Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: December 2020 **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2021.18.1.0054

The role of salt stress on laboratory cultivation of green macroalga Enteromorpha compressa and its antioxidant activity

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Received 4/8/2020, Accepted 15/10/2020, Published Online First 6/12/2020, Published 1/3/2021

Abstract:

Cultivation of the green seaweed *Enteromorpha compressa* was performed under natural laboratory spring environmental conditions of temperature, light intensity and photoperiod to study the salinity tolerance of this intertidal green macroalga. Cultivation was carried out under artificial seawater (ASW) of different concentrations (18, 35, 53 and 106 g/l sea salt) compared to the control using natural seawater (NSW). Growth rate and pigment content of the cultivated alga were recorded at regular intervals during the experimental duration. Antioxidant activity of the crude ethanolic extract and its fractions (petroleum ether, chloroform, ethyl acetate and acetone) was performed against DPPH radical scavenging assay and compared to the standard synthetic antioxidant butylated hydroxy-toluene (BHT). The finding showed that enhancement of algal growth rate under ASW concentrations of 35, 53 and to a lesser extent at 106 g/L during the first 15 days of cultivation were due to the increased pigment biosynthesis, photosynthetic and metabolic activities and followed by gradual retardation due to the impact of prolonged salt stress. Antioxidant activity of alga was found to be concentration, type of extract and incubation time dependent. Acetone fraction of all salt concentrations showed higher antioxidant activity compared to other fractions. Pronounced activity was recorded at higher seawater conc. (106g/l).

Key words: Antioxidant activity, ASW, BHT, Cultivation, Green seaweed, Growth rate, NSW

Introduction:

Macroalgae, known also as seaweeds, can be classified based on the nature of their pigments into brown seaweeds (Phaeophyta), red seaweeds (Rhodophyta) and green seaweeds (Chlorophyta) (**1**). In Asian countries, several species of seaweeds are often used as human food. Fresh and dried seaweeds are extensively consumed, especially by people living in coastal areas. They are of nutritional interest as they are low calorie foods, rich in vitamins, minerals and dietary fibers (**2**). Also, many are used in medical, pharmaceutical, cosmetics, food industry, biotechnology and folk medicine (**3-7**).

The chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions (**8, 9**). Filamentous green macroalgae of the genus *Enteromorpha* grow abundantly in littoral zones of polluted and eutrophicated coastal marine waters. The macroalgae which inhibit the intertidal zone, live in a harish environment where they were subjected to repeated immersion and emersion due to tide, intense light, rapid temperature fluctuation, osmotic stresses and desiccation. Their thalli are attached to hard substrata (Rocks, Stons, Pebbles and Shells). Most *Enteromorpha* species have tubular thalli with hollow spaces that contain nutrient reserve substances and dissolved organics. Many of these species are tolerant to heavy metals, and therefore frequently used as pollution indicators (**10**). The cell wall is rich in sulphated polysaccharides which are strong ion-exchangers (**11**).

The distribution, composition and abundance of benthic macroalgae depend on physical, chemical and biological factors affecting growth and/or replacement by other species (succession) (**12, 13**). Light climate (water clarity), nutrient concentration and salinity are three of the primary growth-controlling factors that have been documented to influence large-scale patterns of

distribution and abundance of macroalgae (**14**). Sousa *et al*., (**15**) reported that the growth of spores from *E. compressa* (opportunistic green macroalga) is strongly salinity-dependant. Consequently, in highly hydrodynamic systems such as most shallow estuaries, salinity variation may play a determinant role in the yearly abundance of green macroalgae.

Chemical analyses indicated that the ether extract of *Enteromorpha spp* has 9-14% protein: 32-36% ash , n-3 and n-6 fatty acids constitute 10.4 and 10.9 g/100 g of total fatty acid respectively. The protein of this seaweed has high digestibility (98%). *Enteromorpha spp* is recommended for human consumption because it has several beneficial components, such as minerals, protein, essential amino acids, essential fatty acid, and fiber (**16**).

Salinity represents one of the most important factors exerting stress injury on the growth and metabolism of plants. Salt stress causes an imbalance of the cellular ions resulting in ion toxicity and osmotic stress, leading to retardation of growth either directly by salt or indirectly by oxidative stress induced by Reactive Oxygen Species (ROS). Salinity can cause significant accumulation of compatible solutes which acts as enzyme producers, stabilizing the structure of macromolecules and organelles (**17, 18**). Salinity stress may alter the metabolic pathways of stressed organism(s) leading to either enhancement or induction of biologically active compounds (**19**). This may be explained and confirmed by the different tolerant *Enteromorpha* and *Ulva* species recorded and fixed on ships traversed various water bodies of variable salinities (Rivers, Seas and Oceans).

