomolecules (Abd El Baky and El-Baroty, 2013a). Thus, *S. obiuuqs* and *S. platensis* were cultured in nitrogen deficient media for induction of antioxidant compounds in algal cells and the data are presented in **Table 1**. The results revealed that the both algal cells showed significant increase in antioxidant molecule when compared with algae grow in medium containing optimum nitrogen levels (data not shown).

However, the concentrations of Ascorbic Acid (AA), Total Carotenoids (T-CAR), tocopherols, phycocyanin, GSH, Total Flavonoids (TFC) and Total Phenolic (TPCs) in algal *S. obliquus* and *S. platensis* (in parentheses) grown under nitrogen stress (nitrogen deficient) were 2.33 (1.28%), 4.54 (1.65%), 1.02 (0.43%), 0.0 (13.51 %), 0.321 (0.245 mM), 0.94 (0.87%) and 1.43(1.73), respectively. These values revealed that *S. obliquus* was characterized by high content of AA and TCO than that found in *S. platensis* with about 2 and 3 times, respectively. On contrary, *S. platensis* was contained much high amounts of phycocynine blue pigments, while *S. obliquus* did not contain this pigment.

3.2. Effect of Algal Extracts on Photosynthetic Pigment Contents of Wheat Plants Cultivated Under Sea Water Stress

The concentration of photosynthetic pigments of wheat plants irrigated sea water (10 and 20%, SW) are shown in **Table 2**. In general, wheat plants irrigated with SW showed significant decreases in the amounts of T-Chl and Chl-a and Chl-b contents when compared with wheat plants irrigated Regular Water (RW). For example, T-Chl contents in wheat plant irrigated 10 and 20% (v/v) SW were 0.652 and 0.597 mg g^{-1} FW, respectively. Whereas the value was 0.822 mg g^{-1} FW, in plants irrigated with RW only. Thus, chlorophyll degradation was dependant on water salinity level. Application of algal *S. obliquus* and *S. platensis* extracts to wheat plants irrigated SW led to significant increase in the concentrations of T-Chl, Chl-a and Chl-b as compared with the values of plants irrigated SW only. The T-Chl content was ranged from 0.903 to 1.037 mg g^{-1} and 0.698 to 0.0721 mg g^{-1} FW in wheat plants irrigated with 10% and 20% (v/v) SW and treated with algal *S. obliuues* and *S. platensis* extracts. While, in non-treated plants, these values were 0.652 and 0.597 mg g^{-1} (FW), respectively. In similar trend for photo-synthetic pigments, T-Chl, Chl-a and Chl-b contents, were observed in plants treated with oxalic acid as a plant growth factor.

However, this increase was less pronounced, when compared with that induced in wheat stressed plant treated with algal extracts. Thus, the level of photosynthetic pigment was found to be restored in treated wheat plants irrigated SW due to application of algal extracts.

3.3. Effect of Algal Extracts on Antioxidant Status of Wheat Plants Cultivated Under Sea Water Stress

As shown in **Table 3**, irrigation of wheat plants with 10 and 20% (in parenthesis) SW caused significantly increase in the accumulation of antioxidant compounds including: TCAR, TOC, AA, TPCs and GSH in wheat plants over than that in plant irrigated RW only (**Table 3**). The levels of these compounds were about 1.3 (1.5), 1.2 (1.5), 1.5 (2.2), 1.2 (1.4) and 1.1 (1.7) times as great as that in wheat plants irrigated RW, respectively.

Application of algal extracts of *S. platensis* and *S. obliquues* contained high level of antioxidants constituents on wheat plants irrigated 10% SW, led to significant increase in antioxidant molecules including: T-CAR, TOC, AA, TPCs and GSH with values being about 2.3 (1.9), 3.3 (2.9), 2.7 (2.4), 1.4 (1.3) and 2.9 (2.3) as greater as that in non-treated plants (irrigated with 10% SW, only), respectively. In plant irrigated 20% SW, these values were about 2.2 (1.9), 2.4 (2.0), 2.2 (1.8), 1.3 (1.3) and 2.32 (1.8) as high as that in plants irrigated with 20% SW, respectively. According to these values, it could be demonstrated that the antioxidant contents in wheat plants irrigated with sea water were positively correlated with the levels of total antioxidant in algae extracts. As compared with foliar application of growth bioregulator oxalic acid (plant growth regulator), the both algal extracts caused great increase in antioxidant compounds in wheat plant irrigated sea wheat. The CAR and TOH contents were slightly increased under salt-stress. This increase was significantly higher than that of negative control plants, but was similar to that in plant treated with bioregulator.

