Protective Role of the Seaweed *Halimeda opuntia* Extract on Cadmium-Stressed *Eruca sativa* (Mill.)

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S EEDS of *E. sativa* were primed with aqueous extract of the chlorophyte *Halimeda opuntia* or with distilled water for 3 h and sown in clay sandy soil (2:1 w/w). Plants were irrigated with tap water for 14 days, then with 20 mM CdCl$_2$ solution or tap water for 21 days. Cadmium stress caused a significant reduction in water content, photosynthetic pigments, soluble sugars, soluble proteins, and caused oxidative damage, as characterized by increasing malondialdehyde content, proline and phenolic compounds. However, presoaking in *H. opuntia* extract enhanced the previous parameters in case of control plants. Additionally, the interactive combination of cadmium stress and algal extract showed a significant amendment of cadmium stress on water content, photosynthetic pigments, sugars and protein contents and relatively declined proline, phenolic compounds and MDA. SDS-PAGE of leaf proteins showed alternation in protein profile in treated plants represented in appearance and disappearance of specific bands.

Keywords: *Eruca sativa*, *Halimeda opuntia*, Cadmium, Photosynthetic Pigments, Proteins, PAGE, Malondialdehyde.

Cadmium (Cd) pollution is increasing day by day due to outflow of sewage sludge, industrial waste water or unfortunate use of phosphate fertilizers to agricultural fields that are progressively increasing the soil Cd level. Consequently, metal accumulator crop species take up and accumulate Cd in their tissues at high level, which not only poses threat to plant endurance, but also induces several toxic responses and raises the danger of food contamination (Irfan *et al*., 2014). Cd is thought to be the most harmful heavy metal because of its high solubility in water (Ali *et al.*, 2014). It is not necessary to plants, but it could be taken up easily by roots and competes with other micronutrients because of their resemblance in physiochemical properties. More than certain limit, it causes reduction in the yield and quality of crops. Cd can cause damage to plant biomembranes, production of ROS, hindrance of photosynthesis (Ali *et al.,* 2013), suppression of seed germination, root extension, and lowered modulation of some enzymes (Tian *et al.*, 2012). Exposure to Cd reduces photosynthetic rate throughout bringing about oxidative stress through promotion of malondialdehyde (MDA) content, and superoxide dismutase (SOD) and peroxidase (POD) activities leading to plant senescence (Ali *et al*., 2013).

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Seaweeds are the macroscopic nautical algae found fixed to the bottom in relatively shallow coastal water. They grow in the seashore, shallow and deep sea areas up to 180 meter depth and also in estuaries and puddles on the solid substrate such as rocks, dead corals and pebbles. Seaweed zone is one of the striking and wide-spread ecosphere in the superficial marine environment. Seaweeds are completely dissimilar to higher plants as they don't have true leaves, stems, roots, vascular system, and specialized sex organs (Thirumaran *et al.,* 2009). Seaweeds comprise mixture of trace elements and growth regulators required by plants. It was also reported that seaweed compost is rich in potassium but poor in nitrogen and phosphorus (Thirumaran *et al.,* 2009). Seaweed liquid fertilizers were used in raising agricultural yield, due to their higher content of phytohormones, trace elements, vitamins, amino acids and minor elements (Orduña-Rojas and Robledo, 2002). Carbohydrates and other organic molecules in seaweeds modify the soil structure and improve its moisture holding capacity (Thirumaran *et al.,* 2009).

Several studies have revealed the beneficial effect of seaweed extracts on plants including early seed germination, enhanced crop performance and productivity, and enhanced opposition to different stresses (Jayaraman *et al.,* 2011). Seaweeds have received increased interest as promising products for providing both novel biologically active substances and compounds necessary for human nourishment, with high potentially economical impact in food and pharmaceutical industry. *Halimeda* (Bryopsidales, Chlorophyta) is a genus of significant importance in coral reef areas including seagrass beds, contributing both organic production and significant amounts of calcareous sediment. *Halimeda* species are distinguished by a segmented thallus. From the holdfast, the alga grows in throbs, at each time producing new green segment. New segments grow from certain points on the distant edge of the mother segment (Verbruggen and Kooistra, 2004). This progressive growth of one segment on top of the former results in a chain appearance. At certain points along the thallus, these rows give lateral branches. The seaweed *Halimeda opuntia* is easily identified by its calcareous and segmental habit (Kooistra and Verbruggen, 2005). This macroalga is capable of trapping organic fractions within its thallus, internal re-mineralization of organic matter, creating nutrient-enriched concentrations of dissolved nutrients that are important for its growth (Lirman and Biber, 2000). However there are no any known reports about the effect of this macroalga extract on the agricultural traits of the crop plants.

Rocket (*Eruca sativa* Mill.) is a member of family *Brassicaceae* that is an important marginal crop grown on soil with reduced fertility and is preferred over other relative species for its tolerance and adjustability to adverse environments (Shinwari *et al.*, 2013). It is a foliage crop with increasing interest in recent years due to the spicy taste of its leaves. Recently, rocket is considered as a new prospective health promoting vegetable owing to the glucosinolates content (D'Antuono *et al.*, 2009). Therefore, this study was designed to determine the outcome of priming by presoaking in the aqueous extract of the calcareous macroalga *H. opuntia* on Cd- stressed *E. sativa* during the vegetative

stage through recording changes in growth, photosynthetic pigments, soluble sugars, soluble proteins, proline content, phenolic compounds, lipid peroxidation and protein pattern.

