Effects of *Sargassum latifolium* Extract on Growth, Oil Content and Enzymatic Activities of Rosemary Plants under Salinity Stress

Fatma Abd El Lateef Gharib; Ibrahim Mohamed Zeid; Olfat Motamed AbdEl-Hameed Salem and Eman Zakaria Ahmed
Botany and Microbiology Department, Faculty of science, Helwan University, Cairo, Egypt
em_7891@yahoo.com; olfatabdelhamed@yahoo.com

Abstract: In the present study, a pot experiment was conducted during 2011-2012 to study the effect of foliar application of seaweed extract (SWE) *Sargassum latifolium* at (0.00, 0.20, 0.40 and 0.60%), alone or under salinity (S) at 100*mM* NaCl on rosemary plants. *S. latifolium* treatment, singly significantly increased growth parameters, oil percentage and yield per plant, microelement content and uptake, photosynthetic pigments and activity as well as activities of peroxidase (POD), polyphenol oxidase (PPO) and ascorbate oxidase (AOD) with maximum promoting effect at 0.20% algal extract, while reduced the activities of catalase (CAT) and indole acetic acid oxidase (IAAO) compared to untreated controls. On the other hand, salinity decreased growth, oil yield, some enzymatic activities, photosynthetic pigments and activity, growth phytohormones and GA_3/ABA ratio while increased abscisic acid (ABA) relative to control plants. Nevertheless, *S. latifolium* especially at 0.40% counter balanced the adverse effects of salinity and stimulated almost measured parameters. GC/MS of essential oils from dry aerial parts of *R. officinalis* revealed that 1,8-cineol (6.67-22.10%), camphor (10.47-14.54%), α-pinene (1.06-13.96%) and borneol (5.64-12.98%) were the main identified oils constituents in rosemary at second cut. Foliar spray with SWE at 0.20%, salinity at 100mM and combination of SWE at 0.40% + salinity decreased α-pinene and 1,8-cineole, while enhanced linalool, α-terpineol, β-caryophyllene, caryophyllene oxide and aromatic compounds.
[Fatma Abd El Lateef Gharib; Ibrahim Mohamed Zeid; Olfat Motamed AbdEl-Hameed Salem and Eman Zakaria Ahmed. **Effects of *Sargassum latifolium* Extract on Growth, Oil Content and Enzymatic Activities of Rosemary Plants under Salinity Stress.** *Life Sci J* 2014;11(10):933-945]. (ISSN:1097-8135). http://www.lifesciencesite.com. 146

Keywords: *Sargassum latifolium*; Salinity stress; *Rosmarinus officinalis*; Antioxidant enzymes; Essential oils.

1.Introduction

Rosemary is one of the most effective spices among the Lamiaceae family commercially available for use as a natural source of antioxidant (Yanishlieva *et al*., 2006; Okoh *et al*., 2011) due to carcinogenic potential and negative health effects of synthetic antioxidants (Botsoglou *et al*., 2002). It is an ancient Mediterranean herb well cultivated in Egypt and available all throughout the year. Rosemary extract may be a good candidate for functional foods as well as for pharmaceutical plant-based products (Moreno *et al*., 2006). Most of the physiological effects of aromatic plants attributed to their content of essential oil and antioxidant compounds. Production of essential oils depends not only on genetic factors and the developmental stage of plants, but also on environmental factors which could result in biochemical and physiological alterations in plants modifying the quantity and quality of the essential oil (Sangwan *et al*., 2001; Prins *et al*., 2010).

Salinity stress is one of the major factors limiting agricultural production, 20% land worldwide is subjected to salinity stress (Emadi and Yassa, 2007). The availability of non-saline water for irrigation is limited and the water quality continues to decline in arid and semi-arid areas. Use of saline water in agriculture now seems inevitable. Salinity reduces the yield of essential oils in plants belonging to the Lamiaceae family, possibly because of the limited supply of calcium (Ca) from the roots to the branches and alterations in the ratio of Ca to ABA in the leaves (Dow *et al*., 1981). Salinity causes oxidative damage through generation of oxygen radicals (Hernández *et al*., 1995), inhibition of antioxidant systems or lipid content in some species (Ali, 2000; 2002). Oxidative stress stimulates synthesis of antioxidant metabolites and enhances antioxidant enzyme activities that could protect plant tissues (Tanou *et al*., 2009). Salt tolerant plants can be used to produce antioxidants and essential oils (Kiarostami *et al*., 2010).

The major components of rosemary essential oil was found to be 1,8-cineol, camphor, α-pinene and borneol (Minaiyan *et al*., 2011, Gharib and Teixeira da Silva, 2012). Different levels of salinity decreased 1,8-cineole, borneol, camphor and α-pinene the main constituents of *R. officinalis* oil in Iran (Langroudi *et al*., 2013). Change in oil composition and antioxidants level in rosemary under different environmental stresses as salinity may changes its efficiency. So there was an urgent need to use inexpensive, non-toxic, non-polluting and non-hazardous way to overcome stress effects on plant like biofertilizers.

On the other hand, seaweed provides an excellent source of bioactive compounds such as

carotenoids, dietary fiber, protein, essential fatty acids, vitamins, amino acids, minerals and growth promoting substances (Bhaskar and Miyashita, 2005). Seaweeds stimulate the growth and yield of plants by increasing nutrient uptake, carbohydrates, proteins, free amino acids, polyphenol (Pise and Sabale, 2010) and enhancing antioxidant properties (Turan and Köse, 2004). Foliar spray of seaweed extract increased yield of cherry tomato (Dobromilska *et al*., 2008), shoot growth and development in *Trigonella foenum-graecum* (Fenugreek) (Pise and Sabale, 2010) and increase plants tolerance to many biotic and abiotic stresses (Mancuso *et al*., 2006; Craigie, 2010).

A literature survey indicated that the degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of antioxidative systems. Our study was performed to evaluate the content and constituent make-up of essential oil extracted from dry rosemary herb foliarly sprayed with different concentrations of *S. latifolium* extract individually or under salinity stress, as well as elements uptake, alterations in phytohormones balance and some enzymatic activities in *R. officinalis*. Such results on the chemical composition of the essential oil, productivity and antioxidant activities of extract would allow for the identification of the best treatment with high potential source of natural antioxidants in rosemary plants.

2. Material and Methods

Plant Material

A pot experiment was conducted at the Experimental Farm of Helwan University, Cairo, Egypt during 2011/2012. Uniform cuttings of rosemary (*Rosmarinus officinalis*) (kindly provided by Medicinal and Aromatic Plants Research Branch, El-Qanatir El-Khairiya, Horticulture Research Institute, Ministry of Agriculture) were transplanted (two cuts/pot) in earthenware pot (40 cm in diameter) of 15 kg of clay loamy soil. Soil samples were digested according to Megroth and Cegarra (1992) and valuated according to Jackson (1973). Physical and chemical properties of the soil used in the experiment were presented as follow:

The soil type was clay loamy in texture (consists of clay 52.96%, silt 28.04%, fine sand 13.14% and coarse sand 5.86%) with pH of 7.8, organic matter 1.05%, E.C. of 7.26 m. mhos cm^{-1} and SP 43. The soil analysis, 5.86 % containing $CaCo_3$, available cations 37.4, 26.24, 19.19 and 0.66 (mlequivelant/l) of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}, respectively and also available anions 54.72, 26.5, 2.28 (mlequivelant/l) soil of SO^{4--}, CL^{-} and $HCO3^{1-}$, respectively.

Sargassum latifolium extract contain 31% alginic acid, 16% Mannitol, minerals (i.e. 11.48% potassium, 0.83% phosphorus, 3.6% calcium , 1.13% magnesium, 1.39% N, 0.005% Zn, and 0.2% Fe) as well as 96.9 µg/100g cytokinin, 0.41 mg/100g indole acetic acid and 3.47 mg/100g gibberellins as growth regulators (Salem, 2007).