Table 1. Antioxidant contents of *Spirulina platensis* and *Scenedesmus obliquus* grown under stress conditions

Compound	*Spirulina platensis*	*Scenedesmus obliquus*
Ascorbic acid (%)	1.280	2.330
Carotenoids (%)	1.650	4.540
Tocopherols (%)	0.430	1.020
Phycocyanin (%)	13.510	0.000
GSH (mM)	0.245	0.321
Total flavonoids (%)	0.870	0.940
Total phenolic (%)	1.730	1.430

Table 2. Influences of *Spirulina platensis* and *Scenedesmus obliquus* extracts spraying on chlorophyll contents of wheat levies irrgated by diluted sea water

Salinity	Treatments	Chl a mg/ g f.w	Chl b mg/ g f.w	Tatal Chl. mg/ g f.w	Ratio[a]	Ratio[b]	Ratio[c]	Chl a/Chl b
Tap water	Negative control (without only spraying)	0.452	0.17	0.622	0.0			2.66
	Sp.	0.997	0.19	1.100	1.8	2.4	3.0	3.7
	Sc.	0.721	0.17	0.891	1.4	2.0	2.4	3.1
	Positive control (Oxalic acid)	0.539	0.18	0.719	1.1	1.6	2.0	3.0
10 % sea water	Negative control (without only spraying)	0.321	0.13	0.451	0.7	0.0		2.5
	Sp.	0.530	0.16	0.690	1.1	1.5	1.9	3.3
	Sc.	0.533	0.15	0.683	1.1	1.5	1.9	5.4
	Positive control (Oxalic acid)	0.491	0.17	0.691	1.1	1.5	1.9	2.9
20 % sea water	Negative control (without only spraying)	0.255	0.11	0.365	0.6	0.8	0.0	2.3
	Sp.	0.501	0.15	0.651	1.0	1.4	1.8	3.3
	sc.	0.695	0.21	0.860	1.4	2.0	2.4	3.3
	Positive control (Oxalic acid)	0.431	0.17	0.601	1.0	1.3	1.6	2.5
LSD at (=< 0.05)		0.11	0.01	0.210				

Ratio[b]: Treatment/Negative control (without only spraying and irrigated by 10% sea water); Ratio[c]: Treatment/Negative control (without only spraying and irrigated by 20% sea water)

In contrast, foliar application of algal extract significant increased antioxidant content in wheat plants grown under low and high SW water levels over than of wheat plants treated with bioregulator. Similar trend was reported in the literature for increased levels of AA, PhOH and GSH in plants grown under stress conditions. For instance, ascorbic acid as one of the two major soluble antioxidants in chloroplast, possess a photoprotective function due to it is antioxidant capacity.

3.4. Antioxidant Enzyme Activities

The change in antioxidant enzyme activities of SOD, POD, APX and CAT (enzyme activity was expressed on a protein basis, specific activity) in wheat leaves was significantly ($p \leq 0.05$) affected by irrigation of SW (**Table 4**). The SOD enzyme activity in wheat plants was increased with an increase of 10% and 20% SW levels, with values of 41.21 and 49.29 U mg^{-1} protein/min, respectively. While, this value was 34.22 U mg^{-1} protein/min in plant irrigated RW. Treated 10 and 20% SW wheat plant with algal extracts of *S. obliquues* and *S. platensis* (in parenthesis) had high SOD activity with values 49.31 (51.52) and 53.21 (U/mg protein/min), 55.21 (U/mg protein/min), respectively. Application of bioregulator significantly increased ($p \leq 0.05$) the SOD activity in both plants irrigated RW and SW. The results revealed that SOD activity in wheat plants irrigated SW exhibited markedly important changes by treated with foliar application of algae extracts. As compared with foliar application of oxalic acid bioregulator, algal extracts caused significant increase of SOD activity in wheat plants irrigated with SW.

POD, APX and CAT activities in wheat plants were also increased due to irrigation both 10% and 20% SW.