Materials and Methods

Preparation of algal extract

The green macroalga *Halimeda opuntia* was collected from the red sea coast, Hurghada, Egypt (27° 15′ N, 33° 48′ E), during autumn season (April, 2014). It was hand collected, washed with seawater to remove sand particles and shells, followed by fresh water. The washed materials were dried in shade at room temperature to constant weight and powdered with an electric mixer and sieved through 2.0 mm sieve. The powdered material was subjected to water extraction with continuous shaking (10g/L) for 24 hr. then filtered using Whatman No. 1 filter paper to obtain the algal extract and stored at 4°C for further analysis and treatments.

Experimental design

Seeds of rocket (*Eruca sativa* Mill.) were kindly provided by the Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt. Seeds were selected for apparent uniformity of size and shape and washed with distilled water, then surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 3 min. washed with tap water twice, then with distilled water. Sterilized seeds were soaked either in distilled water or in the extract of *H. opuntia* for 3 hr. After that, seeds were sown in plastic pots (35 cm diameter and 30 cm depth) containing 10 kg clay-sandy soil (2:1 w/w), irrigated with tap water and left to germinate. After 14 days, plants were divided into four groups. The first group was presoaked in distilled water and considered as control, and the second group was presoaked in *H. opuntia* extract. Both of the two groups received tap water till the end of the experiment (35 days) according to the field capacity. The third and fourth groups were presoaked in distilled water or *H. opuntia* extract, respectively and irrigated with 20 mM $CdCl_2$ solution according to the field capacity till the end of the experiment.

The plants were left to grow at the natural day/night conditions (16 h light/8 h dark) at 25/15°C±2 day/night and relative humidity of 65% at the greenhouse of Botany Department, Faculty of Science, Tanta university. Plants were irrigated whenever needed to the age of 35 days. Samples were collected for the determination of water content, photosynthetic pigments, MDA content, protein profiles by SDS-PAGE, total soluble carbohydrates, total soluble proteins, proline content, and phenolic compounds content.

Phytochemical analysis

Chlorophylls (Chl a and Chl b) and carotenoids contents were determined following the method of Metzner *et al.* (1965) and expressed as mg.g^{-1} f.wt. Total soluble carbohydrate content was determined by the phenol-sulfuric acid

method of Dubois *et al.* (1956) using glucose as a standard and expressed as mg.g^{-1} d.wt. Total soluble protein content was determined as described by Bradford (1976) using a calibration curve constructed by bovine serum albumin as a standard and calculated as mg.g^{-1}d.wt. The separation of the protein of leaves was carried out using SDS-PAGE as described by Laemmli (1970). Scanning of the separated protein bands was analyzed by the gel documentation system (GDS) using Gel Proanalyzer version 3 MEDIA CYBERNE TICE Imaging Exports software (Gel Doc. 2001 Bio Rad System).

Lipid peroxidation was measured by determining the amount of malondialdehyde (MDA), as a product of peroxidation of unsaturated fatty acids, as described by Heath and Paker (1968) and expressed as µmole.g^{-1} f.wt. Proline content was determined according to Bates *et al.* (1973) using a standard curve prepared by proline and expressed as mg.g^{-1} d.wt. Total phenolic compounds content was quantified as mg.g^{-1} d.wt following the method of Jindal and Singh (1975) using Folin-Ciocalteu's reagent and gallic acid as standard phenol. The obtained results were subjected to one way ANOVA analysis to determine the significance of treatment difference using Costate statistical software program for windows.

Results

The analysis results of *H. opuntia* aqueous extract for some primary and secondary metabolites, as well as some macronutrients is illustrated in Table (1). The results reflected that *H. opuntia* is a rich source for soluble sugars, phenolics, N, P, K and Ca. However, it has relatively low content of soluble proteins and flavonoids.

TABLE 1. Phytochemical analysis of *H. opuntia* aqueous extract.

Protein	0.246±0.061 mg/g d.wt
Soluble sugars	16.26±0.32 mg/g d.wt
Flavonoids	3.56±0.11 mg/g d.wt
Phenolics	18.9±1.1 mg/g d.wt
Potassium	65.5±1.9 µg/g d.wt
Nitrogen	66.1±2.3 µg/g d.wt
Phosphorus	547.4±12.9 µg/g d.wt
Calcium	214000±5.9 µg/g d.wt