Application of *Sargassum latifolium* and salinity

Rosemary plants were divided into two groups. The first group were twice foliarly sprayed with freshly prepared solutions of *Sargassum latifolium* extract at (0.00, 0.20, 0.40 and 0.60%) and irrigated with water. The second group were twice foliarly sprayed with *S. latifolium* extract at (0.00, 0.20, 0.40 and 0.60%) and irrigated to near field capacity with saline water at 100*mM* NaCl for 3 weeks. (Initial *S. latifolium* treatments were applied before exposing rosemary plants to salinity to allow for plant uptake of known amount of *S. latifolium* extract). The first spray was done three months after transplanting, while the second spray was applied one week later after the first one. Treatments were repeated again 2 months after the first cut. Control plants were sprayed with distilled water. The pots were arranged in complete randomized blocks design with eight treatments, four replicates per treatment and each replicate represented by 2 plants. Two cuts were taken from rosemary plant; the first one was seven months after transplanting, while the second was carried out after four months from the first cut.

Fertilization was carried out for each pot at proportion of 1 g ammonium nitrate (33.5% N), 2 g calcium superphosphate (15.5% P_2O_5) and 1g potassium sulphate (48% K_2O). These fertilizers were applied in two doses at 60 and 75 days after planting and repeated after the first cut. Irrigation was done regularly to maintain soil field capacity.

At full blooming stage, the The aerial parts (leaves and stems) were harvested by cutting 10 cm above the soil surface and plant growth parameter for the 2 cuts were recorded i.e. plant height (cm), number of branches per plant as well as fresh and dry weights of leaves and stems (g). Plant samples were dried in an oven with drift fan at 70°C until constant dry weight was obtained. Representative fresh samples were taken from each treatment for determination of essential oil content and constituents as well as some metabolic activities.

Isolation of essential oil

Quantitative determination of essential oil (EO) from air-dried parts of different treatment of rosemary was achieved by hydro-distillation for 3 h using a Clevenger-type apparatus. Oil yield per plant was calculated for different treatments during two cuttings. The obtained oil was dried over anhydrous

sodium sulphate and after filtration, stored in a sealed vial at -4°C until tested and analyzed.

GC/MS Analysis of Essential Oil

The EO of dry rosemary developed from the second cut was analyzed by GC/MS at the National Research Centre, Dokki, Cairo, Egypt using Trace GC ultra, Thermo scientific GC equipped with a TG-SMS fused silica column (15 m × 0.25 mm ID × 0.25 µm film thickness).The multi-step temperature program was increased from 40°C (held for 3 min) to 280°C (held for 5 min) at a rate of 5°C min^{-1}. The carrier gas was helium at a flow rate of 1 ml min^{-1}, flow is 0.7 ml min^{-1} (constant flow). Diluted samples (1:1 diethyl ether v\v) and the sample size was 1 µl (injector temperature was 230 °C). The mass spectrometer detector was a SQ single Quadrupole WS, Thermo scientific 120602 operating with an ionization voltage of 70 eV. Scan time and mass range were 5 s and 40-499.9m/z, respectively. FID is set at 280°C, flow is 0.7 ml min^{-1} (constant flow), with a split ratio 1:300 Mass.

Compound Identification

Identification of EO constituents was made by matching their recorded mass spectra with those stored in the Wiley /NBS mass spectral library of the GC-MS data system and other published mass spectra. Retention index was calculated for each compound using the retention times of a homologous series of C_6 - C_{26} *n*-alkanes (Adams, 2001).

Determination of Photosynthetic pigments and Activity (Hill reaction)

Photosynthetic pigments including Chlorophyll (a and b) as well as carotenoid content were determined in fresh rosemary leaves as mg g^{-1} fresh weight according to the procedure achieved by Metzener *et al*. (1965). The photosynthetic activity (Hill reaction) of isolated chloroplasts was determined as adopted by Osman *et al*. (1982) by measuring the concentration of reduced potassium ferricyanide (electron acceptor) and calculated from standard curve as µmol ferricyanide g^{-1} chlorophyll s^{-1} (Arnon and Shavit, 1963).

Determination of Macro and Micro Elements

Determination of N, P, K, Ca, Mg and Na concentrations were carried out in the ground dry herbs at second cut. After wet digestion, total nitrogen was determined using the modified Micro-Kjeldahl method according to AOAC (1980). Phosphorus was determined using vanadate molybdate method (Jackson, 1973). Potassium and Sodium were measured by flame photometer (Atomic spectra AAS vario 6) (Williams and Twine, 1960). Calcium and magnesium were estimated by using Inductively Coupled Spectrometry Plasma (ICP) Model Ultima 2-Jobin Yvon.

Assay of Enzymes Activity

Preparation of Enzyme Extracts

Fresh leaves of rosemary developed from different treatments at the second cut were used for preparation of enzyme extracts according to Kar and Mishra (1976) as follows: 0.5 g fresh leaves were homogenized in mortar with 10 ml cold phosphate buffer (Na/ K phosphate 0.1M at pH 6.8). The homogenate was centrifuged for 10 minutes at 6000 rpm at 4°C.The supernatant was completed to a known volume and used for assaying the activities of the following enzyme spectrophotometrically.

Assay of Enzymatic Activities

Peroxidase activity (POX) was determined according to (Yamane *et al.,* 1999) with slight modifications by recording changes in absorbance for 30 seconds up to 3 minutes at 436 nm. Polyphenol oxidase (PPO) activity was assayed according to Mayer and Harel (1979) by following change in color intensity at 495 nm for 30 seconds up to 5 minutes. Ascorbate peroxidase (AOD) activity was determined using the method of Kiraly and Farkas (1957) with some modification as described by Maxwell and Bateman (1967) and following the rate of disappearance of ascorbate by reading optical densities after 30 sec interval up to 3 minutes at 265 nm. Catalase (CAT) was assayed following the method of Góth (1991). The reaction mixture was initiated by adding H_2O_2 and the residual of H_2O_2 was monitored spectrophotometrically at 405 nm. IAA oxidase (IAAO) activity was determined by a modified method of Gordon and Weber (1951). The developed colour was monitored spectrophotometrically at 562 nm. Activity of different enzymes were expressed as change in optical density g^{-1} fresh weight h^{-1}.

Determination of Endogenous phytohormones

10 grams fresh leaves of rosemary developed from different treatments at the second cut were used for the extraction of phytohormones according to Wasfy and Orrin, (1975). The samples were ground in cold 80% methanol, followed by triple extraction with fresh methanol for 2 hours at 0°C. To estimate the amounts of acidic hormones, the plant hormone fractions and standards were methylated according to Vogel (1975) to be ready for High Performance Liquid Chromatography (HPLC) analysis. The retention time (RT) and the area of peaks of authentic samples were used for the identification and characterization of peaks of samples under investigation.

Statistical Analysis

Analysis of data was carried out according to (Snedecor and Cochran, 1990). Means were compared using least significant difference (LSD at the 5% level) and Duncan's multiple range test at significance $P = 0.05$

3. Results

Growth Parameters

Results in figure (1) indicate that foliar application of *Sargassum latifolium* extract separately at 0.20, 0.40 and 0.60 % promoted all growth criteria (i.e., plant height, number of branches per plant, fresh and dry weights of leaves and stem (g plant^{-1}) compared to the corresponding untreated control plants during the two cutting. The increment in growth characters was significantly greatest with 0.20% SWE except for plant height and number of branches per plant show an insignificant response compared to control plants at first cut.

On the other hand, S (100*mM* NaCl) in irrigation water significantly reduced growth parameters the most in terms of plant height, number of branches per plant fresh and dry weights of rosemary plants compared to their relative controls and other treatments during two cutting (Figure 1). Salinity stress recorded the lowest fresh (43.64, 34.45 g plant^{-1}) and dry weights (18.22, 15.57 g plant^{-1}) of leaves at first and second cuts, respectively compared with (61.17, 49.31 g plant^{-1}) for F.W and (22.70, 21.60 g plant^{-1}) for D.W of controls.

Regarding the combined effect of SWE at any concentration and S at 100m*M* NaCl on vegetative growth, application of SWE ameliorate the negative effect of S and enhanced growth parameters of rosemary plants under stress conditions compared with S treatment during two cutting. The most significant increases in growth parameters were obtained at 0.40% SWE + S.

Oil Content

Figure (1) show that the application of SWE alone increased essential oil (EO) percent and oil yield per plant on a dry weight basis of rosemary plants more than their relative controls especially at the first cut. The greatest increase in essential oil percentage of the two cuts when pooled together (increased 43.68 and 36.07% more than their corresponding controls) and oil yield on a per plant basis (increased 140.17% and 111.80% more than the control) at 0.20, 0.40% SWE, respectively. In all cases, the increments in oil content were often highly significant in comparison with untreated controls.