Table 3. Influences of *Spirulina platensis* and *Scenedesmus obliquus* extracts spraying on antioxidant compounds content of wheat levies irrigated by diluted sea water

		Carotenoids				Tecopherols				Ascorbic acid			
		mg/g				µmol/g				µmol/g			
Salinity	Treatments	F.W	Ratio[a]	Ratio[b]	Ratio[c]	F.W	Ratio[a]	Ratio[b]	Ratio[c]	F.W	Ratio[a]	Ratio[b]	Ratio[c]
Tap water	Negative control	0.492	0.0			0.795	0.0			0.239	0.0		
	Sp.	0.580	1.2	0.933	0.8	1.650	2.1	1.7	1.0	0.452	1.9	1.3	0.8
	Sc.	0.611	1.2	1.000	0.8	1.450	1.8	1.5	0.9	0.396	1.7	1.1	0.7
	Positive control (Oxalic acid)	0.535	1.1	0.900	0.7	0.921	1.2	1.2	0.6	0.314	1.3	0.9	0.6
10 % sea water	Negative control	0.621	1.3	0.000		0.974	1.2	0.0		0.351	1.5	0.0	
	Sp.	0.954	1.9	1.500	1.3	2.120	2.7	2.7	1.3	0.745	3.1	2.1	1.4
	Sc.	0.921	1.9	1.500	1.3	1.930	2.4	2.0	1.2	0.651	2.7	1.9	1.2
	Positive control (Oxalic acid)	0.871	1.8	1.400	1.2	1.210	1.5	1.5	0.7	0.523	2.2	1.5	1.0
20 % sea water	Negative control	0.731	1.5	1.200	0.0	1.620	2.0	1.7	0.0	0.532	2.2	1.5	0.0
	Sp.	1.410	2.9	2.300	1.9	2.420	3.0	3.0	1.5	0.897	3.8	2.6	1.7
	sc.	1.310	2.7	2.100	1.8	2.220	2.8	2.3	1.4	0.784	3.3	2.2	1.5
	Positive control (Oxalic acid)	1.210	2.5	1.900	1.7	1.350	1.7	1.7	0.8	0.698	2.9	2.0	1.3
LSD at (=< 0.05)		0.110				0.090				0.020			

		Phenolic				GSH			
		mg/g				µmol/g			
Salinity	Treatments	F.W	Ratio[a]	Ratio[b]	Ratio[c]	F.W	Ratio[a]	Ratio[b]	Ratio[c]
Tap water	Negative control	1.32	0.0			0.392	0.0		
	Sp.	2.65	2.0	1.7	1.4	0.774	2.0	1.8	1.1
	Sc.	1.98	1.5	1.3	1.1	0.642	1.6	1.5	0.9
	Positive control (Oxalic acid)	1.36	1.03	0.9	0.7	0.521	1.3	1.2	0.8
10 % sea water	Negative control	1.53	1.2	0.0		0.421	1.1	0.0	
	Sp.	1.97	1.5	1.3	1.1	0.978	2.5	2.3	1.4
	Sc.	1.67	1.3	1.1	0.9	0.897	2.3	2.1	1.3
	Positive control (Oxalic acid)	1.64	1.2	1.1	0.9	0.854	2.2	2.0	1.3
20 % sea water	Negative control	1.86	1.4	0.9	0.0	0.681	1.7	1.6	0.0
	Sp.	2.22	1.7	1.5	1.2	1.35	3.4	3.2	2.0
	Sc.	1.99	1.5	1.3	1.1	0.989	2.5	2.3	1.5
	Positive control (Oxalic acid)	1.93	1.5	1.3	1.0	0.978	2.5	2.3	1.4
LSD at (=< 0.05)		0.30				0.020			

Ratio[a]: Treatment/Negative control (without only spraying and irrigated by tap water); Ratio[b]: Treatment/Negative control (without only spraying and irrigated by 10% sea water); Ratio[c]: Treatment/Negative control (without only spraying and irrigated by 20% sea water)

However, the activities of POD, APX and CAT enzyme activities were higher in SW stress wheat plants treated with *S. platensis* than in *S. obliquuss* extract. Also, these activities were significantly increased in plant treated with oxalic acid. In general, the enzyme activities of SOD, POD, APX and CAT in wheat plants treated with algal extracts behaved completely similar trend with significant differences. Similar effect of algal extracts on activities of antioxidant enzymes (SOD, POD, APX and CAT) in wheat plants exposed to salt-stress here had already been observed in some plants treated with some bioregulatores. Looking at antioxidant constituents in algal and antioxidant activities of each enzyme, it is clear that the level of algal constituents caused significant differential role in controlling activities of antioxidant enzymes of wheat plants when irrigated SW, which ultimately resulted in differential response to salt stress.