The results of this study revealed that the water content of *E. sativa* leaves showed a significant reduction (8.5%) under cadmium treatment (20 mM CdCl$_2$) relative to the control. In the meantime, the present results (Fig. 1) indicated that priming of *E. sativa* with *H. opuntia* extract resulted in a slight increase (2%) in leaves water content compared with the control. Furthermore, the interactive combination of *H. opuntia* extract and cadmium treatments resulted in an evident increase in leaves water content (2%) compared with sole cadmium application.
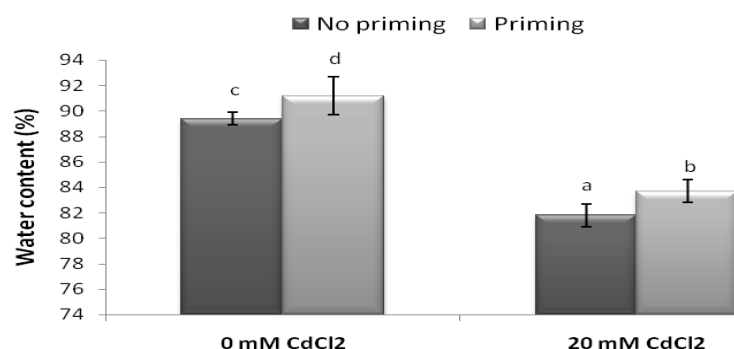

Fig. 1. Effect of cadmium stress (20 mM CdCl$_2$) and presoaking in *H. opuntia* extract on the leaf water content of 35-day-old *E. sativa*. columns with different letters are statistically significant.

A deep insight to the results of photosynthetic pigments showed that cadmium stress (20 mM CdCl$_2$) resulted in a highly significant decrease in Chl a, Chl b and carotenoids of *E. sativa* (Fig. 2).

The decrement was more pronounced with Chl a than Chl b and carotenoids. Meanwhile, the effect of priming with algal extract on the photosynthetic pigment content was reflected in a highly significant increase in Chl a, Chl b and carotenoids content in the plant leaves comparable to their content in the control treatment (unstressed-unprimed). Furthermore, presoaking of *E. sativa* seeds prior application of cadmium stress significantly mitigated the injurious effect of cadmium on photosynthetic pigments content comparable to cadmium single treatment, especially carotenoids.

Sugar analysis data demonstrated that total soluble carbohydrates content of *E. sativa* leaves significantly decreased with cadmium stress in regard to the control (Fig. 3). In the meantime, seeds presoaking in *H. opuntia* extract resulted in a highly significant enhancement in total soluble sugars content of their seedlings compared with control. Also, total soluble sugars content showed a significant increase in leaves of the presoaked seeds in algal extract that exposed to Cd stress compared to Cd individual treatment.
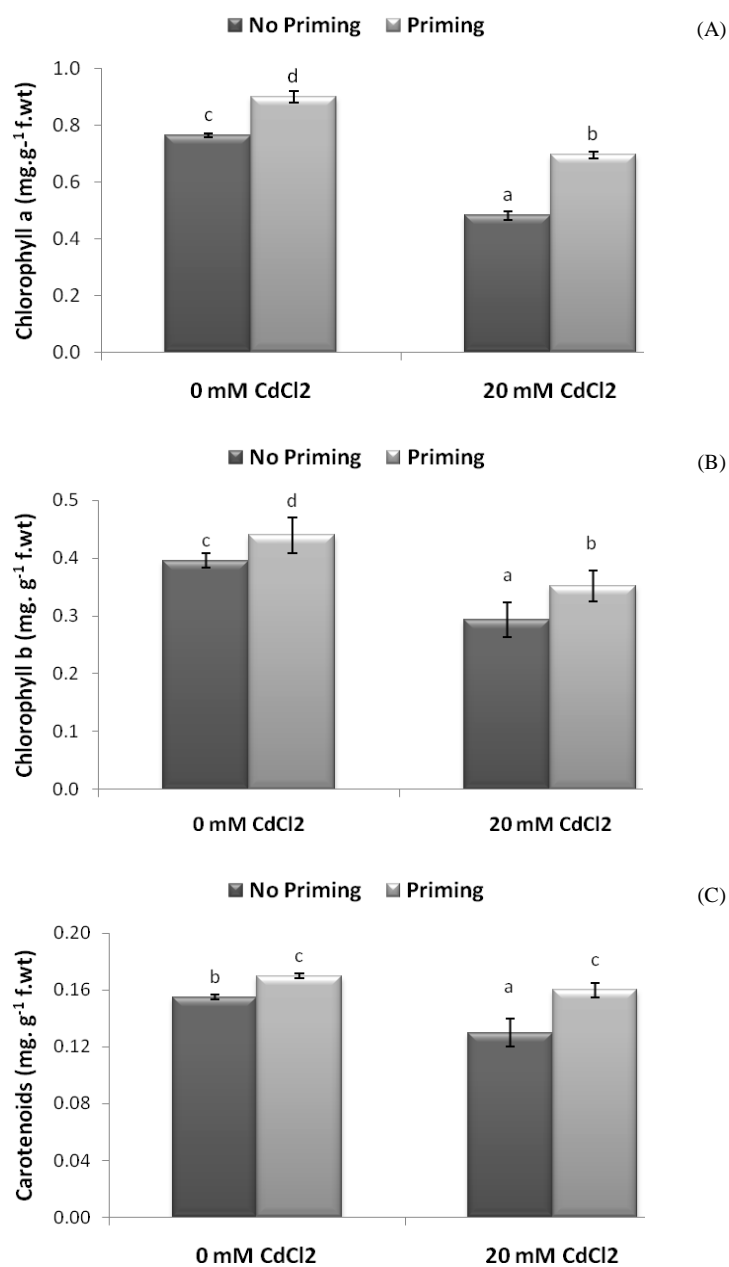
Fig. 2. Effect of cadmium stress (20 mM CdCl₂) and presoaking in *H. opuntia* extract on Chl a (A), Chl b (B) and carotenoids (C) of 35-day-old *E. sativa* leaves. columns with different letters are statistically significant.
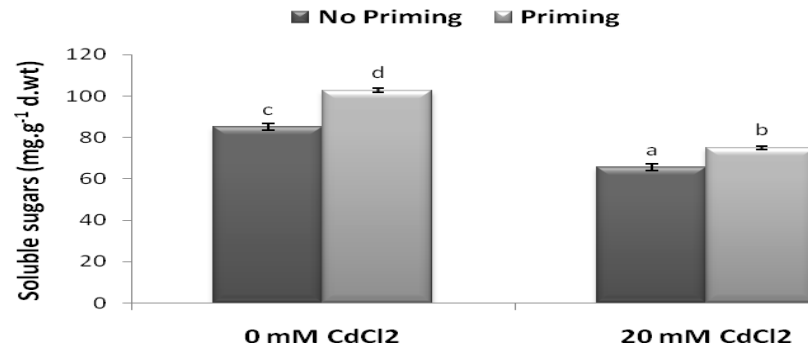
Fig. 3. Effect of cadmium stress (20 mM CdCl₂) and presoaking in *H. opuntia* extract on leaves total soluble sugars content of 35-day-old *E. sativa*. columns with different letters are statistically significant.

