



Physiological changes, antioxidant activity, lipid peroxidation and yield characters of salt stressed barely plant in response to treatment with *Sargassum* extract

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Abstract

This experiment was suggested to evaluate and recognize the ameliorative role of seaweed extract of *Sargassum latifolium* (SAR) as regards some of the physiological and biochemical activities of barley (*Hordeum vulgare* L.) plants grown under salt stress conditions. Two levels of NaCl (75 & 150 mM) and three different concentrations (20, 30 & 40%) of *Sargassum* (SAR) as a water extract (wt./vol.) were applied. At the early stage of growth, significant increases were observed in the activities of superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) in shoots of salt stressed plants then they were decreased in the later stages of plant growth. Application of *Sargassum* extract, especially at 30%, markedly increased the activities of both SOD, POX and CAT in shoots of salt stressed plants. Treatment with *Sargassum* significantly mitigated the adverse effect of salinity as regards, lipid peroxidation (MDA), total antioxidant activity (TAA), proline and phenols in the tested plant. *Sargassum latifolium* caused great variations in the yield characters as well as in contents of soluble carbohydrates and soluble proteins. Furthermore, application of *Sargassum* extract resulted in increasing contents of N, P, K, Ca, Mg and Fe. While, the contents of Na were decreased in the yielded grains of salt stressed barley plants.

Keywords: *Hordeum vulgare*, *Sargassum latifolium*, Salt stress, Lipid peroxidation, Antioxidant enzymes, Physiological changes.

1. Introduction

Much of the injury on plants under abiotic stress is linked to oxidative damage at the cellular level leading to cell death (Mittler, 2002). During optimal growth conditions, balance between ROS formation and consumption is tightly controlled by the plant antioxidant defense system (Hameed *et al.*, 2011). Abiotic stresses, including high soil salinity, significantly reduce crop production worldwide. Decreasing of the acreage of arable land for crop production has become a severe threat to global food

security as more food will be needed to feed the growing population (Ren *et al.*, 2016).

High salt levels generate a two-component stress on plants: an osmotic stress caused by reducing water availability in soil and an ionic stress due to imbalance of solutes in the cytosol (Conde *et al.*, 2011).

Thirumaran *et al.* (2009) stated that recent researches proved that seaweed fertilizers are preferred not only

due to their nitrogen, phosphorus and potassium content, but also because of the presence of trace elements and metabolite similar to plant growth regulators. Recently, seaweed extracts as liquid fertilizers (SLF) has come in the market for the simple reason that they contain many growth promoting hormones like auxin, gibberellin, trace elements, vitamins, amino acids and micronutrients.

Strik *et al.* (2004) reported that the seaweed extracts are effective fertilizers in many crops. El-Barody *et al.* (2007) found that addition of different successive extracts of *Asparagopsis taxiformes* thallus powder to the soil, as a biofertilizer, gave a significant increase in the growth of *Vicia faba*. Lozano *et al.* (1999) stated that the application of an extract of algae to soil or foliage increased ash, protein and carbohydrate content of potatoes. Sabh and Shallan (2008) found that NPK in plants treated with *Sargassum* sp., reached four folds the negative control.

The using of seaweed products improves seed germination, seedling development, increase plant tolerance to environmental stresses (Zhang and Ervin, 2008), liquid extracts obtained from seaweeds have gained importance as foliar sprays and soil drench for many crops including various grasses, cereals, flowers and vegetable species. Also, they apply to stimulate seedling germination and rooting. At present one of the most promising applications of seaweeds is their use as plant biostimulants. The introductory soaking of *Triticum aestivum* seeds in 20% (0.2 mg SW ml⁻¹) extracts of *Sargassum wightii* for 24 h gave an 11% increase in seed germination, a 63% enhance in the number of lateral roots and 46% increase in shoots length in comparison to control (Kumar and Sahoo, 2011). Seaweed extracts are often regarded as soft or natural products that can influence crop growth and development (Norrie and Hiltz, 1999). A wide range of beneficial effects has been observed, including increasing crop yield, nutrient uptake, resistance to frost and stress conditions, longer shelf life of fruit, improved seed germination, and reduced incidence of fungal and insect attack and reduced the effect of salinity stress on membrane permeability (Wang *et al.*, 2005).

Barley (*Hordeum vulgare* L.) is a highly adaptable cereal grain and ranks 5th among all crops for dry matter production in the world. Therefore, barley is an important food source in many parts of the world (Newman and Newman, 2006). Seaweed extracts, which contain a complex mixture of polysaccharides, micronutrients, and plant growth hormones, have been

shown to have a stimulatory effect on plant growth and can enhance plant resistance to abiotic and biotic stresses (Khan *et al.*, 2009; Craigie, 2011; González *et al.*, 2013). Their modes of action are not well understood, but the application of new analytical and molecular tools is providing new insight into their effects on gene expression, biochemical pathways, and physiological processes (Nair *et al.*, 2012; Wally *et al.*, 2012; Laëtitia *et al.*, 2013). Many research studies have shown the beneficial effect of seaweeds extracts in stimulating growth of plants. They contain all major and minor plant nutrients, including bio-control properties; they also contain organic compounds such as auxins, gibberellins and precursors of ethylene and betaine that impact plant growth (Wu *et al.*, 1997; Washington *et al.*, 1999). Beneficial effects from the use of seaweed extracts as natural regulators have induced increased crop yield and plant vigor to withstand adverse environmental effects. It was well reported that seaweed extract contains nutrient of major and minor element, vital amino acid, essential vitamins and plant growth regulators which stimulate the growth and quality yield of crops. Application of seaweed liquid extract stimulate different aspects of plant like good health, development of root system, the absorption of minerals, enlargement of the shoot, increased rate of photosynthesis and crop yield (Sridhar and Rengasamy, 2010). Seaweed liquid extract has newly gained importance as a foliar spray for lots of crops, including various varieties of grasses, flowers, cereals, vegetables and spices (Pramanick *et al.*, 2014).

The present study aimed to evaluate and assess whether *Sargassum latifolium* extract can alleviate the negative effects of salt stress on barley plants through monitoring the different physiological and biochemical processes in plant as well as in the yielded grains of the different treatments.

2. Materials and Methods

2.1. Grains

Grains of barley "*Hordeum vulgare*" (Giza 134) were obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

2.2. Collection of Seaweed

Seaweed *Sargassum latifolium* (Turner) used in the present study was collected from Hurghada Red Sea coast in October 2015. Morphologically distinct thallus of algae were placed in polythene bags and

transported to the laboratory. *Sargassum latifolium* (Turner) was identified and authenticated by Dr. Ehab El-Belely (Lecturer of Applied Phycology, Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt) according to Agardh (1821). Samples were washed thoroughly using tap water to remove the salt.

2.3. Preparation of Seaweed Extract (SWE)

Seaweed was sold dried for seven days, then the materials were crushed and made as coarse powder, was added to distilled water. The extract was filtered through a double-layered cheesecloth. Seaweed liquid extract was prepared with different doses: 20%, 30% and 40% (wt./vol.).

2.4. Grains Treatment

Grains of barley were soaked in different concentration of seaweed extract (20%, 30% & 40%) for 2 hrs before planting, water soaked seeds served as control.