3.5. Effect of Algal Extracts on Lipid Peroxidation of wheat Plants Cultivation under Sea Water Stress

In this study, lipid peroxidation was determined by TBARs content, which is one of the decomposition products of Polyunsaturated Fatty Acids (PUFA) of lipid membrane.

Table 4. Influences of *Spirulina platensis* and *Scenedesmus obliquus* extract spraying on antioxidant enzymes activities of wheat levies irrgated by diluted sea water

		U/mg protein /min				U/mg protein /min				U/mg protein /min				U/mg of protein			
Salinity	Treatments	SOD	Ratio[a]	Ratio[b]	Ratio[c]	POD	Ratio[a]	Ratio[b]	Ratio[c]	APX	Ratio[a]	Ratio[b]	Ratio[c]	CAT	Ratio[a]	Ratio[b]	Ratio[c]
Tap water	Negative control	34.22	0.0			32.41	0.00			282.3	0.0			21.23	0.00	0.79	0.67
	Sp.	48.74	1.4	1.2	1.0	39.15	1.20	1.0	0.9	375.2	1.3	1.2	1.1	26.33	1.24	0.95	0.83
	Sc.	40.51	1.2	1.0	0.8	38.45	1.20	1.0	0.9	352.5	1.2	1.1	1.1	23.54	1.10	0.88	0.74
	Positive control (Oxalic acid)	38.23	1.1	0.9	0.8	37.78	1.20	1.0	0.8	330.9	1.2	1.1	1.0	22.98	1.08	0.86	0.72
10 % sea water	Negative control	41.21	1.2	0.0		39.23	1.20	0.0		310.2	1.1	0.0		26.65	1.25	0.00	0.84
	Sp.	51.52	1.5	1.3	1.0	46.37	1.40	1.2	1.0	437.5	1.5	1.4	1.3	39.87	1.87	1.49	1.26
	Sc.	49.31	1.4	1.2	1.0	45.32	1.40	1.2	1.0	391.4	1.4	1.3	1.2	32.98	1.55	1.23	1.04
	Positive control (Oxalic acid)	45.72	1.3	1.1	0.9	43.25	1.30	1.1	1.0	369.2	1.3	1.2	1.1	29.76	1.40	1.11	0.93
20 % sea water	Negative control	49.23	1.4	1.2	0.0	45.25	1.40	1.2	0.0	335.2	1.2	1.1	0.0	31.65	1.49	1.19	0.00
	Sp.	55.21	1.6	1.3	1.1	51.28	1.58	1.3	1.1	458.9	1.6	1.5	1.4	46.87	2.20	1.76	1.48
	sc.	53.21	1.6	1.3	1.1	49.53	1.50	1.3	1.1	423.7	1.5	1.4	1.3	39.95	1.88	1.49	1.26
	Positive control (Oxalic acid)	51.92	1.5	1.3	1.1	47.47	1.50	1.2	1.1	390.7	1.4	1.3	1.2	34.84	1.64	1.31	1.10
		2.35				2.14				8.54				2.17			

Ratio[a]: Treatment/Negative control (without only spraying and irrigated by tap water); Ratio[b]: Treatment/Negative control (without only spraying and irrigated by 10% sea water); Ratio [c]: Treatment/Negative control (without only spraying and irrigated by 20% sea water)

Table 5. Effect of *Spirulina platensis and Scenedesmus obliquus* extracts on lipid peroxidation in wheat plants irrigated with sea water

Salinity	Treatment	TBARs (MDA mmol/mg protein)	Inhibition (%)
Tap water	Tap water	1.55±0.23	88.87
	Sp.	3.56±0.22	98.65
	Sc.	3.11±0.28	94.63
	(Oxalic acid)	2.35±0.12	90.21
10 % sea water	10% sea water	1.12±0.21	54.65
	Sp.	3.01±0.23	89.54
	Sc.	2.65±0.25	83.87
	(Oxalic acid)	2.12 ±0.13	78.74
20 % sea water	20% sea water	0.98±0.11	45.32
	Sp.	2.87±0.22	74.32
	Sc.	2.54±0.15	67.87
	(Oxalic acid)	1.87±0.21	55.86
LSD at (=< 0.05)		4.65	