The results of total soluble protein content revealed that it has followed the same trend as total soluble carbohydrates, where total soluble protein content showed a significant reduction due to cadmium treatment (20 mM CdCl₂) relative to the control (Fig. 4). Additionally, priming of *E. sativa* in *H. opuntia* extract showed a pronounced increase of total soluble protein content compared with control. Moreover, presoaking of *E. sativa* in algal extract then exposing it to cadmium stress, showed a highly significant increase in their total soluble protein content compared with individual cadmium treatment.
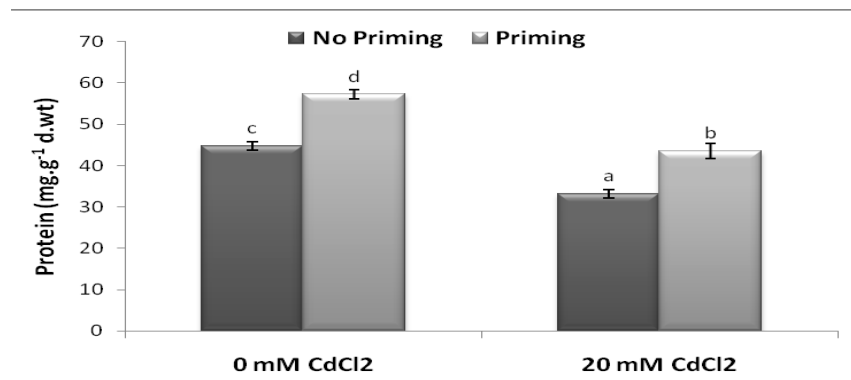

Fig. 4. Effect of cadmium stress (20 mM CdCl₂) and presoaking in *H. opuntia* extract on leaves total soluble protein content of 35-day-old *E. sativa*. columns with different letters are statistically significant.

The SDS -PAGE of the protein extract of leaves of 35-days-old *E. sativa* seedlings under the influence of cadmium stress (20 mM) and *H. opuntia* extract is illustrated in Fig. 5. SDS-PAGE proved that the protein was resolved into

distinct bands that spanned a broad range of apparent molecular masses from 11 kDa to 217 kDa. Some differences in the pattern of the protein bands between the control, cadmium treatment and *H. opuntia* algal extract were observed. Scanning the gel indicated the structural composition of the protein profile of the control was 13, 24, 26, 32, 36, 64, 76, 88,103, 111, 120, 147 and 217 kDa.
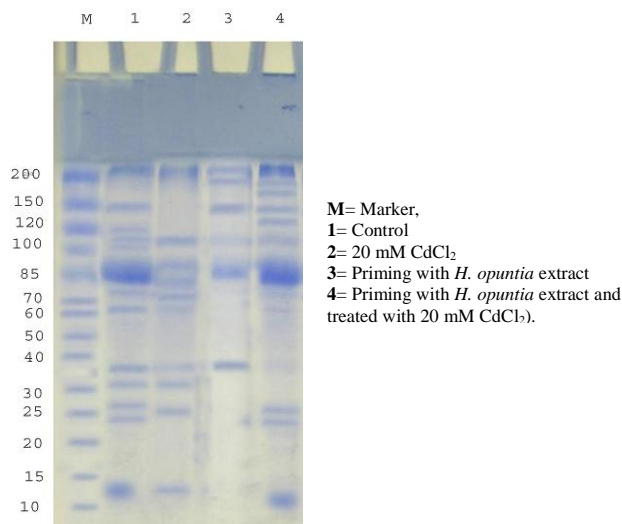

M= Marker,
1= Control
2= 20 mM $CdCl_2$
3= Priming with *H. opuntia* extract
4= Priming with *H. opuntia* extract and treated with 20 mM $CdCl_2$).