Natural cultivation of seaweeds on the sea shore (Mariculture) was performed in many countries, for different industrial applications, by various methods depending on the seaweed species to be cultivated and the nature of the cultivation area (Sandy, Muddy or Rocky) (**20**). It is known that cultivation of seaweed species under laboratory natural conditions is very difficult and rarely successful. An attempt to cultivate the promising alga *E. compressa* of this study under laboratory environmental conditions was evaluated in this study.

This investigation aims to determine the antioxidant activity of successive extracts from the green macroalga *Enteromopha compressa* cultivated in laboratory natural conditions under different artificial sea salt concentrations as compared with sea salts (control treatment).

Materials and Methods:

a. Location of algal collection and its identification

Algal species were collected in April 2018 (Spring season) from Abu Qir bay at Alexandria city in morning time (From 10-12 am). This locality is rich in organic matters and high availability of hard substrata, such structure allowed different species of green seaweeds to grow intensively on the rocky area at the intertidal zone as well as on small stones close to shore line.

The harvested algae, fixed on small stony substrata, were cleaned from sand and foreign materials by washing in situ with sea water, collected in ice box filled with seawater and transported to the laboratory of phycology in Botany and Microbiology Department, Faculty of Science, Cairo University (Figure 1).

The algae were left in natural sea water (NSW) for adaptation in the laboratory natural culture conditions (of spring season) at constant temperature (25±2°C), natural light intensity (≈40μE/m^2/s) and photoperiod (12/12hr).

The alga was identified by Dr. Sanaa Shanab, professor of phycology, as *Enteromorpha compressa* according to Aleem (**21**).

b. Algal cultivation

The adapted thalli of *E .compressa* fixed on its stony substrata were cultivated *in vitro* under salinity stress conditions using different artificial sea salt concentrations [the composition was shown in Table 1, (ASW), 18, 35, 53 and 106 g/L) in 2L beakers at spring environmental conditions (previously mentioned) compared to control sample (cultivated in NSW), Table (2). Cultivation was carried out for 25 days.

Table 1. Composition of artificial sea salt (ASW)

Salts	**g/L**
NaCl	27.0
$MgSO_4.7H_2O$	6.6
$MgCl_2.6H_2O$	5.6
$CaCl_2.2H_2O$	1.5
KNO_3	1.0
Trace elements	**mg/L**
KH_2PO_4	70.0
$NaHCO_3$	40.0
Fe + EDTA (10%) solution (2.4 g $FeCl_3.6H_2O$ + 1g NaEDTA in 500ml H_2O)	1.0 ml
Microelements	**1.0 ml/L**
Salt	**mg/L**
$MnCl_2.4H_2O$	40.0
$ZnCl_2$	4.0
H_3PO_4	6.0