2.5. Methods of planting

A homogeneous grains were sown in pots (30 cm in diameter) containing 9.0 K.g. of clay soil. The pots were divided into twelve groups representing the following treatments, control, 20% *Sargassum* (SAR 20%), 30% *Sargassum* (SAR 30%), 40% *Sargassum* (SAR 40%), 75 mM NaCl, 75 mM NaCl + SAR 20%, 75 mM NaCl + SAR 30%, 75 mM NaCl + SAR 40%, 150 mM NaCl, 150 mM NaCl + SAR 20%, 150 mM NaCl + SAR 30% and 150 mM NaCl + SAR 40%. A homogenous 20 barley grains were sown in each pot. The first four groups irrigated with tap water, the second four groups irrigated with saline water (75 mM NaCl) and the later four groups irrigated with saline water (150 mM NaCl). After 15 days from sowing, the ten uniform seedlings were left in each pot. Plant samples were collected for analysis represented as stage I (37 days), stage II (58 days) and stage III (102 days). Yield components of the different treatments were recorded after 135 days from sowing.

2.6. Phytochemical analysis

2.6.1. Assay of enzyme activities

Antioxidant enzymes were extracted according to the method of Mukherjee and Choudhuri (1983). Superoxide dismutase (SOD) activity was assayed according to the method of Dhindsa *et al.* (1981).

Peroxidase (POX) activity was assayed using the method of Bergmeyer (1974). Catalase (CAT) activity was assayed according to the method of Chen *et al.* (2000).

2.6.2. Determination of lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Hernández and Almansa (2002). Fresh weight samples (500 mg) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 20 min at 4°C. One ml aliquot of the supernatant was mixed with 3 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90°C for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000 g for 5 min. The supernatant absorbance at 532 nm was then measured.

2.6.3. Estimation of Total Antioxidant Activity (TAA)

The total antioxidant activity (TAA) of the freeze-leaf extract was determined by adapting the method used by Govindarajan *et al.* (2003) and Subhasree *et al.* (2009) with slight modifications. In brief, the freeze-leaf extract was diluted with distilled water (60-220 mg/ml). The diluted extract (0.2 ml) was then mixed with 1.8 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a capped plastic tube. The tube was incubated in a water bath at 90°C for 90 minutes, then cooled down to room temperature. The absorbance of this solution was measured at 695 nm using a UV-VIS spectrophotometer against a blank. Ascorbic acid (5-60 mg/ml) was used as the standard. TAA is expressed as equivalent of ascorbic acid.

2.6.4. Determination of proline content

Shoots were hand-homogenized in 3% of sulfosalicylic acid at 4°C for 10 min. The supernatants were used for proline estimation according to Bates *et al.* (1973).

2.6.5. Determination of total phenolic compounds

Total phenolics were measured with the Folin-Ciocalteu reagent (Dai *et al.*, 1994). Twenty five ml of the extract was mixed with 110 ml Folin-Ciocalteu reagent, 200 ml of 20% sodium carbonate and 1.9 ml distilled water, and placed at 60°C for 30 min. Optical density was measured with a spectrophotometer at 750

nm. A standard curve was constructed with different concentrations of gallic acid.

2.6.6. Determination of soluble carbohydrate content

Soluble carbohydrate content was determined in aqueous solution with anthrone sulfuric acid reagent according to Umbriet *et al.* (1969) using glucose as a standard. To extract water-soluble carbohydrates, a known weight (0.1 g dry weight) of tissue powder was boiled in distilled water in a water bath for 1 h. The extracts were then cooled and filtrated through a centered glass funnel. A total of 0.5 ml of each extract was mixed with 4.5 ml of anthrone reagent (0.2 g anthrone, 8 ml absolute ethyl alcohol, 30 ml distilled water and 100 ml sulfuric acid). The mixture was then boiled in a water bath for 7 min. After cooling, the developed blue green color was measured at 620 nm against the blank.

2.6.7. Determination of soluble protein content

The soluble protein content of leaves was determined according to Lowry *et al.* (1951) using Bovine serum albumin as a standard. Samples (0.1 g dry weight) were extracted in 10 ml distilled water for 2 h at 60°C. The extracts were centrifuged and the supernatants were collected. One ml of each extract was added to 5 ml of alkaline reagent (50 ml of 2% Na₂CO₃ prepared in 0.1 N NaOH and 1 ml of 0.5% CuSO₄.5H₂O prepared in 1% sodium potassium tartarate) and mixed thoroughly, then allowed to stand for 10 min. A total of 0.5 ml of folin phenol reagent diluted 1:2 (v/v) was then added and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700 nm.

2.6.8. Determination of Macro and Micro Elements

Determination of Na, N, P, K, Ca, Mg and Fe concentrations were carried out in the yielded grains. Samples (0.5 g) were predigested with 5 ml of HNO₃, cooled, and digested to fumes of HC104 after adding 5 ml of a mixture of HNO₃ and HC104 (3 + 1). After digestion, about 40 ml of distilled demineralized water was added to each flask and the contents were brought to near boiling to ensure complete dissolution of sample except for silica. Samples were cooled, diluted to 50 ml, and filtered. 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 2Jobin Yvon.

2.7. Statistical analysis:

We calculate sample size according to Raosoft, and all statistical calculations were done using SPSS (statistica package for the social science version 20.00) statistical program at the 0.05 level of probability (Snedecor and Cochran, 1982). Quantitative data with parametric distribution were done using analysis of variance the One-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non-significant (NS) at the level of > 0.05, significant at the level of < 0.05, 0.01 and highly significant at the level of < 0.001.

3. Results and Discussion

3.1. Antioxidant enzymes

The production of toxic oxygen derivatives is increased as a result of all types of abiotic or biotic stresses. Plants possess efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions (Foyer *et al.*, 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. In the present study, the obtained results (Fig. 1) revealed that significant increases, at the first stage of plant growth, were observed in the activities of SOD, POX and CAT in the shoots of barley plants grown under the two applied levels of NaCl. However, it was found that the activities of the aforementioned assayed antioxidant enzymes were gradually decreased in the second and third stages of barley growth. The observed decreases were more obvious in plants grown at a higher salinity level (150 mM NaCl). In this regard, changes in the activities of the antioxidant enzymes under saline conditions have been reported by several investigators, Ibrahim (2016) found a marked promotion in the activity of the antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD) was recorded in the salinity stressed wheat plants. Also, increases in the activities of the antioxidant enzymes recorded by Fayez and Bazaid (2014) on barley, Sharaf (2010) on wheat shoot, Meloni *et al.* (2003) on cotton and Hernandez *et al.* (1999) on pea, but is unaffected as in the case of SOD in cucumber (Lechno *et al.*, 1997).

In this respect, the effects of salt stress on the antioxidant enzymes are very complex and depend on the treatment time, plant species and genotypes (Zhu *et al.*, 2004).