Fig. 5. Effect of cadmium stress (20 mM $CdCl_2$) and presoaking in *H. opuntia* extract on protein SDS-PAGE of 35-day-old *E. sativa* leaves.

SDS-PAGE showed the induction of eight polypeptide bands with molecular masses of 11, 23, 25, 72, 91, 128, 170 and 194 kDa. The polypeptide bands of molecular mass 25 and 72 kDa displayed only under cadmium stress treatment; whereas the polypeptide bands with molecular mass of 11, 23, 91,128 and 170 kDa were induced only after presoaking in algal extract then exposure later to Cd-stress. Additionally, the polypeptide band with molecular mass of 194 kDa was detected as a unique band to priming in *H. opuntia* extract as a single treatment, or primed-cadmium-stressed treatment. Moreover, the four polypeptides with molecular mass of 36, 88, 111 and 217 kDa were persistent in all treatments. On the other hand, SDS-PAGE showed the disappearance of the polypeptides with molecular masses of 24, 103 and 120 kDa, which were present in the control, from all treatments.

The present results (Fig. 6) indicated that cadmium stress caused a highly significant increase in malondialdehyde (MDA) content as a stress indicator in *Eruca sativa* leaves compared with the control (40.7% increase). On the other hand, seed priming in *H. opuntia* algal extract followed by 8.3% reduction in MDA content compared to control. Also, seed priming in *H. opuntia* extract prior sowing followed by cadmium application mediated highly significant

decline in MDA content of *E. sativa* seedlings relative to Cd-stressed seedlings (29.9% decline).
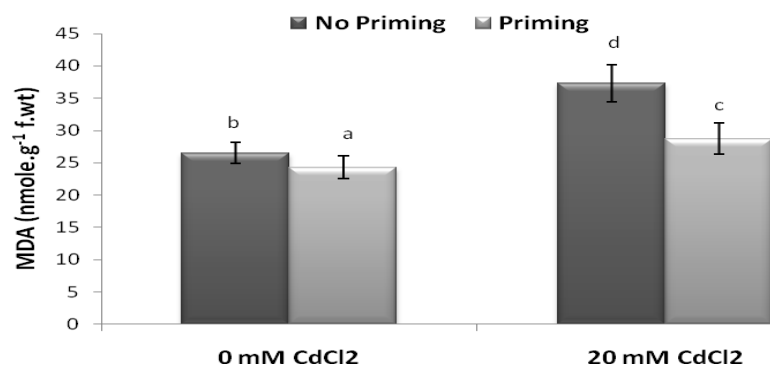

Fig. 6. Effect of cadmium stress (20 mM CdCl$_2$) and presoaking in *H. opuntia* extract on MDA content in leaves of 35-day-old *E. sativa*. columns with different letters are statistically significant.

Figure 7 exposes the variation in total phenolic compounds in *E. sativa* leaves under the experimental treatments. The data revealed that cadmium stress resulted in a highly significant increase in phenolics content compared with control. Nevertheless, seeds presoaking in *H. opuntia* extract yielded a slight decrease in the phenolics content compared with control. In the meantime, presoaking in *H. opuntia* extract prior exposure to Cd-stress produced a pronounced increase in phenolics content relative to individual Cd-treatment.
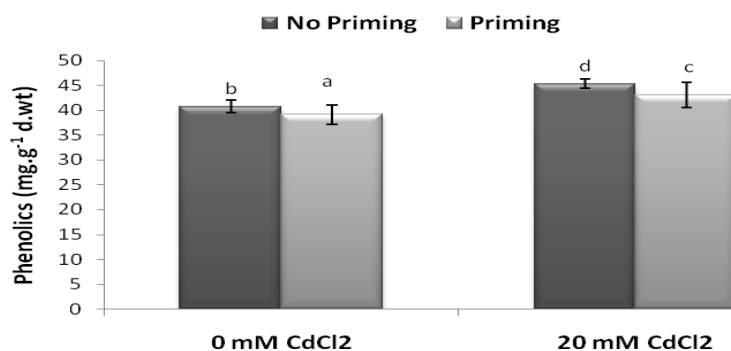

Fig. 7. Effect of cadmium stress (20 mM CdCl$_2$) and presoaking in *H. opuntia* extract on leaves total phenolic compounds content of 35-day-old *E. sativa*. Columns with different letters are statistically significant.