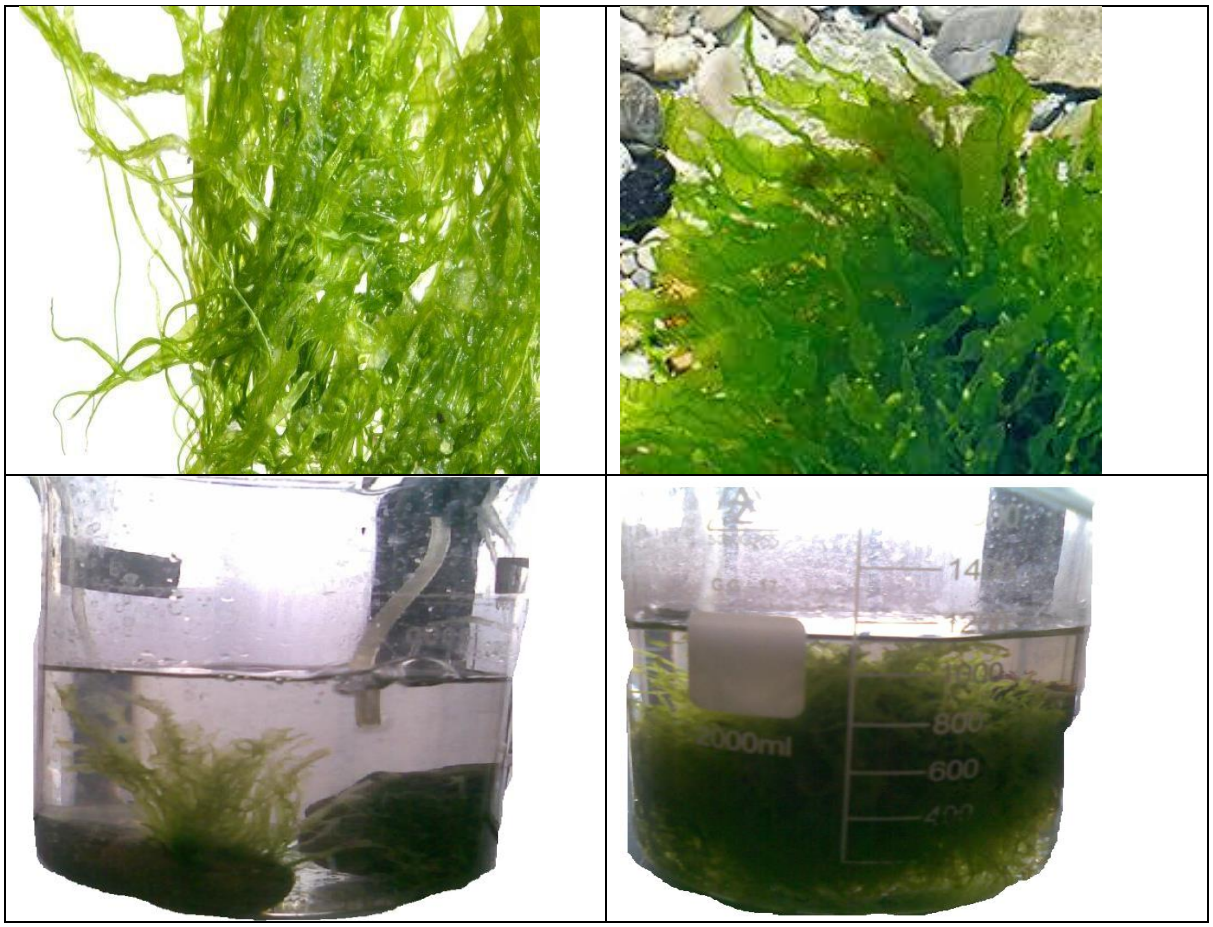

Figure 1. The pictures for E.compressa and during cultivation in lab using artificial sea salt

Table 2. Physical and chemical parameters of natural sea water (NSW) collected from Abu Qir bay at spring season

Parameters	Units	Spring Season
Physical parameters		
Temperature	˚C	20.0
Light intensity	k lux	132.0
	µE/m^2/s	40
EC	mmose/cm	58.00
pH		7.50
Chemical parameters		
Dissolved anions (mg/L)		
Carbonate		0.0
Bicarbonate		2.62
Chloride		705.20
Sulphate		62.18
Nitrate		22.32
Phosphate		0.00
Total anions		792.32
Dissolved cations (mg/L)		
Calcium		30.00
Magnessum		107.00
Sodium		630.00
Potassium		3.00
Amm/nitrogen		6.48
Zinc		0.159
Copper		0.00
Total cations		776.63

c. Algal growth rate:

Algal growth rate was determined as total chlorophylls and total carotenoids (mg/g F.wt) at regular intervals (5 days) during the experimental period (25 days) according to Holden (**22**).

d. Preparation of Algal extracts:

The air dried and grinded algal species were extracted with 70% ethanol (three times). The extracts were filtered, the solvent was evaporated and the obtained residues (Crude extracts) were subjected to fractionation with successive selective solvents of increasing polarity (petroleum ether, chloroform, ethyl acetate and acetone respectively). Residue from each extract was air dried and weighted.

e. Antioxidant activity:

Antioxidant activity of the salinity stressed alga was determined using DPPH (2, 2 diphenyl-1-picrylhydrazyl) method (after 30 and 60 min) in extracts of different polarities after 15 and 25 days of cultivation in different salt concentrations. Butylated hydroxyl toluene (BHT) was used as synthetic antioxidant standard. The scavenging effects of crude ethanolic extract and fractions were determined by the method of Yen and Chen (**23**). The absorbance of all the sample solutions and BHT

were measured at 517 nm. The percentage (%) of scavenging activity was calculated as the following:
% Antioxidant activity = (Control - Sample X 100) / Control

Where: control is DPPH solution (0.16 mM).

f. Physicochemical parameters:

i. Chemical parameters

Natural sea water sample (NSW) was picked up in spring season. NSW and ASW were analyzed according to APHA (**24**). While pH, Temperature, Electric Conductivity (EC) and light Intensity at sea water surface were carried out in situ by pH-meter, Thermometer (Ordinary thermometer graduated from 0-100 °C), Conductivity- meter and luxmeter, respectively.

g. Statistical Analysis:

Statistical analyses using one way ANOVA were carried out for all determinations including the calculation of the mean, standard deviation and Ducan test at $P < 0.01$, according to the method of Armitage (**25**).