In the present study, application of *Sargassum* extract caused great variations in the activities of the measured antioxidant enzymes (Fig. 1). Under saline conditions, treatment with *Sargassum* extract markedly reduced the increases in the activities of SOD, POX and CAT (Fig. 1). The most potent effects were found to be when *Sargassum* applied at 30% and under the first level of salinity (75 mM NaCl). In this concern, Ibrahim (2016) found that algal presoaking of salinity stressed wheat grains demonstrated a highly significant enhancement in the percentage of seed germination and growth parameters especially with the extract of red alga *Laurencia obtusa*. A marked promotion in the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) was recorded in the salinity stressed plants. The occurrence of bioactive compounds in algal extracts such as flavonoids, proline, ascorbic acid, citric acid and plant hormones could potentially participate in the alleviation of salinity stress. Kasim *et al.* (2015) found that seaweed extract of *Sargassum* or *Ulva* antagonizes the oxidative damaging effects of abiotic stress not only directly through activating the antioxidative system, such as catalase and peroxidase. Also, Hemida *et al.* (2014), on wheat plants, abstracted that there was a significant enhancement in Superoxide dismutase (SOD) and Catalase (CAT) activities. These enzymatic activities increased considerably when plants were sprayed with 25% of *Ulva rigida* extract under salt stress Ibrahim *et al.* (2014) found that pre soaking of wheat grains in different concentrations of seaweed extract increased the activities of SOD and CAT with increasing the concentration of algal extract. Increase of the enzyme activities of salinity stressed seedlings presoaked in different concentrations of algal extract could be attributed to the presence of antioxidative compounds such as ascorbic acid (0.146 mg/g), proline (0.78 mg/g), betaine (0.146 mg/g), and glutathione (0.071 mg/g) in *U. lactuca* extract (Tuna *et al.*, 2013).

Gharib *et al.* (2014) in rosemary plants, studied the effect of foliar application of seaweed extract (SWE) *Sargassum* alone or with saline condition at 100 mM NaCl. *Sargassum* with saline condition significantly increased the activities of peroxidase (POD) and polyphenol oxidase (PPO) while, reduced the activities of catalase (CAT) and indole acetic acid oxidase (IAAO) compared to untreated controls. This may be due to different expression in the 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. B2 improved their salinity resistance by increasing the antioxidant enzymes (CAT, POD and glutathione reductase GR) (Azooz, 2009). Hegazi *et al.* (2014) stated that salt stress significantly increased the enzymatic activity of super oxide dismutase (SOD) and ascorbate peroxidase (APX) in eggplants. The increase in enzymatic activity may be due to that plants grown under salt stress have developed a complex antioxidant system to repair the damage initiated by salt stress. The primary components of this system include enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX). Many components of this antioxidant defense system can be found in different subcellular compartments (Vaidyanathan *et al.*, 2003). Antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Yu and Rengel, 1999), and this increase was also evident in the current work. The enhancing effect of seaweed application may be due to that it led to stimulation of the uptake of K and also can alleviate the inhibitory effect of Na toxicity and restore growth. In addition, seaweed application led to an enhancement of antioxidant enzymes (SOD and ASP) for protection against adverse environmental conditions (Schmidt, 2005). Chernane *et al.* (2015) suggested that seaweed extract SWE can improve salt stress tolerance and contributes to the protection of the wheat plant against oxidative deterioration, where they found a significant enhancement in Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX) activities when wheat plants were sprayed with 25% of *Ulva rigida* extract under salt stress.

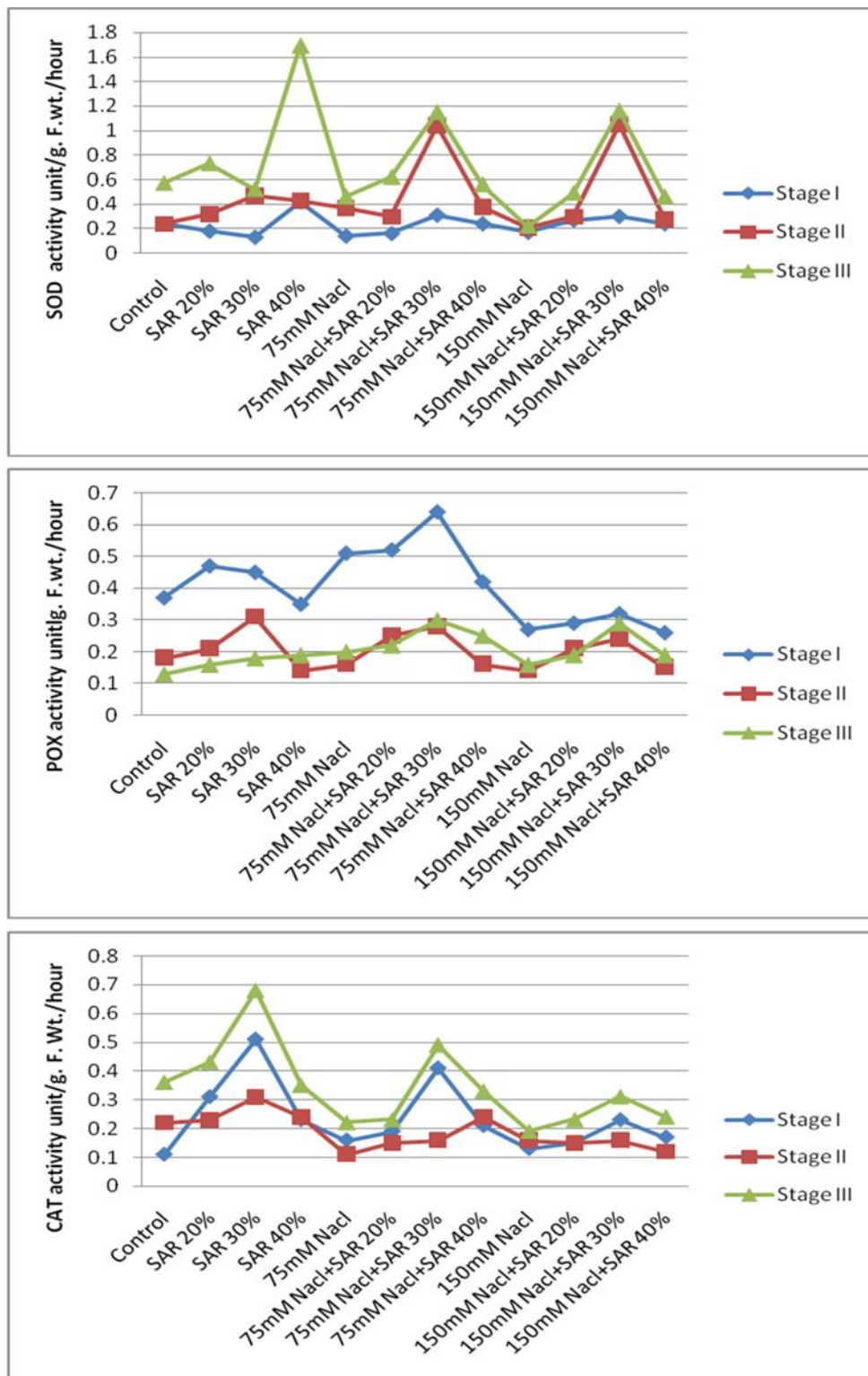


Figure (1): Superoxide dismutase (SOD), Peroxidase (POX) and Catalase (CAT) in response to salinity (75 & 150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant

3.2. Lipid peroxidation:

Lipid peroxidation represented by the determined contents of Malondi Aldehyde (MDA) as shown in figure (2). Significant increases were observed in the

contents of MDA in the shoots of barley plants. This was the case throughout the duration of the experiment and under the two applied levels of salinity (Fig. 2). In this regard, Fayez and Bazaid (2014) found that salt stress and water deficit caused increases in lipid

peroxidation (measured as MDA) in barley leaves. The level of MDA is often used as an indicator of oxidative damage due to enhanced generation of ROS (Mittler, 2002; Miller *et al.*, 2010). Kasim *et al.* (2015) showed that abiotic stress led to an increase in malondialdehyde (MDA) content (lipid peroxidation product) and membrane leakage in the abiotic stress treated wheat leaves during the vegetative stage, which might be attributed to peroxidation of membrane lipids that could be monitored as increased MDA content. Naureen and Naqvi (2010) measured H₂O₂ and MDA concentrations in salt stressed wheat plants that are oxidative stress indicators. H₂O₂ caused membrane damage fasten the Haber-Weiss reaction by production of hydroxyl radicals to increasing lipid peroxidation. Hameed *et al.*(2011) found that, under salinity; MDA content increased significantly during the experimental period in sesame cultivars as compared to control groups. The increase in membrane damage (lipid peroxidation) with increasing water stress levels has been also reported in wheat (Ezzat-Ollah *et al.*, 2007).