Moreover, S stress (100m*M* NaCl) in irrigation water significantly increased oil percentage (32.33%) although reduced oil yield (-22.80%) on a per plant basis of the two cuts when pooled together relative to their corresponding controls (Figure 1).

Considering, the combined effect of SWE and S, the most promising values of oil yield per plant and oil percent of the two cuts when pooled together increased 43.52% and 10.76% more than their controls, respectively was obtained by foliar application of SWE at 0.40% + S followed by SWE at 0.20% + S for oil content. In most cases, the increase in oil percent were often non significant compared to controls.

EO Composition

The essential oil obtained by hydro-distillation of dry *R. officinalis* herb at second cut was analyzed by GC/MS (Table 1). In *R. officinalis* 23, 26, 28 and 28 components were identified in the EO of untreated plants, plants foliar sprayed with SWE at 0.20%, S treatment, SWE at 0.40% + S, respectively. The identified components representing (94.67-96.53%) of the total oil with 1,8-cineol (6.67-22.10%), camphor (10.47-15.05%), α-pinene (1.06-13.96 %), borneol (5.64-12.98%), as the main constituents followed by citronellol (2.32-8.27%), berbenone (6.31-7.32%), endoborneol acetate (4.59-7.32%), linalool (1.79-7.06%), endoborneol (2.09-6.65%), camphene (0.36-6.22%), β-caryophyllene (0.85-3.80%) and α-myrcene (0.51-2.73%).

1,8-cineol (22.10%) was the major compound in the EO of untreated rosemary followed by camphor (14.54%), α-pinene (13.96%) and borneol (8.48%), respectively.

Foliar spray of SWE at 0.20% increased monoterpene hydrocarbons (25.82%) including camphene (6.22%) and myrcene (2.73%); sesquiterpene hydrocarbon (3.49%) including β-caryophyllene (2.61%). Oxygenated sesquiterpenes (2.10%) including caryophyllene oxide (1.24%) as well as aromatic compounds (0.67%). On the contrary, oxygenated monoterpenes (58.75%) decreased at 0.20% SWE including low 1,8-cineol (6.67%), camphor (9.90%) and borneol (2.04%) as well as aliphatic esters (6.46%) as the common factors for the EOs compared with untreated rosemary plants.

On the contrary, S decreased monoterpene hydrocarbons (4.37%) including very low α-pinene (1.06%) and camphene (0.36%), while, oxygenated monoterpenes recorded the highest value (72.77%) including high camphor (15.05%) and borneol (12.98%), but low 1,8-cineol (11.17%) compared with untreated rosemary plants.

Moreover, SWE at 0.40% + S treatment showed nearly similar values for monoterpene hydrocarbons and oxygenated monoterpenes being 20.88% and 64.31% compared with 22.04% and 64.44% for untreated plants, respectively. However, aliphatic esters (5.00%) decreased, while sesquiterpene hydrocarbon (2.35%) including β-caryophyllene (1.94%), oxygenated sesquiterpenes (2.16%) including caryophyllene oxide (0.67%), aromatic compounds (0.80%) increased in rosemary plants under study at 0.40% SWE + S, compared with untreated plants.

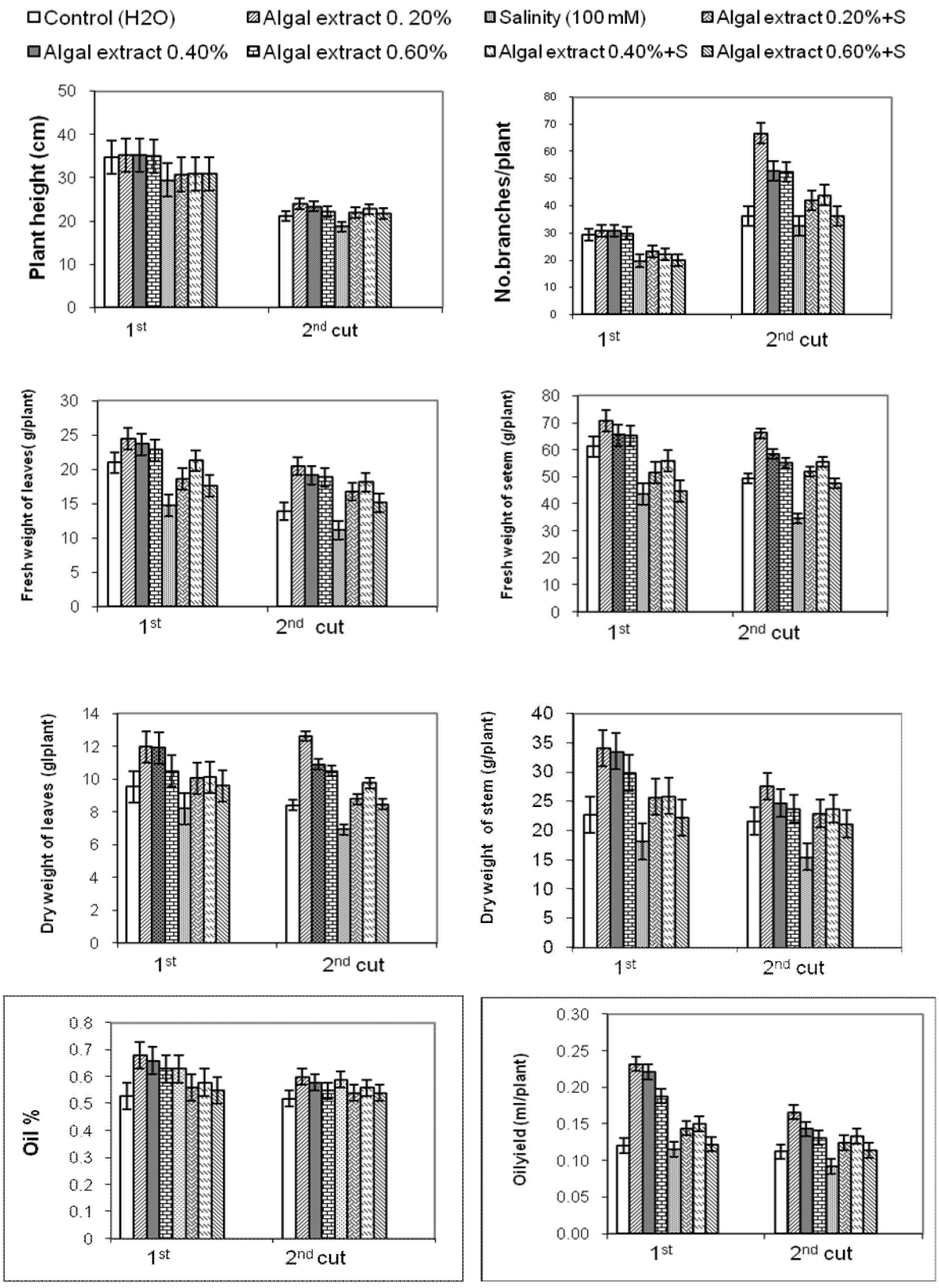

Figure 1: Effect of foliar spray with *Sargassum latifolium* extract on the vegetative growth and oil content of rosemary plants under normal and salinity (S) (100 *mM* NaCl) during two cuttings. Vertical bars represent LSD at 5%

Table 1: Composition of the essential oil of dry rosemary plants as affected by foliar spray with *Sargassum latifolium* extract under normal and salinity (100 *mM* NaCl) at second cut