Results and Discussion

Diversity, distribution, abundance and community structure of seaweeds are influenced by a number of abiotic such as seawater characteristics, light, temperature, wave action, nutrient availability and biotic factors represented by competition between species, grazing. These factors affect propagule dispersal, fertilization, settlement, and recruitment (**26**).

Previous study carried out by Shanab *et al*. (**27**) reported that the green macroalga *E. compressa* dominated all over the year with high relative abundance in spring season where optimum light intensity, temperature and nutrients were available after the turnover in spring and autumn seasons leading to algal growth in considerable biomasses.

The rate of light absorption is greater in surface water than in deeper ones. So algal species inhabiting the supralittoral and the intertidal zone receives and at the same time can tolerate the exposure to high light intensities than algae inhabiting the sublittoral zone (**28-31**). Temperature governs the growth of algae and their distribution. Certain species show a limited temperature range (termed stenothermal) while others, the eurythermal, can grow in a wide range of temperature (**31, 32**). The less abundant and decreased growth of *Enteromorpha sp.* in other seasons (summer, autumn and winter seasons), may not only be due to the unfavorable environmental conditions characterizing these seasons (**27**), but also due to production of biflagellated gametes (sexual units) and quadriflagellated zoospores (asexual units) during early and late summer (**33, 34**); as it has isomorphic digeneretic life cycle . It was reported that *Enteromorpha* released an appreciable great number of gametes at 20°C than at higher temperatures. In addition, the highest spore and adult biomass were recorded in spring and early summer (**35**). This may be one of the explanations for the presence of *E. compressa* all over the year (in different developmental stages).

In addition, the intertidal inhabiting alga showed high tolerance to the adverse summer and winter environmental conditions and continue to grow and reproduce with slow rates. These tolerances may also be due to the seasonal alteration in hormones involved in the regulation of physiological processes (**36**).

El Shobary (**37**) and Osman *et al*., (**38**), collected (seasonally) different seaweed species (Red, Brown and Green) from Abu Qir bay at Alexandria to study their antimicrobial activity. These authors reported that the growth, distribution and abundance of the green seaweed species (*E. compressa*, *E. linza*, *Ulva fasciate*, *U. lactuca*) were seasonally influenced. while *E. Compressa* and *U. fasciata* dominated in all seasons, while, the alga *E.linza* was only recorded by the authors in spring and summer seasons, and *U. lactuca* was collected only in spring. The authors explained these differences in green algal abundance to the specific species requirement not only of certain range of temperature and light intensity but also to the increase in Gas than auxin concentrations to grow in massive quantities (**39**). These findings confirmed to a great extent our observation and results concerning the apparent stability of the cover of *E. compressa* which result from the continuous recruitments of young plant and prolific output of biflagellated gametes and quadriflagellated zoospores (It has an isomorphic digeneretic life cycle). The disappearance of the macroscopic alga from a particular level may in fact means that its microscopic stages (of the life cycle) persist at the same shore level cannot be observed and only develop fully with the return of favorable conditions.

So, the dominated intertidal alga: *E. compressa* was selected as a promising alga and collected in great biomass, fixed on their stony support, in spring season for investigating its salinity tolerance and antioxidant activity.

Growth rate

The obtained results (Table 3, Fig. 2 and Table 4, Fig. 3) clearly indicated that, total chlorophylls and carotenoids contents followed the same trend of progressive increase significantly in the first fifteen days of experiment under all salt concentrations (ASW and NSW) followed by gradual decrease till the end of experiment (25 days).

Table 3. Growth rate of cultivated *E. compressa* during 25 days (under natural conditions) using different artificial sea salt concentrations (ASW) determined as total chlorophylls as mg/g (F.wt).