In the present study, application of *Sargassum* extract caused great variations in lipid peroxidation in shoots of barley plants (Fig. 2). Treatment with *Sargassum* extract caused significant reduction in lipid peroxidation in shoots of salt stressed barley plants. This was the case throughout the experimental period. These results are more obvious in plants grown at the first level of NaCl (75 mM) and with the application

of the *Sargassum* extract at 30%. Interestingly, Kasim *et al.* (2015) concluded that seaweed extract of *Sargassum* or *Ulva* pretreatment led to decreased levels of MDA contents in the abiotic stressed wheat plant. These results were in agreement with those of Mansori *et al.* (2014) who reported that spraying of bean plants with seaweed extract could alleviate the inhibitory effect of abiotic stress. The positive anti-stress effects of seaweed extract may be related to the cytokinin activity of seaweed extract as reported by Zhang and Ervin (2004). Cytokinins mitigate stress-induced free radicals by direct scavenging and by preventing reactive oxygen species (ROS) formation by inhibiting xanthine oxidation (Fike *et al.*, 2001). Auxins diminished lipid peroxidation through the stimulation of non-enzymatic (ascorbate, glutathione) and enzymatic (SOD, CAT, APX) antioxidants tightly regulating ROS homeostasis (Bajguz and Piotrowska-Niczyporuk, 2013). Kasim *et al.* (2016) state that the increased lipid production due to seaweed treatments in salt stressed radish plants may be more due to the stabilizing effect of the extract on membrane lipids and the enhanced activity of lipids synthesizing enzymes. Also, the increased lipid production as a result of the combination of the priming with seaweed extract and salt stress might be due to the decreased generation of ROS which cause lipid peroxidation and disrupts enzyme activity. Coinciding with these results, Rani and Usha (2013) reported that the seaweed treatment increased the accumulation of total lipid content in *Cassia angustifolia*.

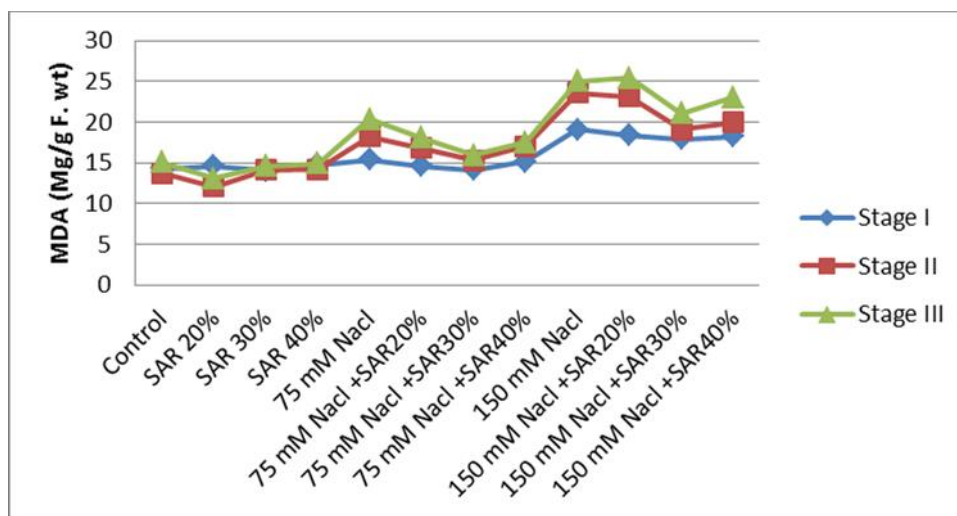


Figure (2): Lipid peroxidation (MDA) in shoot response to salinity (75 & 150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant

3.3. Total Antioxidant Activity:

In the present investigation, the total antioxidant activity (TAA) was significantly affected in response to salt stress conditions (Fig. 3). The magnitude of increasing in was found to be more obvious at the first stage in plants grown under the second salinity level then they were decreased in the later growth stages (stage II & III). At the first level of salinity (75 mM NaCl), TAA was increased throughout the first and second growth stages, then they well decreased at the third stage (Fig. 3). Fayez and Bazaid (2014) found that salt and water stresses significantly increased the TAA of barley leaves, which increased with increasing the stress. Khan *et al.* (2009) state that seaweed extracts have a multitude of effects on plant metabolism, and recent gene expression analyses have provided further insight into the pathways involved. Fan *et al.* (2013) observed increases in total soluble protein content, antioxidant capacity, phenolics, and flavonoid content in spinach treated with brown algal extracts. These effects were correlated with increases in transcript abundance of key enzymes involved in nitrogen metabolism (cytosolic glutamine synthetase),

antioxidative capacity (glutathionereductase), and glycine betaine synthesis (betaine aldehyde dehydrogenase and choline monoxygenase). Chalcone isomerase activity, a key enzyme in the biosynthesis of flavanone precursors and phenylpropanoid plant defense compounds, also increased following treatment with seaweed extract.

The results of the present study (Fig. 3) revealed that treatment with *Sargassum* extract caused great variations in TAA in shoots of barley plants. Treatment with *Sargassum* extract caused significant increases in TAA in shoots of barley plants. This was the case throughout the experimental period. These results are more obvious in plants grown at the first level of NaCl (75 mM) and with the application of the *Sargassum* extract at 30%. Abiotic stress is shown to exhibit high levels of antioxidant activity and polyphenol content. Thus, the effectiveness of seaweed extracts (*Ascophyllum nodosum*) in enhancing growth in abiotic stress conditions may be associated with the high antioxidant content in the extract which may act to reduce oxidative damage and increase abiotic stress tolerance in plants (Guinan *et al.*, 2013).