Oil components (%)	Control (H_2O)	Algal extract 0.20%	Salinity (100 *mM* NaCl)	Algal extract 0.40% + Salinity	RI
Monoterpene hydrocarbons					
α-pinene	13.96	12.75	1.06	11.45	939
Camphene	5.58	6.22	0.36	5.03	954
Limonene	0.85	0.0	0.0	0.0	978
α-myrcene	0.72	2.73	0.51	1.72	991
δ-3-carene	0.29	1.21	0.38	1.02	1004
α-terpinene	0.39	1.96	1.56	1.10	1018
γ-terpinene	0.25	0.95	0.50	0.56	1062
Total	**22.04**	**25.82**	**4.37**	**20.88**	
Oxygenated monoterpenes					
1,8-cineol	22.10	6.67	11.17	10.46	1030
Linalool	1.79	7.06	6.44	4.41	1085
Camphor	14.54	9.90	15.05	10.51	1122
Endoborneol	7.32	2.04	2.42	2.09	1169
Borneol	8.48	5.64	12.98	11.45	1165
Berbenone	6.31	7.53	6.87	7.95	1194
Piperitenone	0.11	0.27	0.44	0.42	1245
α-terpineol (*p*-Menth-1-en -8-ol)	0.15	3.84	4.91	3.62	1295
Myrtenol	1.32	9.76	4.22	9.31	1324
citronellol	2.32	6.04	8.27	4.09	1344
Total	**64.44**	**58.75**	**72.77**	**64.31**	
Sesquiterpene hydrocarbons					
β-caryophyllene	0.85	2.61	3.80	1.94	1421
α-humulene	0.11	0.38	0.63	0.27	1454
α-cadinene	0.00	0.41	0.61	0.32	1538
γ -cadinene	0.00	0.00	0.30	0.00	1543
Total	**0.96**	**3.40**	**5.34**	**2.53**	
Oxygenated sesquiterpenes					
Caryophyllene oxide	0.23	1.24	1.90	0.67	1581
Veridiflorol	0.00	0.31	0.59	0.44	1633
α-Eudesmol	0.28	0.55	1.10	0.86	1652
α-bisabolol	0.0	0.0	0.30	0.19	1662
Total	**0.51**	**2.10**	**3.89**	**2.16**	
Aliphatic esters					
Endobornyl acetate	7.32	5.82	7.09	4.59	1259
Geranyl actate	0.22	0.64	0.86	0.41	1383
Total	**7.54**	**6.46**	**7.95**	**5.00**	
Aromatic compounds					
Eugenol	0.00	0.00	0.35	0.17	1364
Methyl Eugenol	0.00	0.67	0.83	0.63	1407
Total	0.00	0.67	1.18	0.80	
Unidentified compounds	**4.51**	**3.47**	**5.33**	**4.95**	

Photosynthetic Activity and Pigments

Data presented in Table (2) show that foliar application of SWE at any concentration or combined with salinity increased photosynthetic activity (PA) and the total photosynthetic pigments (TPP) in rosemary leaves more than controls at second cut. The most effective concentrations were SWE at 0.20%, followed by SWE at 0.40%, whereas S significantly decreased PA and TPP.

Foliar spray of rosemary plants with SWE at 0.20, 0.40 and 0.60% significantly increased PA, Chl *a* and *b*, carotenoids and consequently TPP. The greatest recorded values of PA (2.32 µmol ferricyanide g^{-1} chlorophyll s^{-1}), carotenoids and TPP (12.03 and 21.38 mg g^{-1} F. W) in the leaves of rosemary plants were obtained with 0.20% SWE compared to PA (0.74µmol ferricyanide g^{-1} chlorophyll s^{-1}), carotenoids and TPP (5.65 and 12.16 mg g^{-1} F. W) for untreated plants, respectively.

On the other hand, salinity significantly decreased PA, Chl *a* and *b*, carotenoids and TPP in rosemary leaves compared to untreated plants and other treatments (Table 2). Moreover, the interaction between SWE and S slightly increased these pigments, with the most effective treatment being SWE at 0.40% + S.

Table (2): Effect of foliar spray with algal (*Sargassum latifolium*) extract on photosynthetic activity (µmol ferricyanide g^{-1} chlorophyll s^{-1}) and pigments (mg g^{-1} F.W) in the leaves of rosemary plants under normal and salinity (S) (100 *mM* NaCl) for 3 weeks at second cut. Different letters indicate significant differences between treatments (Duncan test, $P \leq 0.05$)

Treatments	Photosynthetic activity (µmol ferricyanide g^{-1} chlorophyll s^{-1})	Photosynthetic pigments (mg/ g fresh weight)					
		Ch.*a*	Ch.*b*	Ch*a*/*b*	Ch.*a*+*b*	Carotenoids	Total pigments
Control (H_2O)	0.74 b	4.79 c	1.72 a	2.78 e	6.51 ab	1.88 a	8.39 b
Algal extract 0.20%	2.32 e	5.96 g	3.39 f	1.76 a	9.35 e	4.01 f	13.36 f
Algal extract 0.40%	2.04 d	5.47 f	3.22 e	1.70 a	8.68 d	3.58 e	12.26 e
Algal extract 0.60%	1.40 c	5.09 e	2.38 d	2.14 b	7.46 c	2.73 d	10.19 d
Salinity (100 *mM)*	0.51 a	4.29 a	1.81 b	2.37 c	6.09 a	1.85 a	7.94 a
Algal extract 0.20%+ S	0.88 b	4.60 b	1.85 bc	2.49 cd	6.46 ab	2.00 c	8.46 bc
Algal extract 0.40%+ S	1.47 c	4.91 d	1.88 c	2.61 d	6.78 b	2.02 c	8.80 c
Algal extract 0.60%+ S	0.77 b	4.74 c	1.85 bc	2.56 d	6.59 b	1.94 b	8.53 b
L.S.D. at 0.01	0.13	0.14	0.07	0.17	0.50	0.06	0.56

Macro- and Micro Elements

In rosemary plants, foliar application of SWE at any concentration or combination with S increased the content and uptake (mg $plant^{-1}$) of N, P, K, Mg and Ca except Na content decreased compared with controls at second cut (Table 3). The most effective treatments were SWE at either 0.20% or 0.40% combined with S, respectively. On the contrary, S decreased the content and uptake of these elements except Na content, showing the highest content (22.85 ppm) and its uptake (51.4 mg $plant^{-1}$) unaffected compared to (17.21 ppm, 51.6 mg $plant^{-1}$) for their respective controls (Table 3).

Furthermore, the results obtained indicate that the greatest and lowest level of N, P, K, Mg and Ca content and up take were obtained for either SWE at 0.20% or S, respectively.

Changes in Enzymes Activities

Data presented in Table 4 show that foliar application of SWE separately at 0.20, 0.40 and 0.60%, significantly increased the activity levels of POD, PPO and AOD (g^{-1} F.W $hour^{-1}$) in the leaves of rosemary compared with controls at 2nd cut. This, promoting effect was maximal for three enzymes at 0.20% SWE and decreased thereafter; a more or less opposite trend to that obtained with activity levels of CAT and IAAO (g^{-1} F.W $hour^{-1}$) which decreased in response to application of SWE, especially at 0.20%.

Moreover, S stress, singly or combined with foliar application of SWE at any concentration increased the activity levels of POD, PPO, AOD, CAT and IAAO in the leaves of rosemary relative to untreated controls. The greatest activity levels of these enzymes were obtained for S stress, followed by SWE at 0.40 % + S, except for AOD the opposite was obtained.

Changes in Endogenous Phytohormones

Application of SWE either separately at 0.20% or at 0.40% combined with S, increased the contents of IAA, GA_3, GA_3/ABA ratio, and total cytokinins as compared with corresponding untreated control rosemary plants (Table 5).

On the contrary, S decreased these phytohormones and GA_3/ABA ratio to 200.14 while, increased ABA to 3.52 mg 100 g^{-1} F.W in comparison with 253.96 and 2.78 mg 100 g^{-1} F.W for control plants, respectively.

Table (3): Effect of foliar spray with *Sargassum latifolium* extract on macro and microelements content and uptake of rosemary plants under normal and salinity (S) 100 ***mM*** NaCl at second cut

Treatments	Macro-and Microelements content						Macro-and Microelements uptake (mg/plant)					
	N%	P%	K%	Ca (ppm)	Mg (ppm)	Na (ppm)	N	P	K	Ca	Mg	Na
Control (H_2O)	1.91	0.287	0.98	30.35	4.58	17.21	573.2	86.1	294.1	91.1	13.7	51.6
Algal extract 0. 20%	2.41	0.342	1.45	41.19	5.35	14.05	968.8	137.5	582.9	165.6	21.5	56.5
Algal extract 0.40%	2.41	0.314	1.41	41.04	5.17	16.47	858.4	111.8	502.2	146.2	18.4	58.7
Algal extract 0.60%	2.33	0.305	1.14	39.52	4.79	17.20	797.3	104.4	390.1	135.2	16.4	58.9
Salinity (100 mM)	1.87	0.274	0.81	21.60	3.33	22.85	420.8	61.7	182.3	48.6	7.5	51.4
Algal extract 0.20%+ S	1.98	0.291	0.99	36.22	4.87	17.96	628.3	92.3	314.1	114.9	15.5	57.0
Algal extract 0.40%+ S	2.11	0.311	1.25	40.98	5.07	17.37	708.1	104.4	419.5	137.5	17.0	58.3
Algal extract 0.60%+ S	1.97	0.291	0.98	33.57	4.61	20.15	583.3	86.2	290.2	99.4	13.7	59.7

Table (4): Effect of foliar spray with algal (*Sargassum latifolium*) extract on changes in enzymatic activities (g^{-1} fresh weight hour^{-1}) of rosemary plants under normal and salinity (S) 100 *mM* NaCl for 3 weeks at second cut.