Sea salt	Sea salt conc. (g/l)	Total chlorophyll(mg/g)				
		Experimental duration /days				
		5	10	15	20	25
NSW	Control	3.77±0.86	3.93±0.65	5.29±0.56	4.34±0.86	1.36±0.64
ASW	18	2.55±0.65	3.5±0.32	3.16±0.25	1.62±0.85	1.47±0.36
	35	4.74±0.96	5.07±0.65	5.00±0.68	3.25±0.48	0.06±0.02
	53	4.58±0.67	5.72±0.51	3.84±0.68	2.70±0.36	0.41±0.08
	106	2.75±0.63	4.42±0.32	2.67±0.67	1.56±0.76	0.04±0.01
L.S.D		0.0285	0.0290	0.0285	0.0246	0.0212

Each value is presented as mean of triplicate treatments, LSD: Least significant difference (LSD) at $P \leq 0.01$ according to Duncan's multiple range tests, NSW: natural sea water, ASW: artificial sea water

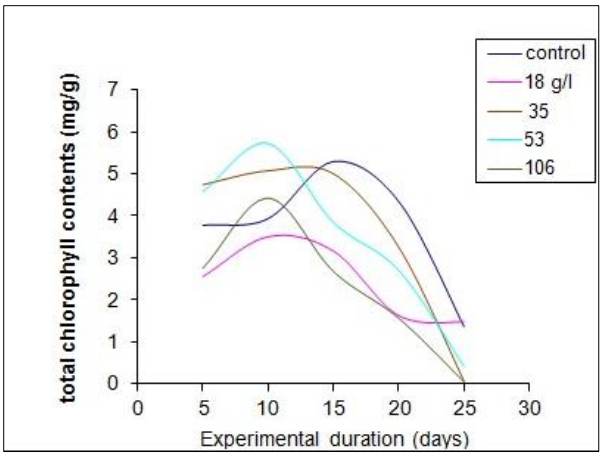

Figure 2. Growth rate of cultivated *E. compressa* during 25 days (under natural growth conditions) using different artificial sea salt concentrations (ASW) determined as total chlorophylls (mg/g F.wt).

Table 4. Growth rate of cultivated *E.compressa* during 25 days (under natural growth conditions) using different artificial sea salt concentrations (ASW) determined as carotenoids (mg/g F.wt).

Sea salt	Sea salt conc. (g/L)	Carotenoids (mg/g F.wt)				
		Experimental duration /days				
		5	10	15	20	25
NSW	Control	0.32±0.11	0.89±0.12	0.91±0.321	0.73±0.15	0.32±0.02
ASW	18	0.38±0.08	0.41±0.08	0.93±0.325	0.33±0.02	0.27±0.03
	35	0.74±0.22	1.1±0.25	1.03±0.24	0.18±0.02	0.25±0.05
	53	0.51±0.05	0.92±0.12	0.90±0.25	0.26±0.04	0.11±0.02
	106	0.64±0.06	0.96±0.22	0.15±0.05	0.1±0.05	0.08±0.01
L.S.D		0.0201	0.0255	0.02651	0.025501	0.02851

Each value is presented as mean of triplicate treatments, LSD: Least significant difference (LSD) at $P \leq 0.01$ according to Duncan's multiple range tests, NSW: natural sea water, ASW: artificial sea water

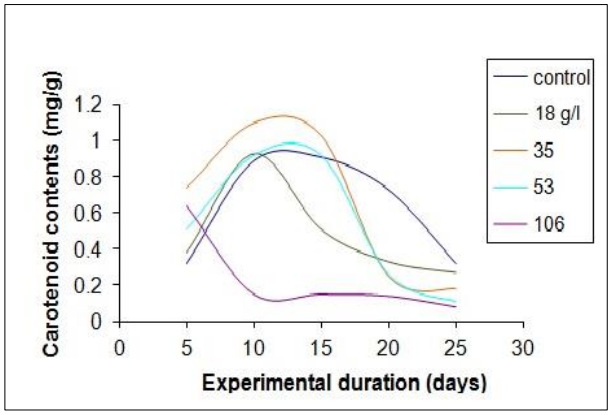

Figure 3. Growth rate of cultivated *E.compressa* during 25 days (under natural growth conditions) using different artificial sea salt concentrations (ASW) determined as Total carotenoids (mg/g F.wt).

An obvious enhancement of growth was recorded at salt conc. 35 and 53 g/L followed, in descending order, by those of the control, 18 g/L and 106 g/L sea water concentrations. The increased growth rate of the alga under the available laboratory conditions (Growth chamber) similarity of the spring season (temperature 20°C, light intensity of 2000 lux (40 $\mu E/m^2/s$), 12/12 light/ dark cycles, aeration) and the available anions and cations in the sea water [natural (NSW, Table 1) and artificial ASW, Table 2] provided more or less some of the necessary nutrients needed for algal growth. The observed significant increase in algal growth rate in the artificial sea water (35 and 53 g/L) concentration may be due to an enhancement of pigment synthesis, photosynthetic rate and primary metabolites production (Fig 2, 3 and Table 3,4). It is important to appreciate that the effect of external factors showed complex interactions and that an optimum level of one factor under certain conditions may be sub- optimum under other conditions. A good example of this is the finding that the optimum temperature for growth may vary depending on the physiological state of the experimental alga and weather it is unadapted or adapted (**28, 40, 41**).