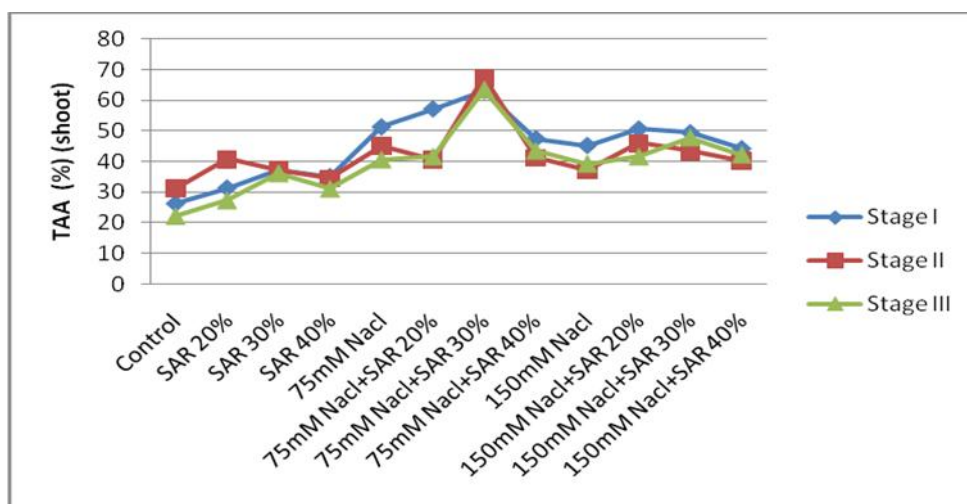


Figure (3): Total Antioxidant Activity (TAA) in response to salinity (75 &150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant

3.4. Proline

Results in figure (4) showed that the contents of proline in shoots of barley plants were increased significantly in response to salt stress conditions. The magnitude of increasing was found to be more obvious in plants grown under the second salinity level (150 mM NaCl). This was the case throughout the different stages of growth. Li *et al.*, investigators has demonstrated that protein and amino acids metabolism is strongly influenced by changes in the salinity concentrations (Li *et al.*, 2010). Proline accumulation in response to abiotic stresses is widely reported, and may play a role in plant stress adaptation within the cell (Fayez, 2000; Ashraf and Foolad, 2007; Shahbaz *et al.*, 2011; Sperdoui and Moustakas, 2012). In particular, different amino acids are accumulated at different rates under a salt-stressed condition; for example, proline, which forms a minor component of the pool of free amino acids in glycophytes, accumulates under stress conditions (Khedr *et al.*, 2003). Depending on these possible mechanisms by which proline protects plants against abiotic stress, our results suggested that the increase of proline in stressed barley plants may be at least partially responsible for the alleviated lipid peroxidation and photosynthetic pigments. Some biochemical indices of

water deficit injury, proline accumulation and decline in protein synthesis in plants have been reported (Irigoyen *et al.*, 1992). Different roles have been presented related to the proline gathering in plant tissues in environmental stress that could lead to adjust osmosis, integration of plasma membrane, energy source, carbon and nitrogen source, destroyer of free radicals of hydroxyl and creation of structural proteins (Dhanapackiam and Ilyas, 2010).

The obtained results (Fig. 4) revealed that treatment with *Sargassum* extract caused great variations in proline contents in shoots of barley plants. Treatment with *Sargassum* extract significantly increased proline contents in shoots of barley plants. This was the case throughout the experimental period. These results are more obvious in plants grown at the first level of NaCl (75 mM) and with the application of the *Sargassum* extract at 30 %. Nair *et al.* (2012) determined that the lipophilic components (LPC) of the seaweed extract increased proline content in Arabidopsis plants undergoing freezing stress and that this increase was associated with increased expression of proline synthesis genes (Saleh *et al.*, 2009). Proteins and proline accumulation is one of the most frequently reported modifications induced by salinity in plants and involved in stress resistance mechanisms.

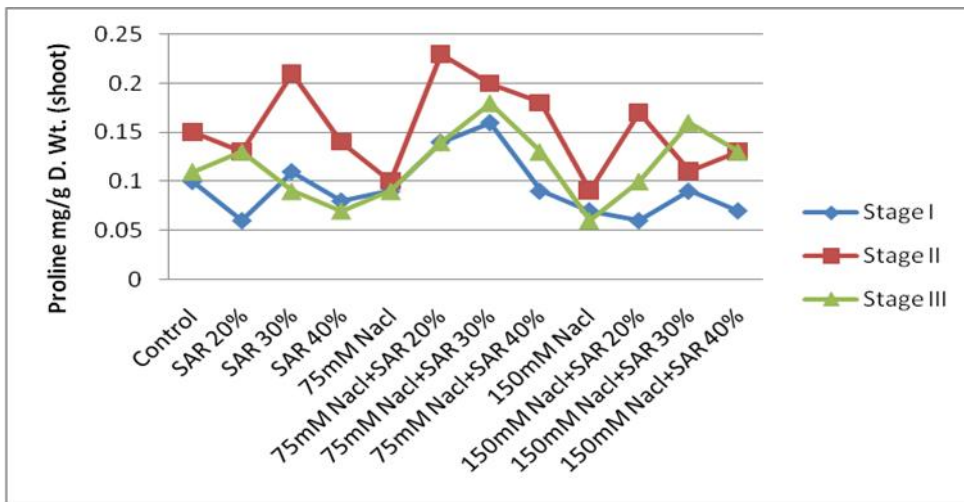


Figure (4): Free proline in response to salinity (75 &150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant

3.5. Phenols

Results in figure (5) showed a significant increase, at the first stage of plant growth, in phenols contents in shoots of barley plants grown under the two applied levels of salt stress. The contents of phenols were decreased in barley shoots at the later stages of growth, especially under the second salinity level (Fig. 5). Several studies illustrate the increases of phenols in plants grown under salt stress conditions, in cucumber (Tiwari *et al.*, 2009), potato (Daneshmand *et al.*, 2010) and wheat (Keles and Oncel, 2004; Wael *et al.*, 2014). Also, Petridis *et al.* (2012) showed that the total phenols increased in the salt-stressed olive trees. Kasimet *al.* (2016) indicate that NaCl stress significantly stimulated the accumulation of phenolic compounds in radish leaves and this was more pronounced by 200 mM NaCl.

In the present study, treatment with *Sargassum* extract markedly increased the total phenols in salt stressed plants (Fig. 5). The most potent effects were found to be when *Sargassum* applied at 30% and under the first level of salinity (75 MmNaCl). In this regard, Kasim *et al.* (2016) recorded an increase in the total phenolic compounds in the seaweed primed-stressed radish plants. Our results are also consistent with the results

of Lola-Luz *et al.* (2014), who reported that seaweed extract resulted in increasing of total phenolic compounds in broccoli. This increase could be due to osmotic stress or an increase in plant hormone activities. Thus, the induction of secondary metabolism is one of the defense mechanisms adapted by the plants to realize saline environment (Radi *et al.*, 2013). Halima *et al.* (2015) found that salt stress, increased significantly the phenolic compounds in wheat plants. In fact, total phenolic content (TPC) was two folds higher in plant submitted to salt stress compared to the control plants. Treatment with SWE at all concentration enhances significantly the Total phenolic content in barley plants cultivated under different growth conditions. Polyphenols represent a large family of plant secondary metabolites. The synthesis of these compounds is induced in response to biotic and abiotic stimuli such and may act as antioxidants to protect the plant against oxidative stress. Increase in total phenolic content by application of SWE in barley plants can be explained by enzyme activation. It was reported that treatment with SWE caused significantly enhanced activities of phenylalanine ammonia lyase (PAL) the most important enzyme responsible for the biosynthesis of polyphenols (André *et al.*, 2009).