The data in Fig. 8 revealed that cadmium stress produced a highly significant increase in free proline content of *E. sativa* compared with control (more than two-fold increase). On the other hand, presoaking of the seeds in *H. opuntia*

extract resulted in 8.9% decline in free proline content comparable to that of the control. In the same time, presoaking of *E. sativa* seeds in *H. opuntia* extract exerted a slight decrease (3%) in free proline content in comparison with Cd-stressed treatment.
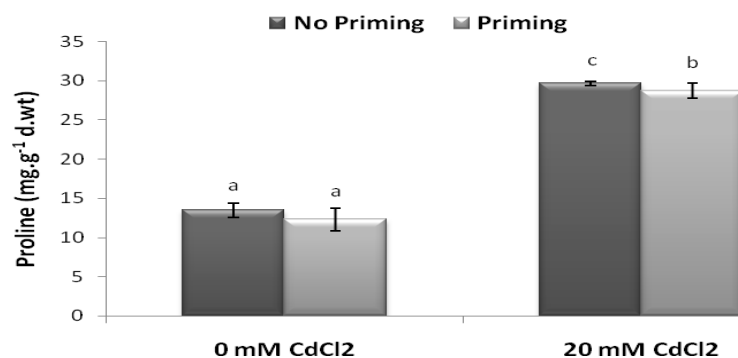

Fig. 8. Effect of cadmium stress (20 mM CdCl$_2$) and presoaking in *H. opuntia* extract on free proline content in leaves of 35-day-old *E. sativa*. columns with the same letter are non-significant, while columns with different letters are statistically significant.

Discussion

This study aimed at finding out how the presoaking of forage crop, *Eruca sativa*, seeds in the aqueous extract of the green macroalga *Halimeda opuntia* alleviates the deleterious Cd-induced changes in plant growth, photosynthesis, protein, free proline, lipid peroxidation and protein profile during the vegetative stage. As indicated by our results, cadmium has resulted in a significant decrease in the shoot water content. Similar reduction in water content was observed in sunflower (De Maria *et al.*, 2013) and wheat (Alayat *et al.*, 2014). The reduced water content following cadmium exposure is attributed to its toxic and osmotic effects on shoot growth represented in reduced turgor pressure and reduced cell wall elasticity as a consequence of disturbed water balance, stomatal conductance and water transport (De Maria *et al.*, 2013). Due to its high mobility and water solubility, Cd freely enters the roots through the cortical tissue and can reach the xylem via an apoplastic and/ or a symplastic pathway, forming complexes with organic acids or phytochelatins (Salt *et al.*, 1995). It can then reach the stele causing imbalance in root and shoot water relations.

At the same time, plants treated with presoaking in the aqueous extract of *H. opuntia* experienced significant improved water content compared to the control. Also, presoaking of seeds in the algal extract before its exposure to Cd stress reduced the negative effect of Cd toxicity on the shoot water content compared to individual Cd treatment. The augmenting effect of seaweeds on plant growth

may attributed to the trace amounts of macro- and micronutrients, amino acids, vitamins, cytokinins, auxins, abscisic acid-like compounds, and quaternary ammonium compounds (Stirk *et al.*, 2003). Also, the high content of the macronutrients (K, N, P and Ca) in *H. opuntia* extract might be responsible for improving the water status of the plant, hence increasing its growth rate under normal and stress conditions.

The study results showed remarkable reduction in total chlorophyll content (Chl a and Chl b) and carotenoids as a result of cadmium stress application. These results are consistent with the results of many authors who concluded that Cd toxicity promoted chlorophyll degradation, inhibition of chlorophyll biosynthesis (Hsu and Kao, 2003) and decreased PSII light harvesting complexes (LHCII) that is related to reaction centers resulting in reduced photosynthetic electron transport chain. Another convenient reason for the reduction of chlorophyll content is the ability of some heavy metals like Cd, Pb and Ni to substitute the central Mg atom in chlorophyll molecule.

The recorded increase in chlorophyll content (Chl a and Chl b) as a result of priming with *H. opuntia* aqueous extract in this study is in accordance with the earlier findings of many authors, who reported general increment in chlorophyll content after seaweed application in *Arachis hypogea* (Selvam and Sivakumar, 2014). The increase in photosynthetic pigments may be due to the presence of high betaines content in the seaweeds extract which cause slowing down the degradation of leaf chlorophyll rather than increasing its content (Blunden *et al.*, 1997). Also, it could be attributed to the high nitrogen content in the seaweed extract, which is a key component in chlorophyll molecule.

Carotenoids, as an outstanding antioxidant compounds, showed a significant decrease with $CdCl_2$ treatment in comparison with the control. Such diminish in carotenoids content was observed in *Brassica napus* (Ali *et al.*, 2013). The decline of carotenoids as a response to cadmium toxicity should be attributed to the imbalance between carotenoid biosynthesis and carotenoid breakdown or might be due to cadmium-induced inhibition of carotenoids biosynthesis.

In contrast, pre-soaking of *E. sativa* seeds in *H. opuntia* aqueous extract resulted in a slight increase in carotenoids content. Also, priming of the seeds with algal extract relatively raised the level of carotenoids than that of $CdCl_2$ treatment. Such increase in carotenoids under abiotic stress conditions may support the hypothesis that it can protect photosynthetic machinery against reactive oxygen species generate under stress conditions (Parviz and Satyawati, 2008).