Treatments	Peroxidase	Polyphenol oxidase	Ascorbic oxidase	Catalase activity	IAAO
	g^{-1} fresh weight hour^{-1}				
Control (H_2O)	36.0 a	66.8 a	54.0 a	172.5 d	7.7 d
Algal extract 0.20%	61.2 d	77.6 de	126.0 c	101.0 a	1.6 a
Algal extract 0.40%	57.6 c	74.4 c	120.0 b	123.0 c	3.2 b
Algal extract 0.60%	39.6 b	71.2 b	119.0 b	106.5 b	5.6 c
Salinity (100 ***mM***)	108.0 h	104.0 g	138.0 d	561.0 g	18.5 g
Algal extract 0 20% +S	73.8 f	79.2 e	198.0 f	321.0 f	12.1 e
Algal extract 0.40% + S	84.6 g	94.0 f	234.0 g	322.0 f	13.8 f
Algal extract 0.60%+ S	64.8 e	75.6 cd	156.0 e	208.5e	7.8 d
L.S.D. at 0.01	3.60	3.20	6.00	6.00	1.30

Table (5): HPLC analysis for endogenous hormone concentrations (mg 100 g^{-1} F. W) of rosemary plants foliary sprayed with *Sargassum latifolium* extract under normal and salinity (S) 100 ***mM*** NaCl at second cut

Treatments	Concentrations (mg/100 g F.w.)						
	IAA	GA_3	ABA	GA_3/ABA	Kinetin	Benzyl adenine	Total cytokinins
Control	7.59	706.01	2.78	253.96	0.14	5.79	5.93
Algal extract 0.20%	12.03	1029.59	2.55	403.76	0.20	9.12	9.32
Salinity (100 mM NaCl)	6.40	704.50	3.52	200.14	0.14	5.27	5.41
Algal extract 0.40% + S	8.01	838.01	2.92	286.99	0.15	8.86	9.01

Moreover, when considering SWE as a single factor, it was more effective than combinations of SWE at 0.40% + S in increasing IAA, GA_3, GA_3/ABA and total cytokinins.

On the bases of these results, it could be concluded that, the GA_3/ABA ratio might represent the primary hormonal signal; the increase in GA_3/ABA ratio seemed to correlate with the improved metabolic activity and subsequent growth.

4. Discussion

The present study indicates that the application of SWE up to 0.60%, individually or in combination with S, greatly promotes the vegetative growth, fresh and dry-matter production of rosemary plants, possibly through participation in the synthesis of auxin and/or cytokinin, enhancement of cell division, root growth, number of branches and leaves as well as leaf area, leading to increased nutrient uptake, photosynthetic activity and Chl accumulation compared to respective controls at 1st and 2nd cuttings. This agrees with Crouch and van Staden, (1992) who reported that Seaweed concentrates (SWC) stimulated root growth, enhanced both root: shoot ratios and biomass accumulation in tomato seedlings. In grapes, marine extract improved leaf content of macronutrients, promoted growth, and impart resistance to drought stress (Mancuso *et al*., 2006). Also, *Ascophyllum Nodosum* extracts at 0.1g L^{-1} affected root growth, whereas 1.0g L^{-1} affected arabidopsis height and number of leaves (Rayorath *et al*., 2008).

In the present study, S stress (100 m*M* NaCl) showed pronounced significant decrease in vegetative growth of rosemary plant by decreasing plant height, number of branches and leaves consequently decreased fresh and dry weight of rosemary plant. Similarly, increasing S stresses reduced growth parameters of *Salvia officinalis* at 100 m*M* NaCl (Ben-Taarit *et al*., 2009), peppermint (Khorasaninejad *et al*., 2010) and *R. officinalis* at 150 m*M* NaCl (Kiarostami *et al*., 2010). Suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride (Munns, 2003).

Intuitively, the combination of SWE and S showed in this study a much better ability than single S (100 m*M* NaCl) to promote growth of rosemary plants. Similarly, Salicylic acid (SA) application increased shoot and root dry weights of rosemary under stress conditions compared to salt stressed untreated plants (Najafian *et al*., 2009). Also, Foliar applications of kinetin at 10 μ*M* improved ion, pigment contents and counterbalance the stress symptoms induced by salinity in common sage (*Salvia officinalis* L.) (Tounekti *et al*., 2011).

In our study, the quantity of oils were significantly enhanced by application of SWE, especially at 0.20% at first cut due to enhance nutrients uptake, photosynthetic activity, dry matter production/ plant as well as change in leaf oil gland population, which consequently increased EO percent and oil yield per plant on a dry weight basis. According to Czepak (1998), the higher the dry matter yield of plants the higher their EO yield. Bottcher *et*

al., (1999) found that *M. hortensis* herbs (1st cut) contained 22% more essential oils than physiologically younger plants (2nd cut).

In rosemary, S stress significantly increased oil percentage, while reduced oil yield on a per plant basis by reducing photosynthetic pigment and activity, dry matter production/ plant, number of leaf oil gland population, reduction in biosynthesis of monoterpenes and consequently low EO yield per plant on a dry weight basis. Similarly, salinity decreased essential oil yield in *Mentha piperita* (Tabatabaie and Nazari, 2007), *Thymus maroccanus* (Belaqziz *et al*., 2009) and *Salvia officinalis* fruits at 100 m*M* NaCl (Ben-Taarit *et al*., 2010) due to disturbance in photosynthesis, carbohydrate production (Flexas and Medrano, 2002) and suppression of the plant growth under stress condition in peppermint (*Mentha piperita* L.) (Aziz *et al*., 2008; Khorasaninejad *et al*., 2010). On the contrary, salinity has been reported to increase essential oil percent of sage (*Salvia officinalis*) (Hendawy and Khalid, 2005), thyme (*Thymus vulgaris*) (Ezz El-Din *et al*., 2009) and basil (*Ocimum basilicum*) plants (Said-Al Ahl and Mahmoud, 2010). Stimulation of essential oil production under a moderate degree of salinity could be due to a higher oil gland density and an increase in the absolute number of glands produced prior to leaf emergence as a result of a stress induced reduction in leaf area (Tabatabaie and Nazari, 2007), secondary metabolites synthesis and accumulation as self-defense components to cope with stressful conditions (Ezz *et al., 2*009).

In this study, the combination of SWE especially at 0.4% + S increased oil yield more effectively than S by enhancing photosynthetic activity, dry matter accumulation and consequently oil yield of rosemary plants.

In the present study, the EO of rosemary was hydro-distillated to evaluate the EO composition (Table 1). The major constituents of *R. officinalis* EO detected in this study was 1,8-cineol followed by camphor, α-pinene and borneol, respectively. Previous reports showed chemical composition closer to our findings in the EO of fresh and dried *R. officinalis* leaves although the relative quantities of individual components varied; 1,8-cineole was the predominant compound (Yang *et al*., 2010; Gharib and Teixeira da Silva, 2012) but in another study, α-pinene was the main compound followed by camphor and 1,8-cineol (Bernstein *et al*., 2009). Differences in the volatile components percent in our plant material might have been caused by climatic and seasonal factors, the origin and stage of distillation.

Salinity decreased 1,8-cineol (11.17%), α-pinene (1.06%) and camphene (0.36%), while, increased camphor (15.05%) and borneol (12.98%), compared with untreated rosemary plants. According to Langroudi *et al*., (2013) different levels of salinity decreased 1,8-cineole, borneol, camphor and α-pinene the main constituents of *R. officinalis* essential oils in Iran.