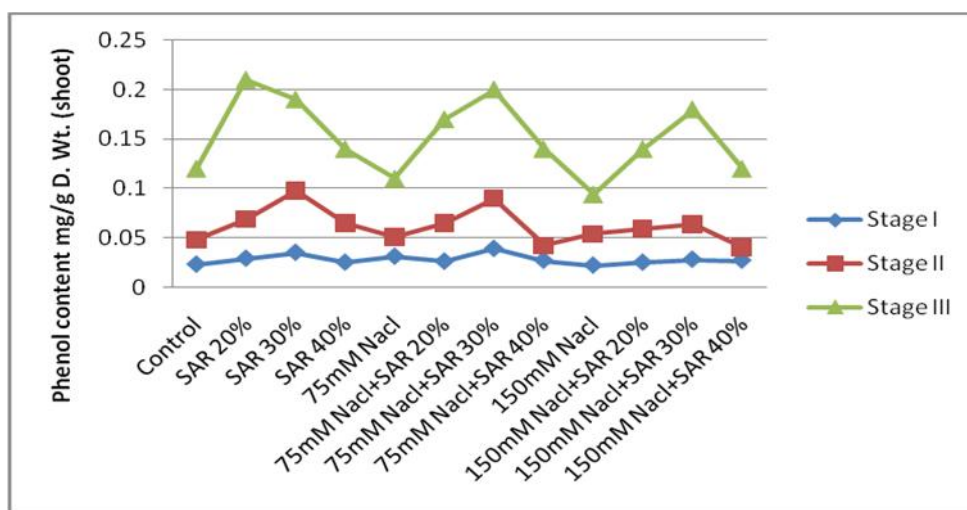


Figure (5): Total Phenol in response to salinity (75 &150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant

3.6. Yield and yield components

Figures (6, 7, 8) showed that NaCl significantly reduced number of both spikes per plant, number & weight of grains per plant and weight of 1000-grains. The magnitude of reduction was increased with increasing salinity level. According to Munns (2002) found that reduction in grain yield of stressed barley plants might be attributed to the rapid reduction in leaf photosynthetic assimilates from stem to grains is the main source as well as limiting factor for growth and development of grain and also salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves.

Results of the present investigation (Figs. 6, 7, 8) revealed that application of *Sargassum* 30% resulted in increasing the yield and its components of the tested plants than that of the other applied concentrations.

This was the case with plants grown in either absence or presence of NaCl. There are numerous reports of beneficial effects of seaweed extracts on crop yield reviewed by Stirk and van Staden (2006), Khan *et al.* (2009) and Craigie (2011). Recent studies have shown enhanced growth and yield in agricultural and horticultural crops such as apple (*Malus domestica*) (Basak, 2008), wheat (Kumar and Sahoo, 2011), tomato (Kumari *et al.*, 2011), okra (Zodape *et al.*, 2011), strawberry (Alam *et al.*, 2013), winter rapeseed (Laëtitia *et al.* 2013), spinach (Fan *et al.*, 2013), broccoli (Mattner *et al.*, 2013). The obtained results are in agreement with the work of Mercedes *et al.* (2006) on tomato. Seaweed extracts also act as biostimulants, enhancing seed germination and establishment, improving plant growth, yield, and fruit production, increasing resistance to biotic and abiotic stresses, and improving postharvest shelf life (Craigie, 2011; Mattner *et al.*, 2013).

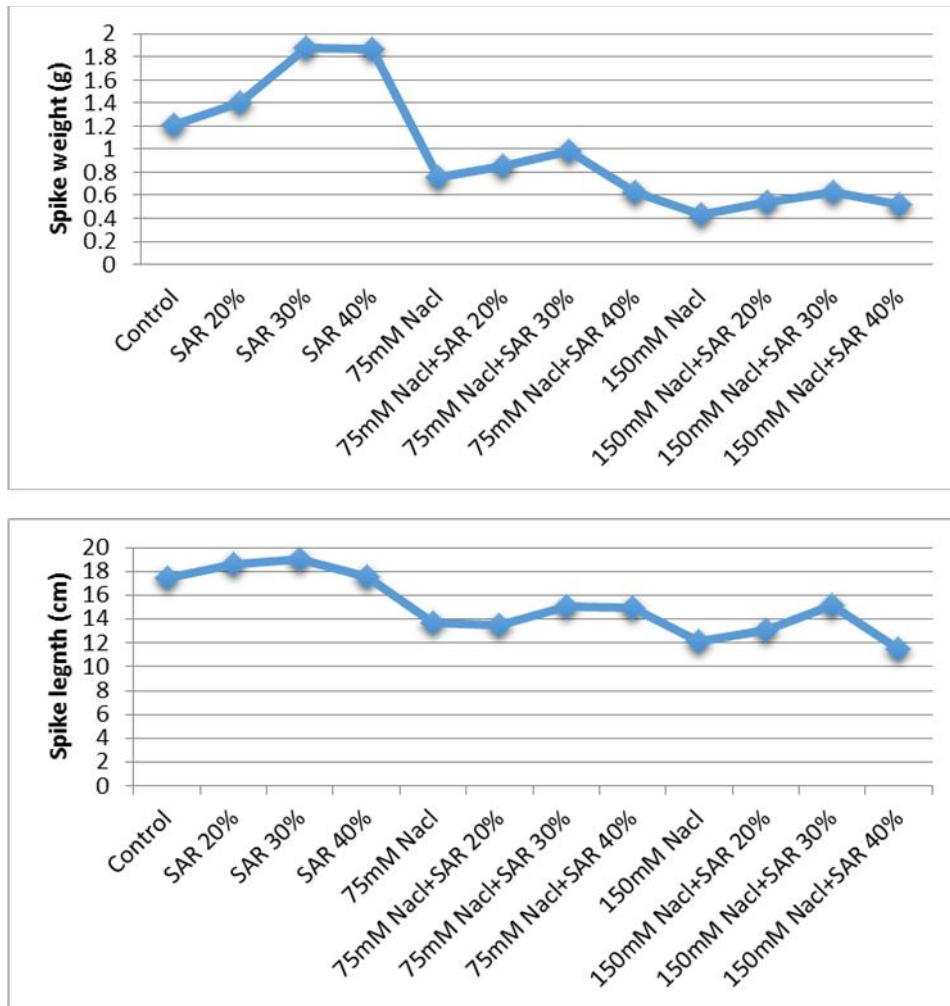


Figure (6): Spike weight (g/plant), Spike length (cm/plant) in response to salinity (75 &150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant