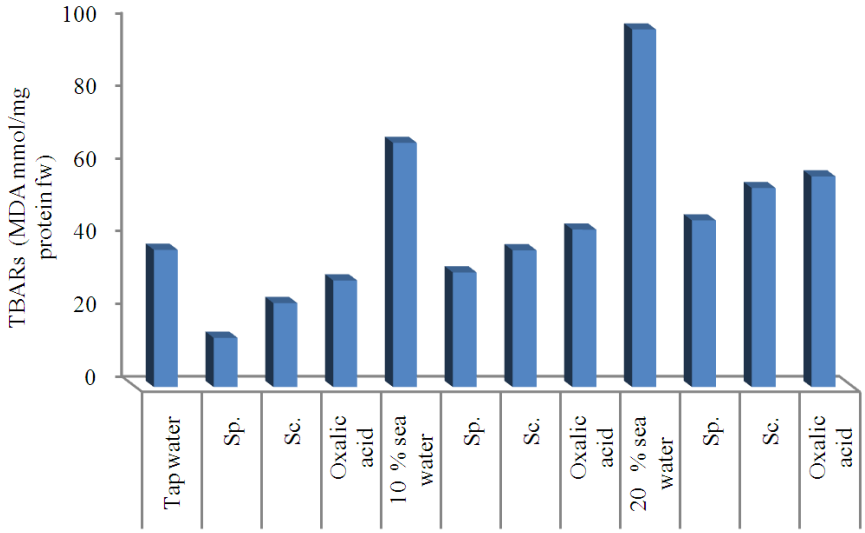

Fig. 1. Effect of *Spirulina platensis* and *Scenedesmus obliquus* extracts on lipid peroxidation in wheat plants irrigated with sea water

Table 6. Effect of *Spirulina platensis and Scenedesmus obliquus* extracts on dry weight and yield in wheat plants irrigated with sea water

Salinity	Treatment	Whole plant dry weight (g)	100 grain weight (g)
Tap water	Negative control	1.55±0.23	3.45±0.16
	Sp.	3.56±0.22	4.35±0.16
	Sc.	3.11±0.28	4.11±0.21
	Positive control (Oxalic acid)	2.35±0.12	3.87±0.24
10 % sea water	Negative control	1.12±0.21	3.1±30.22
	Sp.	3.01±0.23	4.01±0.25
	Sc.	2.65±0.25	3.75±0.27
	Positive control (Oxalic acid)	2.12±0.13	3.24±0.31
20 % sea water	Negative control	0.98±0.11	2.85±0.19
	Sp.	2.87±0.22	3.83±0.17
	Sc.	2.54±0.15	3.08±0.22
	Positive control (Oxalic acid)	1.87±0.21	2.76±0.32
LSD at (=< 0.05)		0.13	0.15

As shown in **Table 5 and Fig. 1**, the high level of TBARs in wheat plants irrigated with 10 (67.23) and 20% SW (98.45 MDA mmol/mg protein fw) than that of plant irrigated RW (37.77 MDA mmol/mg protein fw). Whereas, these levels were decreased significantly in plants treated with algal extracts of *S. platensis* (31.56-45.87) and *S. obliquus* (37.65-54.78 MDA mmol/mg protein fw) when compared with untreated plants. Generally, these results revealed that algal extracts induced protection action against oxidative damage caused by salt stress.

3.6. Effect of Algal Extracts on Dry Weight and Grain Yield of wheat Plants Cultivation under Sea Water Stress

The irrigation wheat plants with 10 and 20% SW showed adverse effect on the overall growth parameters, that caused significant reduction of plant height, shoot fresh, spike length and spikelet's/spike (data not showed). Dry Weight (PDW) and Grain Yield (GY) of wheat plants irrigated RW were significantly reduced by 1.55 g and 3.45, respectively (**Table 6**). Treated wheat plant irrigated 10 and 20% SW with of *S. platensis* and *S. obliquus* algal extracts showed markedly significant increase of both PDW and GY, with values ranged (2.87-3.01 and 2.54-2.65 g) and (3.83-4.01g and 3.03-3.75g), respectively. The elevation in PDW and GY of treated plant with algal extracts may be due to their improvement in all growth parameter. Similar effect was noted in plant treated with oxalic acid, but it was less than that in plants treated with algal extracts.

4. DISCUSSION

Treated wheat plants with algae extract contained high amounts of antioxidant constituents was positive correlated with increasing of photosynthetic pigments restored in wheat plants irrigated SW. In other words, algal extracts might improve the salt tolerance of wheat plants by restoring the main photosynthetic pigments. Similar results were reported by Abd El Baky *et al.* (2010), those chlorophylls in photosynthetic membranes could be protected the photosynthetic apparatus from excessive ROS by quenching of singlet oxygen and other radicals.