Generally, Foliar spray of *R. officinalis* with SWE at 0.20%, S and combination of SWE at 0.40% + S changed the composition of rosemary essential oil by enhancing linalool, α-terpineol, β-caryophyllene, caryophyllene oxide and aromatic compounds, while decreasing α-pinene and 1,8-cineole.

Photosynthetic activity and pigments of rosemary leaves were enhanced by the application of SWE and combination with S, while single treatment with S, significantly decreased PA, Chl *a* and *b*, carotenoids and TPP in rosemary leaves more than controls (Table 2).

The SWE might increase cell metabolic rate and retard senescence by protecting and preventing chloroplasts from senescing and retarding Chl destruction and/or increase Chl biosynthesis due to high content of N (1.0%) and Fe (0.20%) in SWE, which increased N and Fe levels (structural component of Chl) and enhanced Chl accumulation leading to a greater rate of photosynthesis. Similarly, the promotive effects of *Sargassum polycystum* at low concentrations on chlorophyll *a* and *b* were observed for *Cajanus Cajan* (Erulan *et al*., 2009). SWE significantly increased carotenoids content in rosemary leaves. Carotenoids can protect photosynthetic system against reactive oxygen species generate under salt stress (Parviz and Satava Waiz, 2008). Whereas S at 100 m*M* NaCl significantly decreased PA and Photosynthetic pigments, working on rosemary and sage (*Salvia officinalis* L.), salt stress at 100 m*M* NaCl caused significant reduction in photosynthetic activity and pigment contents with the most adverse effect on Chl *b* (Kiarostami *et al*., 2010; Tounekti *et al*., 2011). The decrease in chlorophylls levels may be due to reduction in pigment biosynthesis or enzymatic chlorophyll degradation (Yang *et al*., 2009).

Intuitively, the combination of SWE and S might concomitantly alleviate the adverse effect of S and improved photosynthetic activity and pigments contents. In this study, SWE at 0.20% followed by SWE at 0.40% + S increased kinetin, benzyl adenine and total cytokinins contents in rosemary plants. Spraying kinetin at 10 μ*M* alleviate the stress symptoms induced by salinity (100 m*M* NaCl) and improved pigment contents in sage (*Salvia officinalis* L.) (Tounekti *et al*., 2011).

A foliar application of either SWE or combination with salinity increased the content and uptake (mg plant^{-1}) of N, P, K, Mg and Ca except Na content in rosemary plants, possibly due to increase

root growth, enhance nutrient uptake which seems to be involved in the mechanism of stress tolerance and played an important role in enhancing the activity of enzymes responsible for salinity resistance. *Ascophyllum nodosum* extract at 0.1g L^{-1} increased root growth in *Arabidopsis* (Rayorath *et al*., 2008). Whereas, marine extract improved macronutrients content of grape leaf and promoted growth (Mancuso *et al*., 2006). Increased mineral nutrient content seem to be involved in stress-tolerance mechanism and play an important role to enhance the activity of enzymes responsible for salinity resistance (Cherki *et al*., 2002). Thus, combined treatments of SWE, especially at 0.40% + S may be effective for enhancing nutrient uptake and typically alleviates the effects of salt stress in rosemary.

On the other hand, salinity decreased the content and uptake of N, P, K, Mg and Ca except Na, showing the highest content and unaffected its uptake due to reduction in dry matter accumulation in rosemary plants. Na^+ accumulation lead to low water potential, change in essential ion uptake, ionic imbalance, reduces leaf expansion, photosynthetic rate and limit growth (Zaho *et al*., 2007). In strawberry, salinity increased the uptake of Na^+ and Cl^- and decreased the uptake of N, P, K, Ca^{2+}, Mg^{2+} and P levels due to competition of Na^+ and Cl^- with other mineral ions (Yildirim *et al*., 2009). Progressive increase in NaCl concentration (50-150 m*M*) increased Na^+, whereas Ca^{2+} and K^+ content decreased in rosemary leaves (Kiarostami *et al*., 2010) and sage (*S. officinalis* L.) (Tounekti *et al*., 2011) leading to reduction of K^+/Na^+ and Ca^{2+}/Na^+ ratios and Na^+ toxicity (Ramezani *et al*., 2011).

A foliar application of either SWE separately at any concentration significantly increased the activity levels of POD, PPO and AOD, opposite results were obtained with activity levels of CAT and IAAO which decreased in response to application of SWE, especially at 0.20% in the leaves of rosemary compared with controls. In turf grasses, application of a seaweed extract increased the activity of superoxide dismutase (SOD) (Fike *et al*., 2001) and in spinach leaves, *A. nodosum* extract increased antioxidant levels (Fan *et al*., 2011).

The greatest activity levels of POD, PPO, AOD, CAT and IAAO in the leaves of rosemary were obtained for salinity stress singly, followed by combination with SWE, especially at 0.40 % except for AOD the opposite trend was obtained. In shoot of mung bean plant, activity of POD and RNase were significantly increased with increasing NaCl concentration (Abdel Haleem, 2007). CAT responses to saline stress was variable from large increase in activity (Gossett *et al*., 1994) to no changes (Fadzilla *et al*., 1997) and lastly salt stress caused severe deactivation of catalase (Shalata and Neumann, 2001).

In rosemary plants, the activities of antioxidant enzymes, were higher, in response to combined treatments of SWE + S compared with SWE, singly or untreated controls and this may be due to different expression in activities of antioxidant enzymes in response to salt stress to get rid of reactive oxygen species and increase their salinity tolerance. Spraying, *H.sabdariffa* seedlings with vit. B_2 improved their salinity resistance by increasing the antioxidant enzymes (CAT, POD, AOD and glutathione reductase GR) (Azooz, 2009).

In rosemary plants, phytohormones were enhanced by the application of SWE separately or combined with salinity. SWE contain 0.001% cytokinin and 0.0002% IAA and its application may be involved in gene expression regulating the signaling activities or levels of growth regulating substances through direct impact on the activities of oxido-reductive enzymes related to the hormonal metabolism and promoted almost all growth criteria. In bean plants (*Phaseolus vulgaris*), foliar application of seaweed concentrate (Kelpack 66) increased growth and cytokinin content (Featonby-Smith and van Staden, 1984), a close correlation was found between the use of kinetin and seaweed extract on potato yield (Blunden and Wildgoose, 1977). Growth promoting hormones (IAA, IBA and cytokinins) in seaweed concentrates cause many beneficial effects on plants (Tarakhovskaya *et al*., 2007).

On the contrary, salinity decreased growth promoting phytohormones while, increased ABA and reduced growth in comparison with control rosemary plants, possibly due to enhance biosynthesis of growth inhibitors. In *Arabidopsis* and soybean cultivar, salinity declined cytokinin, IAA, GA and SA, while increased ABA and Jasmonic acid contents resulting in reduced growth and yield (Wang *et al*., 2001; Hamayun *et al*., 2010). ABA is responsible for the alteration of salt-induced genes, and these genes are predicted to play an important role in the mechanism of salt tolerance (Omami *et al*., 2006).

Decrease in other growth hormones detected under salinity due to decrease in photosynthesis so the carbon source needed for growth decreased. Under stress, plants need energy to adapt to the stress rather than increase in growth. Seaweed extract helps rosemary plants to adapt to salinity and retain its hormonal balance translated to increase in vegetative growth.

Conclusion

In our study, Application of *Sargassum latifolium* extract improved physiological performance in terms of dry matter yield, photosynthetic

production, nutrient uptake, essential oil content, growth promoting phytohormones and activities of some antioxidant enzymes which can be related to eliminate the adverse effects of salinity stress and enhance rosemary growth. *S. latifolium* extract at 0.40 + salinity was the most promising concentration for growing at highest rate under salinity stress. Generally, SWE and combination with salinity improved activities of some antioxidant in rosemary extract to be a good candidate for functional foods as well as for pharmaceutical plant-based products.

However, further investigation is required to elucidate the possible role of *S. latifolium* extract on plant productivity in relation to different salinity stress, to provide insight into molecular mechanisms governing salinity tolerance in rosemary plants, facilitate genetic engineering of plants to tolerate salinity stress and extend cultivation of tolerant rosemary plant at saline area in Egypt.