The variable salinity concentrations used in this study may influence the photosynthetic and metabolic processes which led to either an enhancement or retardation of algal growth depending on the sea salt concentration. Marine seaweeds were habituated and adapted on the salinity range of the sea, ocean, estuaries, where they live (Mediterranean Sea, from which *E.compressa* was collected, has salinity of 38.4-39%). Salinity variation and osmotic tolerance shown by algae were closely associated with intertidal habitat. Algae inhabiting this zone tolerate sea water concentrations ranged between 0.1 to 3.0 times that of the sea water (**28**). It is of wider interest to know the ways by which seaweeds adapt themselves physiologically to withstand stress without irreversible damage.

It has been found that intertidal algae exhibit only reversible inhibition of their photosynthetic activity when immersed in fresh water or dilute sea water while the sublitoral algae suffered from rapid and irreversible damage. The marked decrease in algal growth rate at lower salinity level (18 g/L of ASW) compared to those of the control (NSW), 35 g/l (approach to the salinity range of the Mediterranean Sea), 53 and 106 g/L sea salt concentrations, go parallel with these findings. There is evidence that some seaweed cells have the property to accumulate salt against diffusion gradient showing some osmoregulatory activity. Boney (**28**) confirmed, by experimental work, that *E. clathrata* cells are able to control their osmotic pressure to balance that of the surrounding medium (either by passive diffusion or active uptake) when transferred to water salinity greater or smaller than that of its normal medium.

Therefore, the enhanced algal growth of *E. compressa* in this study, under artificial sea salt concentrations of 35, 53 and to a lesser extent of 106 g/L during the first fifteen days of experiment, may be due to an increased pigment biosynthesis and consequently increased photosynthetic and metabolic activities with maintaining a constant ionic environment with the experimental algal cells. The gradual decrease in algal growth rate of *E.compressa* (determined as total chlorophylls and total carotenoids) after the first fifteen days of enhancement may be due to reduction in chlorophyll content due to prolonged salt stress as a result of inhibition of chlorophyll biosynthesis brought about by inhibition of protochlorophyllide reductase and δ-aminolevulinic acid dehydrogenase as reported by Church *et al.* (**42**) and Ji *et al.* (**18**).

Salinity also induced a decrease in chlorophyll content due to damage of the thylakoid membrane which is a major target of salt stress (**43**). In addition, inhibition of growth of salt cultivated alga may be due to pronounced inhibition of nutrient uptake by NaCl which induced alteration in the membrane permeability as well as disruption of cellular homeostasis and osmoticum (**43, 44**). Moreover, salt stress may induce inhibition of oxygen evolution

indicating the damage of PS II reaction center as reported by Ji *et al* (**18**) and Church *et al*. (**42**).

The problem of adaptation to a marked salinity changes is associated with ionic transport and maintained constant ionic environment within the cell which is in turn in equilibrium with surrounding medium (cellular homeostasis). This includes principle of sodium pump that may occur in algal cells as that already known in animal cells. This mechanism works at full capacity in normal sea water salinity, while in an increased salt concentration, there will be some sodium ion accumulation since the pump is unable to cope (**28**). Experimental work with *Porphyra perforata* and *Ulva lactuca,* performed by Boney (**28**) and Ji *et al* (**18**), revealed that respiration and photosynthesis are involved. In periods of darkness, there are a steady loss of potassium ions and gain of sodium ones and the effects are immediately reversed when the alga is again illuminated. Also the retention of one ion and exclusion of the other is dependent on the presence of oxygen and on the process of oxidative-phosphorylating reactions. Thus, it becomes apparent that metabolic work must be done to counteract the flow of ions within their respective concentration gradients and so maintain cell homeostasis.

Antioxidant Activity (by DPPH method)

E. compressa was chosen as a promising alga, for the study of antioxidant activity, on the bases of its preliminary higher activity (55.7 to 58.1% **27**) and its abundance in large masses in spring season. One may think that the tolerance of this alga to different environmental conditions and its persistence all over the year must be due to its internal defense mechanism and special metabolism which enable this seaweed species not only to tolerate the harsh conditions of the habitat, but also to grow and dominate its inhabiting intertidal zone. The seasonal antioxidant activity of the crude ethanolic extract of *E. compressa* may be attributed to the presence of antioxidant active compounds and /or enzymes which belongs to the defense system of the alga against the harmful stress effects, the polarity of extracting solvent, (petroleum ether, chloroform, ethyl acetate and acetone) the incubation period (30 and 60 min.) and the duration of experiment.