The results of total soluble sugars revealed that the used cadmium concentration (20 mM) had resulted in a pronounced decline in soluble sugars content. Such result was previously obtained by John *et al.* (2008) in *Lemna polyrrhiza*. They attributed that cadmium stress provoked inhibition of

photosynthesis rate and stimulation of respiration, consequently decrease in total soluble sugar content. The deleterious impact of cadmium on carbon metabolism may be a repercussion of the toxic effect of ROS generated under cadmium stress, which inactivate enzymes involved in carbohydrate metabolism as Rubisco. Nevertheless, the stimulatory effect of *H. opuntia* extract on total soluble sugars may be due to enhancement of chlorophyll which is followed by promoting photosynthetic rate. Papenfus *et al*. (2013) concluded that organic acids and methionine in seaweeds extract could enhance nutrient absorption in plants which result in better carbohydrates accumulation. Another possible explanation for increased carbohydrates accumulation after seaweeds application is the high content of inorganic nutrients as potassium, calcium, nitrogen and phosphorus, which regulate the activity of sugar-synthesizing enzymes.

A significant reduction in total soluble protein content was recorded after cadmium treatment throughout this study. Balestrasse *et al*. (2003) showed that the reduction in protein content could be due to the decrease in protein biosynthesis or the increase in the activity of protein degrading enzymes. However, priming with *H. opuntia* extract resulted in an enhanced protein accumulation in *E. sativa* leaves and mitigated the detrimental effect of cadmium on protein content. Such results support the earlier findings of Selvam and Sivakumar (2014) on *Arachis hypogea*. The increase in protein content may be assigned to the high content of available macronutrients in *H. opuntia* extract (Ca, K, N, and P) that enhanced the activity of protein synthesizing enzymes.

Proteins that have a role in resisting stress are well defined as heat-shock proteins (HSPs). SDS-PAGE of *E. sativa* leaves revealed that cadmium and *H. opuntia* extract treatments resulted in significant variations in the protein pattern represented in appearance of the polypeptide bands with molecular mass of 25 and 72 kDa. These two bands related to HSP70 family and small HSP family (ranging from 10- 42 KDa), respectively. This result has been earlier demonstrated by Reddy and Prasad (1993) who reported the synthesis of 70 kDa phosphoprotein (HSP70 family) in Cd-treated maize plants. The HSP70 functions as molecular chaperones for the newly synthesized proteins to prevent their aggregations during their transfer to their final location (Su and Li, 2008). HSP70 participates also bind to protein precursor facilating its transfer through the membranes into the organelles such as chloroplast (Soll, 2002). The present results also showed the disappearance of specific bands under cadmium stress. The disappearance of some electrophoretic bands could be attributed to the loss of the genetic materials due fragmentation and laggards in chromosomes or due to the deletion of their corresponding genes (Kumar, 2012). Moreover, the induced disappearance of some polypeptides in response to cadmium stress may be due to the inhibitory effect of cadmium on protein biosynthetic pathway and the induced proteolysis due to oxidative damage caused by cadmium.

The disappearance of some polypeptides as a result of seed priming in *H. opuntia* extract might be due to the high content of this extract in flavonoids and phenols, which play an important role in the protection against oxidative stress

and dispense the role of such polypeptides. However, induction of new polypeptides by seeds priming in the algal extract could be attributed to the high calcium content in *H. opuntia* extract, which plays a key role in regulating the biosynthesis of some enzymes, beside its vital role as a signaling molecule.

Considering the deleterious effect of cadmium on membrane integrity, treatment of *E. sativa* with 20 mM $CdCl_2$ resulted in highly significant increase in the level of MDA in the plant leaves. MDA is a well-known index for oxidative stress severity and the elevation of MDA indirectly indicates an increase in ROS. The increase in MDA content in Cd-stressed plants was observed in a number of plant studies (Hashema *et al*., 2016). The accumulation of MDA, which is the decomposition product of polyunsaturated fatty acids of bio-membranes refer to the insufficiency of suitable antioxidant system to overcome the over-production of ROS generated under cadmium stress.

Nevertheless, priming of *E. sativa* with *H. opuntia* aqueous extract prior sowing resulted in reduction of MDA level compared by the control and Cd-stressed treatments. These results support the findings of Mansori *et al*. (2015), who reported that the extract of *Fucus spiralis* and *Ulva rigida* decreased the MDA level in leaves of water-stressed *Phaseolus vulgaris* plants. Fike *et al*. (2001) demonstrated that protective anti-stress effect of seaweeds may be associated with cytokinin activity, which mitigate stress-induced free radicals by direct scavenging of ROS and by inhibiting xanthine oxidation preventing ROS formation. Also, *H. opuntia* extract is rich in mineral nutrients as calcium, which has a specific role in controlling membrane structure and function by binding to phospholipids that stabilizes lipid bilayers and thus provides structural integrity to cellular membranes against oxidative damage (Hirschi, 2004).

Phenolic compounds as powerful antioxidants were accumulated in the leaves of *E. sativa* after exposure to cadmium stress. Dai *et al*. (2006) attributed that the increased activity of phenyl-alanine ammonia lyase (PAL) under cadmium stress enhance phenolic metabolism to produce antioxidant phenolics. Also, Wada *et al*. (2014) concluded that the exposure of plants to stress conditions up-regulates the activity of enzymes involved in biosynthesis of phenolic compounds resulting in efficient scavenging of toxic radicals and improved growth performance of plants.