References

1. Abdel Haleem, M.A., 2007. Physiological aspects of mungbean Plant (*Vigna radiata* L.wilczek) in response to salt stress and gibberellic acid treatment. *Res. J. Agric. & Biol. Sci*., 3(4): 200–213.
2. Adams, R.P., 2001. Identification of Essential Oil Components by Gas Chromatography/ Quadrupole Mass Spectroscopy, Allured Publishing Corp., Carol Stream.
3. Ali, R.M., 2000. Role of putrescine in salt tolerance of *Atropa belladonna* plant. *Plant Sci*., 152: 173–179.
4. Ali, R.M., 2002. Effect of nicotinic acid and nicotinamide adenine dinucleotide on growth and content of oil, glycerol and ricinine alkaloids of salinity stressed *Ricinus communis* L. *Phyton*., 42: 269–277.
5. AOAC., 1980. Official Method of Analysis of the Association of Official Analytical Chemist. Washington DC, USA.
6. Arnon, M.M. and N. Shavit, 1963. A sensitive and simple methods for determination of ferricyanide. *Anal. Biochem*., 6: 549–554.
7. Aziz, E., H. Al-Amier and E. Craker Lyle, 2008. Influence of salt stress on growth and essential oil production in peppermint, Pennyroyal and apple mint. *J. Herbs, Spices & Med. Plants*. 14(1,2): 77–87.
8. Azooz, M.M., 2009. Foliar application with riboflavin (vitamin B_2) enhancing the resistance of *Hibiscus sabdariffa* L. (Deep Red Sepals Variety) to salinity stress. *J. Biol. Sci*., 9: 109–118.
9. Belaqziz, R., A. Romane and A. Abbad, 2009. Salt stress effects on germination, growth and essential oil content of an endemic thyme species in Morocco (*Thymus Maroccanus* Ball.). *J. Appl. Sci. Res*., 5(7): 858–863.
10. Bernstein, N., D. Chaimovitch and N. Dudai 2009. Effect of irrigation with secondary treated effluent on essential oil, antioxidant activity, and phenolic compounds in oregano and rosemary, *Agronomy J.* 101: 1–10.
11. Ben-Taarit, M.K., K. Msaada, K. Hosni, M. Hammami, E. Kchouk and B. Marzouk, 2009. Plant growth, essential oil yield and composition of sage (*Salvia officinalis* L.) fruits cultivated under salt stress conditions. *Ind Crops Prod*., 30 (3): 333–337.
12. Ben Taarit, M.K., K. Msaada, K. Hosni and B. Marzouk, 2010. Changes in fatty acid and essential oil composition of sage (*Salvia officinalis* L.) leaves under NaCl stress. *Food Chem*., 9 (3): 951–956.
13. Bhaskar, N. and K. Miyashita, 2005. Lipid composition of *Padina tetratomatica* (Dictyotales, Pheophyta), a brown seaweed of the west coast of India. *Ind. J. Fisheries*., 52: 263–268.
14. Blunden, G. and P.B.Wildgoose, 1977. The effect of aqueous seaweed extract and kinetin on potato yields. *J. Sci. Food Agric*., 28: 121–125.
15. Botsoglou, N.A., E. Christaki, D.J. Fletouris, P. Florou-Paneri and A.B. Spais, 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Sci*., 62: 259–265
16. Bottcher, H., I.Gunther and U. Bauermann, 1999. Physiological postharvest responses of marjoram (*Majorana hortensis* Moench). *Postharvest Biol. Technol*., 15: 41–52.
17. Czepak M.P, 1998. Produção de óleo bruto e mentol cristalizável em oito freqüências de colheita da menta (*Menta arvensis* L.). In: Ming L.C, Scheffer M.C, Correa Júnior C, Barros I.B.I and Mattos J.K.A (Eds) Plantas Medicinais, Aromáticas e Condimentares. Avanços na Pesquisa Agronômica (1st Edn), FCA-UNESP, Botucatu, Brasil, pp 53-80.
18. Cherki, G., A. Foursy and K. Fares, 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivers. *Environ. Exper. Bot*., 74: 39–50.
19. Craigie, J., 2010. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol*., 23: 371–393.
20. Crouch, I.J. and J. Van Staden, 1992. Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. *J. Appl Phycol*., 4: 291–296.
21. Dobromilska, R., M. Mikiciuk and K. Gubarewicz, 2008. Evaluation of cherry tomato yeilding and fruit mineral composition after using of Bio-algeen S-90 preparation. *J. Elementology*., 13: 491–499.
22. Dow, A.I., T.A. Cline and E.V. Horning, 1981. Salt tolerance studies on irrigated mint. *Bulletin of Agricultural Research Centre.* Washington State University, Pullman., pp: 906.
23. Emadi, F. and N.A. Yassa, 2007. Analysis of *Rosmarinus officinalis* essential oil at different times. Abstract Book of the 3 Congress of Medicinal Plants. Shahed University. Tehran. Iran., pp: 184–185.
24. Erulan, V., G. Thirumaran, P. Soundarapandian and G. Ananthan, 2009. Studies on the effect of *Sargassum polycystum* (*C. Agardh*, 1824) extract on the growth and biochemical composition of *Cajanus Cajan* (L.)

Mill sp, *American-Eurasian J. Agric. & Environ. Sci.*, 6 (4): 392–399.
25. Ezz El-Din, A.A., Aziz, E.E., S.F. Hendawy and E.A. Omer, 2009. Response of *Thymus vulgaris* L. to salt stress and alar (B_9) in newly reclaimed soil. *J. Appl. Sci. Res.*, 5(12): 2165–2170.
26. Fadzilla, N.M., R.P. Finch and R.H. Burdon, 1997. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. *J. Exper. Bot.*, 48: 325–331.
27. Fan, D., M.D. Hodges, J. Zhang, C.W. Kirby, X. Ji, S.J. Locke, A.T. Critchley and B. Prithiviraj , 2011. Commercial extract of the brown seaweed *Asophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects Caenorhabditis elegans against oxidative and thermal stress. *Food Chem.*, 124: 195–202.
28. Featonby-Smith, B.C. and J. Van Staden, 1984. The effect of seaweed oncentrate and fertilizer on growth and endogenous cytokinin content of *Phaseolus vulgaris*. *South Afr. J. Bot.*, 3: 375–379
29. Flexas, J. and H. Medrano, 2002. Drougth-inhibition of photosynthesis in C-3 plants: Stomatal and non-stomatal limitations revisited. *Ann. Bot.*, 89(2): 183–189
30. Fike, J.H., V.G. Allen, R.E. Schmidt, X. Zhang, J.P. Fontenot, P. Bagley, R. Ivy, R. Evans, R. Coelho and D. Wester, 2001. Tasco-Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. *J. Anim. Sci.*, 79: 1011–1021
31. Gharib, F.A. and J.A. Teixeira da Silva, 2012. Composition, total phenolic content and antioxidant activity of the essential oil of four lamiaceae herbs. *Med & Aroma. Plant Sci. & Biotech.*, 7 (1): 19–27.
32. Gordon, S.A. and R.P. Weber, 1951. Colorimetric estimation of indole acetic acid. *Plant Physiol.*, 26(1): 192–195
33. Gossett, D.R., E.P. Millhollon and M.C. Lucas, 1994. Antioxidant response to NaCl stress in salt tolerant and salt sensitive cultivars of cotton. *Crop Sci.*, 34: 706–714.
34. Góth L., 1991. Serum catalase: reversibly formed charge isoform of erythrocyte catalase. *Clin. Chem.*, 37 (12): 2043–2047.
35. Hamayun, M., S.A. Khan, A.L. Khan, Z.K. Shinwari, I. Iqbal, E.Y. Sohn, M.A Khan and I.J. Lee, 2010. Effect of salt stress on growth attributes and endogenous growth hormones of soybean cultivar Hwangkeumkong. *Pak. J. Bot.*, 42 (5): 3103–3112.
36. Hendawy, S.F. and K.A. Khalid, 2005. Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. *J. Appl. Sci. Res.*, 1: 147–155.
37. Hernández, J.A., E. Olmos, F.J. Corpas, F. Sevilla and L.A. Del Rio, 1995. Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Sci.*, 105: 151–167.
38. Jackson, M. I., 1973. Soil Chemical Analysis. Prentice Hall of India Pvt Ltd., New Delhi, India, pp. 111–204
39. Khorasaninejad, S., A. Mousavi, H. Soltanloo, K. Hemmati and A. Khalighi, 2010. The Effect of salinity stress on growth parameters, essential oil yield and constituent of peppermint (*Mentha piperita* L.). *World Appl. Sci. J.*, 11 (11): 1403–1407.
40. Kiraly, Z. and G.L. Farkas, 1957. On the role of ascorbic oxidase in the parasitically increased respiration of wheat. *Arch. Bioch. & Biophy.*, 66: 474–485.
41. Kiarostami, K., R. Mohseni and A. Saboora, 2010. Biochemical changes of *Rosmarinus officinalis* under salt stress. *J. Stress Physiol. & Biochem.*, 6 (3): 114–122.
42. Langroudi, M.E., S. Sedaghathoor and S. Bidarigh, 2013. Effect of different salinity levels on the composition of rosemary (*Rosmarinus officinalis*) essential oils. *American-Eurasian J. Agric. & Environ. Sci.*, 13 (1): 68–71
43. Mancuso, S., E. Azzarello, S. Mugnai and X. Briand, 2006. Marine bioactive substances (IPA extract) improve ion fluxes and water stress tolerance in potted *Vitis vinifera* plants. *Adv. Hortic. Sci.*, 20: 156–161.
44. Maxwell, D.P. and D.F. Bateman, 1967. Changes in the activity of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. *Phytopath.*, 57: 132–136.
45. Mayer, A.M. and E. Harel, 1979. Polyphenol oxidases in plants. *Phytochem.*, 18: 193–215.
46. Megroth, S.P. and J. Cegarra, 1992. Chemical extractability of heavy metals during and after long–term applications of sewage sludge to soil. *J. Soil Sci.*, 43: 313–321.
47. Metzener, H., H. Rauand and H.Senger, 1965. Unter suchungen zur synchronisier barteit einzelner pigmentan angel mutanten von chlorella. *Planta*. 65: 186.
48. Minaiyan, M., A. Ghannadi, M. Afsharipour and P. Mahzouni, 2011. Effects of extract and essential oil of *Rosmarinus officinalis* L. on TNBS-induced colitis in rats. *Res. Pharma Sci.*, 6 (1): 13–21.
49. Moreno, S., T. Scheyer, C.S. Romano and A.A. Vojnov, 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research*, 40: 223–231.
50. Munns, R., 2003. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239–50.
51. Najafian, S., M. Khoshkhui and V. Tavallali, 2009. Effect of salicylic acid and salinity in rosemary (*Rosmarinus officinalis* L.): Investigation on changes in gas exchange, water relations, and membrane stabilization. *Adv. Environ. Biol.*, 3(3): 322–328.
52. Okoh, O.O., A.P. Sadimenko and A.J. Afolayan, 2011. Antioxidant activities of *Rosmarinus officinalis* L. essential oil obtained by hydro-distillation and solvent free microwave extraction. *Afr. J. Biotech.*, 10 (20): 4207–4211.
53. Omami, E.N., P.S. Hammes and P.J. Robbertse, 2006. Differences in salinity tolerance for growth and water-use efficiency in some amaranth (*Amaranthus* spp.) genotypes. *New Zeal. J. Crop Hort. Sci.*, 34: 11–22.
54. Osman, M.E.H, H. Metzner and F. Karin, 1982. Effect of nitrate on thylakoid reaction. I. Influence of photosynthetic electron transport. *Photosynthetica*, 16 (1): 7–12.