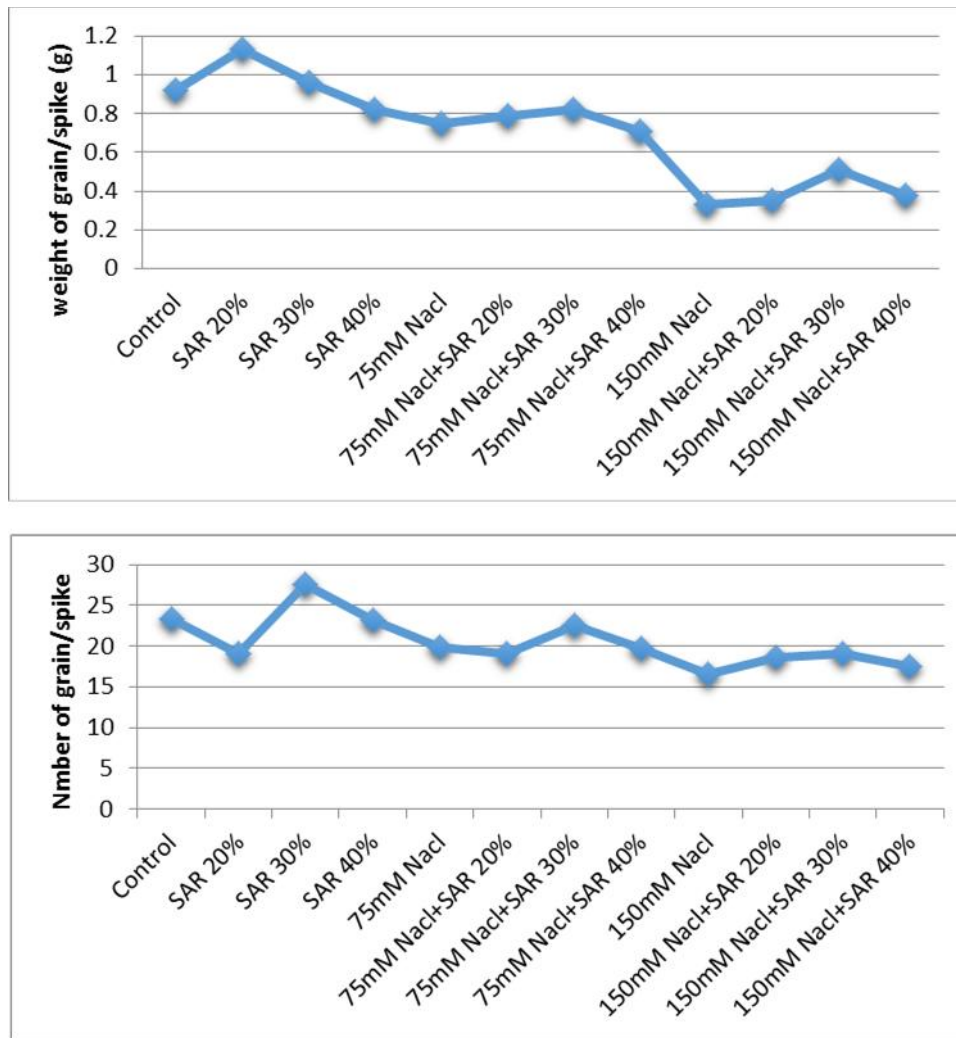


Figure (7): Weight of grain/spike (g/plant), number of grain/spike in response to salinity (75 & 150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant.

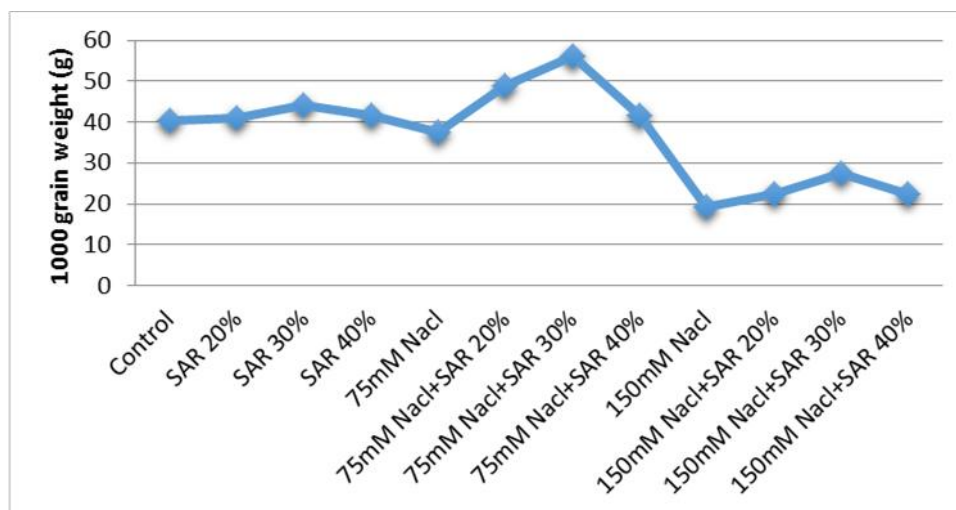


Figure (8): 1000 grain in response to salinity (75 & 150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant.

3.7. Soluble carbohydrates and soluble proteins

In the present investigation, contents of soluble carbohydrates as well as soluble protein in the yielded grains of barley plants were significantly decreased with increasing salinity level (Fig. 9). Similar results were observed by other investigators (Koyro and Eisa, 2008; Abeer, 2009; Sharaf, 2010). One approach for gaining a better understanding of the mechanisms by which plants can respond to salt stress is to study those proteins that are specifically accumulated after exposure of the plants to salinity (Parida *et al.*, 2004). Saleh *et al.* (2009) reported that proteins and proline accumulation is one of the most frequently reported modifications induced by salinity in plants and involved in stress resistance mechanisms. Fayez and Bazaid (2014) reported that salt and water deficit stresses caused a significant increase in the soluble carbohydrate content of barley leaves with increasing salt doses, the rate of increase in soluble carbohydrate content was increased due to indicating a role of soluble carbohydrate in the osmotic adjustment. The accumulation of sugars in plants under stress

conditions might be involved in the osmotic adjustment was reported (Pérez-López *et al.*, 2010).

On the other hand, the obtained results showed that *Sargassum* supplementary resulted, in most cases, in a significant increase as regards the contents of soluble carbohydrates and proteins in the yielded grains of salinized and non-salinized plants. These results are in agreement with other investigators (Lozano *et al.*, 1999; Safinaz and Ragaa, 2013). In this regard, Salah El Din *et al.* (2008) studied the effect of seaweed extracts of *Sargassum latifolium* and *Halimeda opuntia* as a liquid fertilizer on growth, yield and biochemical constituents of faba bean where noted that the tiller number was positively affected by the foliar spray in all concentrations and with all three studied algal species. While, the pods number was positively affected only at 0.6% concentration and *H. opuntia* species recorded better pod number than that of the two other studied species. Also, they found that seaweed extracts increased significantly the yields of seed index, total seed carbohydrate and protein increased by foliar spray at concentration 0.6% for all the three studied algal species.