The results of our study pointed out that combined effect of cadmium and *H. opuntia* aqueous extract resulted in pronounced accumulation of phenolic compounds. André *et al*. (2009) explained such increment in phenolic content by application of seaweed extract in wheat plans by enhanced activities of PAL, the most important enzyme responsible for biosynthesis of polyphenols. Also, the increase in phenolic content after seaweed application might be due to increased osmotic stress or an increase in plant hormone activities caused by seaweed application (Lola-Luz *et al*., 2014). Meanwhile, the individual treatment with *H. opuntia* extract resulted in the reduction of leaf phenolic content in *E. sativa*

seedling. This could be explained by the higher phenolic content in the algal extract (18.9 mg/g d.wt) which provides extra-protection for the plant by preventing the accumulation of ROS, as well as, feed-back inhibition of phenolic compounds biosynthesis in plant leaves.

Proline is a protective osmoticum and has a role in Redox homeostasis against ROS generated during heavy metals stress showed a significant increase in *E. sativa* leaves under cadmium stress. Showalter (1993) hypothesized that the increase of proline in Cd-treated plants was attributed to the active proteolysis of cell wall glycoproteins. Proline acts directly as an antioxidant to protect the cell from free radical damage and maintains a more reducing environment that is favorable for phytochelation synthesis and cadmium sequestration (Alayat *et al*., 2014). Also, accumulation of proline apparently protects the metabolic machinery, stabilizes the bio-membranes and macromolecules to prevent water loss and supported nutrient uptake to support growth performance in cadmium-stressed plants (Irfan *et al*., 2014). Finally, proline acts as a molecular chaperone capable of defending protein integrity and improving the activities of different enzymes (Szabados and Savoure, 2009).

The relative reduction in proline content as a result of pre-soaking in *H. opuntia* extract compared with unprimed treatments was observed in *Amaranthus tricolor* irrigated with *Ascophyllum nodosum* extract (Abdel Aziz *et al*., 2011). The decrement of endogenous proline level after seed priming in *H. opuntia* aqueous extract could be attributed to the presence of antioxidative compounds as flavonoids (3.56 mg.g^{-1} d.wt) and phenolic compounds (18.90 mg.g^{-1} d.wt) in addition to the high concentration of soluble sugars (16.26 mg.g^{-1} d.wt) which serves as osmoprotectants. All of these compounds exert protective effects against cadmium-induced toxicity.

Conclusion

Anthropological activities result in production of huge amounts of dangerous pollutants, which in turn get into the food chain through the agricultural soils and irrigation waters. Nowadays, it has become an urgent need to find eco-friendly solutions for heavy metals agricultural pollution. The most effective solution is represented in the use of naturally occurring resources like seaweeds. The results of this study concluded that using the aqueous extract of the macroalga *H. opuntia* could rectify the toxic effects of cadmium stress on the foliage crop *E. sativa*.

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الدور الوقائى لمستخلص العشب البحرى هالميدا اوبينتا على نبات الجرجير المجهد بالكادميوم

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تم نقع بذور نبات الجرجير فى مستخلص مائى لطحلب الهالميدا اوبينتا التابع لقسم الطحالب الخضراء، أو فى الماء المقطر لمدة ثلاثة ساعات. زرعت بذور الجرجير بعد فترة النقع فى أصص بلاستيكية تحتوى على تربة عبارة عن خليط من الطين و الرمل بنسبة ٢ : ١ بالوزن، ثم رويت الأصص بماء صنبور عادى لمدة ١٤ يوما بعد الزراعة. بعد هذه الفترة تم تقسيم الاصص السابقة الى مجموعتين، المجموعة الاولى رويت بمحلول من كلوريد الكادميوم (٢٠ ملى مولر)، أما المجموعة الثانية فقد رويت بماء الصنبور حتى عمر ٣٥ يوم. أظهرت النتائج ان الإجهاد بالكادميوم تسبب فى انخفاض ملحوظ في المحتوى المائى، أصباغ التمثيل الضوئى، والسكريات الذائبة، والبروتينات الذائبة. و من الاضرار الناجمة عن الإجهاد بالكادميوم ذيادة فى نسبة مؤشرات الضرر التأكسدي مثل أكسدة الدهون الغير مشبعة، محتوى البرولين، بالإضافة الى كمية المركبات الفينولية. ومع ذلك، فإن النقع المسبق لبذور النبات فى مستخلص طحلب الهالميدا اوبينتا نتج عنه تحسن واضح فى المؤشرات السابقة مقارنة بالمعاملة المنفردة لكلوريد الكادميوم. كما أظهرت النتائج أن النقع المسبق لبذور الجرجير فى مستخلص الهالميدا أوبينتا قبل التعرض للإجهاد بالكادميوم تحسنا كبيرا فى المحتوى المائى، أصباغ البناء الضوئي، محتوي السكريات والبروتينات، كما نتج عن هذة المعاملة إنخفاض نسبى فى محتوى البرولين و المركبات الفينولية و كذلك التأثير الضار للكادميوم على الدهون الموجودة فى الاغشية. وأظهرت نتائج التفريد الكهربى للبروتينات الموجودة فى أوراق النبات تباين في الأنماط البروتينية نتيجة للمعاملات السابقة متمثلة في ظهور واختفاء بعض السلاسل الببتيدية.