Application of algal extracts increased the levels of phenolic compounds, ascorbic acid and α-tocopherol, in wheat plants irrigated sea water to protect the membrane by preventing or reducing oxidative damage by ROS (Abd El Baky and El-Baroty, 2008). However, it is hypothesized a cycle where H_2O_2 scavenged by phenolic compounds to produce phenoxyl radicals, this radical reduces the ascorbic acid into mono (OH)-dehydroascorbate (Abd El Baky and El-Baroty, 2008).

It has been known that, salt stress leads an extensive lipid peroxidation, which is a good indicator of salt-induced oxidative damage at the cellular level (Hernandez and Almansa, 2002). Also, Elstner (1991) reported that salt stress causes an oxidative stress due to induction high amount of ROS in plants such as superoxides ($^{-}O2$), Hydroxyl (^{-}OH) and peroxy radicals (OOH).

Moreover, algae could improve protection defense in wheat plant with increase efficiency either through non-enzyme or enzyme antioxidative systems, as marker by increased antioxidant compounds (CAR, AA, GSH, TOC

and phenolic) and higher activity of SOD, CAT, POX and POX and GR antioxidant enzymes. Similar results obtained with Abd El Baky *et al*. (2010), demonstrated that significant decrease in TBARs level in leaves of wheat plants grown under salt stress and treated with algal extracts rich in antioxidant compounds. In contrast, increased MDA content in wheat plants irrigated 10 and 20% SW may indicate a higher oxidative damage due to inadequate response of the endogens antioxidative systems. In this respect, Hernandez *et al*. (2000) demonstrated that plants defend against reactive oxygen species by induction of certain antioxidative enzyme activities such as CAT, PEX, GR and SOD, which scavenge their reactive oxygen species. Also, Muthukumarasamy *et al*. (2000) and Rios-Gonzalez *et al*. (2002) stated that salt tolerance is often correlated with increasing the activity of antioxidative enzymes such as APX, GR and SOD, in wheat grown under sea water stress. The higher antioxidant enzyme activities of SOD, POD, APX, GR and GST were detected in tomato, barley, maize and sunflower plants grow under salt stress (Liang, 1999; Rodriguez-Rosales *et al*., 1999). Raza *et al*. (2007) reported that the salt tolerance in many plants is correlated with a more efficient antioxidative system.

In general, similar response of wheat plants to application both algal extracts of *S. obliquue* and *S. platensis* caused protective varied effect on wheat plant due to reduction of ROS levels with associated higher quantity of antioxidant compounds. Abd El Baky *et al*. (2012) reported that algal rich content of antioxidant is algal known to scavenge hydroxy radicals and other reactive oxygen species in plants exposed to salt stress and their effect coupled with elevation in the activities of all antioxidant enzymes.

However, low FW, DW and GY in wheat and barley plants irrigated SW has been reported in literature (Sairam and Srivastava, 2002; Francois *et al*., 1984). Abd El Baky *et al*., 2010) stated that improve of PDW and SY as one of the parameters of salt tolerance in wheat irrigated SW treated with algal extracts indicated that metabolic and photosynthetic processes was restored.

Here again, application of algal extracts seemed to reduce SW salinity stress of wheat plants by restored the photosynthetic pigments and increased the antioxidant defense abilities included non-enzymatic and enzymatic antioxidant systems, which led to reduce the oxidative damage of functional molecules and maintenance of many physiological processes of wheat plants such as photosynthetic activity and productivity (Abd El Baky *et al*., 2010).

Finally, the *S. platensis* and *S. obliquus* extracts could be contain some bioactive components act as growth regulator substances such as auxine and cytokinins, in additional to antioxidant compounds (**Table 1**) which lead to mitigate the effect of sea water salinity stress on wheat plant metabolic pathway. The results of Molnar and Ordog (2005) and Abd El Baky *et al*. (2010) reported that some plant growth regulators found in microalgae possessed beneficial effects on tissue cultures of recalcitrant plants.

5. CONCLUTION

The present results indicate that, treated wheat plants irrigated SW with *S. platensis* and *S obliquus* extracts exhibited a protection action against oxidative stress induced by salt stress due to elevation the levels of non-antioxidant constituents and enzyme antioxidant protective systems. The later substances are known to be responsible for salt tolerance of wheat plants. Therefore, the irrigation of wheat plants by mean of brackish water at 20% (v/v) is possible when treated with algal extracts.

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