The obtained results (Table 5, 6) revealed that acetone extract recorded the highest antioxidant activity especially of the elevated artificial salt conc. (ASW 106 g/ L sea salt) followed in descending order by and contributed to decreased salt conc. of 53 , 35 and 18 g/ L sea salt (81.5, 77, 78.4 and 74.2 % respectively) after 25 days of experiment. The lowest activity was shown by acetone extract of the control alga cultivated with NSW (66.3, 69.20 %, after 30 and 60 min. of incubation). This means that by increasing salt stress conditions, polar antioxidant active substances of as phenolic compounds (in addition to the algal pigments) were induced. These substances synergistically exhibited the pronounced antioxidant activity.

Table 5. Antioxidant activity (%) of successive extracts of cultivated *E. compressa* after 15 days of cultivation using different artificial sea water conc.
(Using DPPH radical scavenging method) after 30 and 60 min. of incubation.

Sea	Sea salt conc.	Petrolium ether		Chloroform		Ethyl acetate		Acetone	
		30min	60min	30min	60min	30min	60min	30min	60min
NSW	Control	50.1±1.25	53.1±1.54	39.0±1.32	35.4±1.1	48.5±1.42	50.5±1.35	66.3±1.48	69.2±1.3
ASW	18	47.5±1.24	44.8±2.5	48.7±2.20	44.2±2.51	54.8±1.6	57±1.041	66.8±1.6	71.5±1.9
	35	32.5±1.54	36.6±1.5	39.5±1.62	34.2±2.32	49.7±1.54	51.0±2.1	72.3±1.8	84±1.22
	53	34.0±1.62	44.2±1.65	49.2±1.85	50.0±1.51	43.3±1.65	44.12±1.5	64.2±1.7	67.6±1.6
	106	48.8±1.36	44.0±1.84	38.6±1.62	29.5±1.1	44.2±1.6	43.7±1.7	64.1±1.9	70±1.87
BHT		89.2±2.10	88.0±2.51	89.2±2.10	88.0±2.32	89.2±2.52	88.0±2.14	89.2±2.60	88±2.41
L.S.D		2.32	3.65	2.66	2.67	2.71	1.98	2.41	2.43

Each value is presented as mean of triplicate treatments, LSD: Least significant difference (LSD) at $P \leq 0.01$ according to Duncan's multiple range tests, BHT: Butylated hydroxy toluene.

Table 6. Antioxidant activity (%) of successive extracts of cultivated *E.compressa* after 25 days of cultivation (using DPPH radical scavenging method) after 30 and 60 min.of incubation.

Sea salt	Sea salt conc. (g/L)	Petrolium ether		Chloroform		Ethyl acetate		Aceto	
		30min	60min	30min	60min	30min	60min	30min	60min
NSW	Control	66.6±1.5	66.5±1.1	42.2±1.6	40.2±1.7	45.3±1.2	50.0±1.02	65.3±1.3	71.7±1.33
ASW	18	50.0±1.27	51.5±1.25	34±1.42	27.3±1.13	45.5±1.0	50.0±1.54	66.0±1.22	74.2±1.4
	35	47.5±1.6	54.5±1.6	43.5±1.6	41.2±1.5	40.0±1.5	45.7±1.1	68.0±0.92	78.4±1.5
	53	56.6±1.4	56.7±1.8	45.0±1.47	43.5±1.2	49.0±1.87	52.±1.58	64.2±1.01	77±1.69
	106	57.5±1.6	57.5±1.9	39.8±1.2	40.4±1.4	51.1±1.1	52.3±1.5	71.0±1.58	81.5±2.1
BHT		89.2±2.5	88.0±2.41	89.2±2.1	88.0±2.23	89.2±2.6	88.0±2.41	89.2±2.6	88±2.45
L.S.D		2.61	2.01	2.41	2.56	2.76	2.431	2.615	2.98

Each value is presented as mean of triplicate treatments, LSD: Least significant difference (LSD) $P \leq 0.01$ according to Duncan's multiple range test, BHT: Butylated hydroxy toluene

Higher plants were recorded to accumulate soluble sugars and polyols at the expense of other sugar fractions in response to salinity (**45**). Similarly, the accumulation was also reported in fresh water algae as well as in marine species in response to salinity stress (**46, 47**). The capacity of *Dunaliella* for osmoregulation has been attributed to the accumulation of glycerol in the cells (**48**). Cultivation of *Spirulina platensis* under salt stress conditions revealed that remarkable alteration of algal metabolism as well as an enhancement of biologically active compounds were recorded (**49**). Salt stress caused a decrease in chlorophyll content, increase in β-carotene and antioxidant compounds, lipid and Poly unsaturated fatty acids (PUFAs) (**50, 51**).