55. Parviz, A. and S. Satyawati, 2008. Salt stress and phtobiochemical responses of plants- A review. *Plant Soil Environ*., 54: 89–99.
56. Pise, N.M. and A.B. Sabale, 2010. Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella Foenum-Graecum* L. *J. Phytology*, 2: 50–56.
57. Prins, C.L, I.J.C. Vieira and S.P. Freitas 2010. Growth regulators and essential oil production. *Brazilian J. Plant. Physiol.*, 22: 91-102.
58. Ramezani, E., M.G. Sepanlou and H.A.N. Badi, 2011. The effect of salinity on the growth, morphology and physiology of *Echium amoenum* Fisch. and Mey. *Afr. J. Biotech.*, 10 (44): 8765–8773.
59. Rayorath, P., J.M. Narayanan, A. Farid, W. Khan, R. Palanisamy, S. Hankins, A.T. Critchley and J.B. Prithivira, 2008. Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynh. *J. Appl. Phycol*., 20: 423–429.
60. Said-Al Ahl, H.A.H. and A.A. Mahmoud, 2010. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. *Ozean J. Appl. Sci*., 3(1): 97–111.
61. Salem,O.M.A. 2007. Studies on some Egyption seaweed and their effect on the improvement of broad bean and wheat yield. Ph.D thesis. Egypt, Helwan University- Faculty of Science- Botany and Microbiology Department.
62. Sangwan, N.S., A.H.A. Farooqi, F. Shabih and R.S. Sangwan, 2001. Regulation of essential oil production in plants. *Plant Growth Regul*., 34: 03-21.
63. Shalata, A., P.M. Neumann, 2001. Exogenous ascorbic acid (vitamin-C) increases resistance to salt stress and reduces lipid peroxidation. *J. Exp. Bot*., 52: 2207–2211.
64. Snedecor, G.W. and W.G. Cochran, 1990. Statistical methods, 8th ed. Ames: Iowa State University Press.
65. Tabatabaie, J. and j. Nazari, 2007. Influence of nutrient concentrations and NaCl salinity on the growth, photosynthesis and essential oil content of peppermint and lemon verbena. *Turk. J. Agric. For*., 31: 245–253
66. Tarakhovskaya, E.R., Y.I. Maslov and M.F. Shishova, 2007. Phytohormones in Algae. *Russ. J. Plant Physiol*., 54: 163–170.
67. Tanou, G., A. Molassiotis and G. Diamantidis, 2009. Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. *Environ. Exp. Bot*., 65: 270–281.
68. Tounekti, T., I. Hernández, M. Müller, H. Khemira and S. Munné-Bosch, 2011. Kinetin applications alleviate salt stress and improve the antioxidant composition of leaf extracts in *Salvia officinalis*. *Plant Physiol. Biochem*., 49 (10): 1165–1176.
69. Turan, M. and C. Köse, 2004. Seaweed extracts improve copper uptake of grapevine. *Acta Agriculturae Scandinavica*: Section B, *Soil & Plant Sci*., 54: 213–220.
70. Vogel, A.J., 1975. A Text Book of Practical Organic Chemistry, 3rd ed., English Language Book Society and Longman Group Ltd., pp. 483–485.
71. Wang, X.Q., H. Ullah, A.M. Jones and S.M. Assmann, 2001. G protein regulation of ion channels and abscisic acid signalling in *Arabidopsis* guard cells. *Sci*., 292: 2070–2072.
72. Wasfy, W.S and E.S.Orrin, 1975. Identification of plant hormones from cotton ovules. *Plant Physiol*., 55: 550–554
73. Williams, V. and S. Twine, 1960. Flame photometeric method for sodium, potassium, and calcium. In: Modern Methods of plant Analysis (Paech, K. and Tracey, M.V. eds.), Vol. V. pp. 3–5 Springer-Veralg, Berlin.
74. Yamane, K., S. Kawabata and N. Fujishige, 1999. Changes in activities of superoxide dismutase, catalase and peroxidise during senescence of gladiolus florets. *J. Japan. Soc. Horticul. Sci*., 68: 798–802.
75. Yang, C.W., H.H. Xu, L.L.Wang, J. Liu, D.C. Shi and D.L. Wang, 2009. Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. *Photosynthetica*, 47: 79–86.
76. Yang, S.A., S.K.Jeon, E.J. Lee, C.H. Shim and S.I. Lee, 2010. Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. *Nat. Product Res*., 24:140–151.
77. Yanishlieva, N.V., E. Marinova and J. Pokorny, 2006. Natural antioxidants from herbs and spices. *Eur. J. Lipid Sci. & Tech.*, 108: 776–793.
78. Yildirim, E., H. Karlidag and M. Turan, 2009. Mitigation of stress in strawberry by foliar K, Ca and Mg nutrient supply. *Plant Soil Environ*., 55: 213–221.
79. Zaho, G.Q., B.L. Mab and C.Z. Ren, 2007. Growth, gas exchanges, chlorophyll fluorescence and ion content of naked oat in response to salinity. *Crop. Sci*., 47: 123–131.

9/23/2014