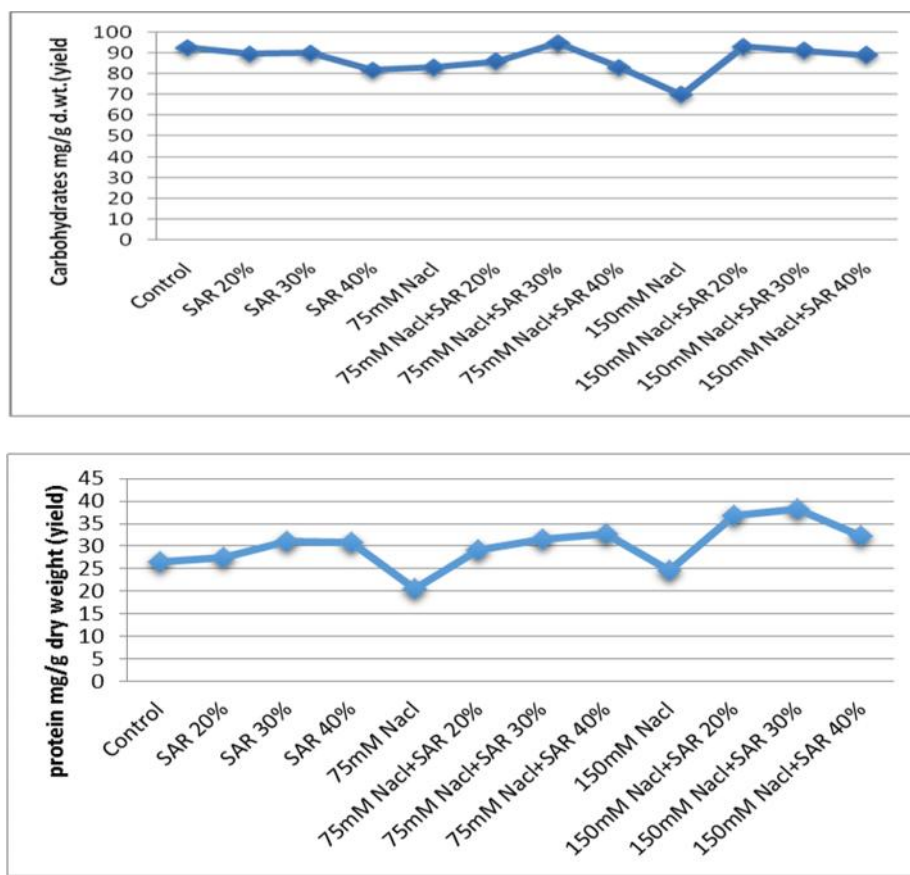


Figure (9): Total soluble carbohydrates, soluble protein (mg/g.d.wt./grains) in response to salinity (75 & 150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant.

3.8. Mineral contents:

Regarding the contents of different determined elements in the yielded barley grains of the different treatments, the obtained results (Fig. 10) revealed that salinity stress caused a significant increase in Na⁺ contents. The magnitude of increase was consistent with increasing salinity level. On the other hand, contents of N, P, K, Ca, Mg and Fe significantly decreased. The magnitude of reduction was increased with increasing salinity level. At the same time, the obtained results (Fig. 10) showed that application of *Sargassum* extract resulted in increasing contents of N, P, K, Ca, Mg and Fe while contents of Na were decreased. These results are more obvious in plants grown at the first level of NaCl (75 mM) and with the application of the *Sargassum* extract at 30 %. In this regard, Fayez and Bazaid (2014) reported that salinity stress caused a significant increase in Na content and a considerable decrease in K content, resulting in a significant increase in the Na/K ratio. The Na content was increased in barley leaves with increasing NaCl doses and water deficit. In contrast, the K content decreased with increasing NaCl doses. According to Blumwald *et al.* (2000), the decrease in K concentration due to NaCl may be attributed to a high external Na concentration. Wakeel *et al.* (2011) suggested that the Na toxicity affects plant growth,

increased Na/K ratio and thus displacement of K by Na in the plant cell affects the activity of plasma membrane (PM) H⁺-ATPase. Salah El Din *et al.* (2008) faba bean, showed that treatment with seaweed extracts of *Sargassum latifolium* and *Halimeda opuntia* as a liquid fertilizer improved the concentration level of NPK. Selvam and Sivakumar (2014) found that the leaf of 2% seaweed liquid fertilizer (SLF) treated *Arachis hypogea* L. has subjected to scanning electron microscopy with energy dispersive spectroscopic analysis, it revealed that the presence of ten elements in the following order: Ca>P>N>Na>K>Mg>Mn>S>Fe>Zn in treated and Ca>N>P>Na>Mg>Mn>K>Zn>S>Fe in control plant, reduced uptake of NaCl while increased K and Ca content in the leaves (Demir *et al.*, 2004). Stimulation the uptake of N, P, K, Mg, Ca, Zn, Fe and Cu by the plants that alleviate the inhibitory effect of Na toxicity and restored growth (Nelson and Van-Staden 1984). Seaweed extract application also stimulated mineral nutrient uptake in plants such as grape (Mancuso *et al.*, 2006), soybean (Rathore *et al.*, 2009), tomato (Zodape *et al.*, 2011), and winter rapeseed (Laëtitia *et al.*, 2013) with increased accumulation of both macro-(N, P, K, Ca, S) and micro-nutrients (Mg, Zn, Mn, Fe) reported by Mancuso *et al.* (2006), Rathore *et al.* (2009) and Zodape *et al.* (2011).

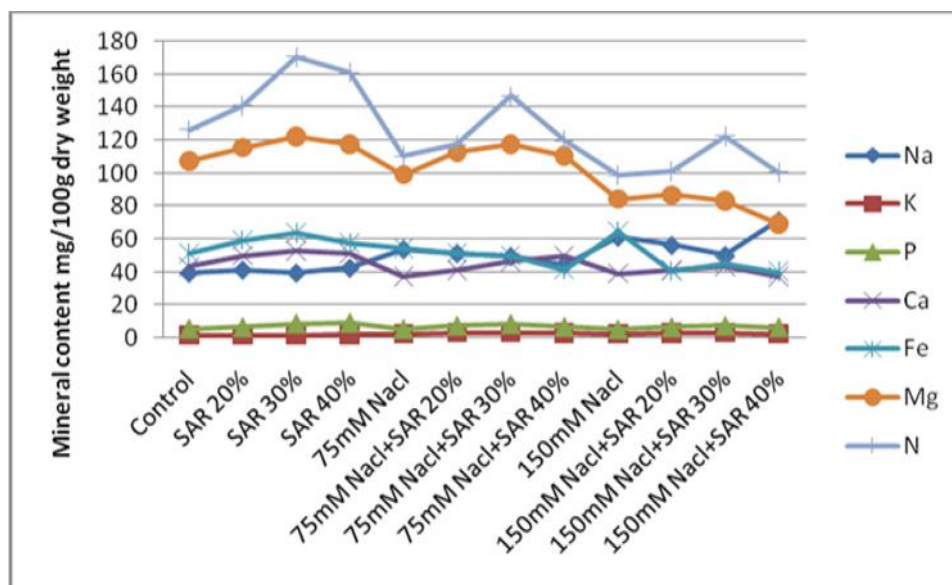


Figure (10): Mineral contents (mg/100g.d.wt./grains) in response to salinity (75 & 150 mM NaCl), *Sargassum* extract (SAR) (20%, 30%, 40%) and their interactions with barley plant.

4. Conclusion

From the outcome of the obtained results, it could be concluded that the application of the *Sargassum latifolium* greatly improving most of the growth characteristics of barley plants grown in either saline or non-saline conditions. This may be due to that, these treatments, participate in different metabolic processes. Also, seaweed extract of the *Sargassum latifolium* has a beneficial regulatory role in barley plants grown under salt stress conditions via antagonizes the oxidative damaging effects of salinity not only directly through activating the antioxidative system, such as catalase, peroxidase and ascorbate, but also through improving the yield characters and yield component including soluble proteins, soluble carbohydrates and minerals contents in the yielded grains. Nabti *et al.* (2016) recorded that the benefits of seaweeds application in agricultural field are numerous and diverse such as stimulation of seed germination, enhancement of health and growth of plants namely shoot and root elongation, improved water and nutrient uptake, frost and saline resistance, biocontrol and resistance toward phytopathogenic organisms, remediation of pollutants of contaminated soil and fertilization.

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