The polar ethanolic extract of the naturally collected *E. compressa* (**27**) showed the highest antioxidant activity (with DPPH and ABTS [2, 2'-azino-bis ethylbenzthiazoline-6-sulfonic acid]). These results are in conformity with the obtained results concerning the laboratory cultivated salt stressed alga. The polar acetone extract exhibited higher antioxidant activities at all the salinity stress levels used, which may be due to the increased synthesis of carotenoids and the polar antioxidant substances under salinity stress conditions as reported by Shanab *et al*. (**27**), Christaki *et al*. (**50**) and Martel *et al*. (**52**).

It is known that algal cultivation under salt stress conditions, physiological and metabolic alterations may occur accompanied by an increased production of reactive oxygen species (ROS). Under salinity stress or other stresses, the efficiency of defense system of an alga (antioxidant substances and/or enzymes) was highly increased to scavenge and /or overcome the harmful effects of ROS (**53**).

Successive extracts of the cultivated *E.compressa* under natural sea water (NSW, control) or artificial sea water (ASW) of different concentration, exhibited variable antioxidant activities depending on the salinity concentration.

Conclusion:

Short time exposure to salt stress induces an enhancement of algal growth due to increased pigment synthesis and photosynthetic activity progressively decreased with prolonged exposure to salt concentrations. Antioxidant activity increased with the increment of NaCl concentrations and acetone extract exhibit the highest activity at all salt concentrations used. This may be due to increased production of polar antioxidant substances to combat the adverse effect of salinity stress.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Egypt.

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Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: December 2020 **E-ISSN: 2411-7986**

دور الإجهاد الملحي في الزراعة المختبرية للطحلب الأخضر انتيرومورفا كومبريسا وفعاليته المضادة للأكسدة

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الخلاصة:

تم إجراء زراعة العشب البحري الأخضر Enteromorpha compressa في ظل ظروف بيئية طبيعية خلال موسم الربيع من درجة حرارة ,وشدة وفترة الإضاءة لدراسة قدرة هذا الطحلب على تحمل الملوحة. تمت التجربة بالزراعة باستخدام مياه البحر الاصطناعية (ASW) بتركيزات مختلفة (18 ، 35 ، 53 و 106 جم / لتر ملح البحر) مقارنة بالزراعة باستخدام مياه البحر الطبيعية. تم تسجيل معدل النمو ومحتوى الصبغة في الطحالب المزروعة على فترات منتظمة خلال فترة التجربة. تم إجراء النشاط المضاد للأكسدة للمستخلص الإيثانولي الخام وجزيئاته (الأثير البترولي ، والكلوروفورم ، وخلات الإيثيل والأسيتون) ضد الشق الحر DPPH ومقارنتها بمضادات الأكسدة الاصطناعية المعيارية هيدروكسي تولوين (BHT)، وقد أظهرت النتائج أن زيادة معدل نمو الطحالب تحت تركيزات 35 ASW، 53 وبدرجة أقل عند 106 جم / لتر خلال الأيام الخمسة عشر الأولى من الزراعة بسبب زيادة التخليق الحيوي للصبغة ، والتمثيل الضوئي والأنشطة الأيضية. تبع ذلك إنخفاض تدريجي بسبب تأثير إجهاد الملح لفترات طويلة. واوضحت النتائج أن النشاط المضاد للأكسدة يعتمد على التركيز ونوع المستخلص ووقت الحضانة. وقد اعطى مستخلص الأسيتون في جميع تركيزات الملح نشاطًا مضادًا للأكسدة أعلى مقارنة بالمذيبات الأخرى، وقد تم تسجيل نشاط واضح عند ارتفاع تركيز الملح في مياه البحر. (106 جم / لتر).

الكلمات المفتاحية: العشب البحري الأخضر، زراعه، محلول ملحي صناعي، محلول ملحي طبيعي ، مضاد الاكسده القياسي، معدل النمو ، نشاط مضاد للاكسده