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Impact of Some Halophytic Extracts on the Antioxidant System of Salt-Stressed Safflower (*Carthamus tinctorius* L.)

El-Shourbagy, M. N. ; K. M. Saad-Allah ; S. A. Foda and Esraa O. Razzaky*
Botany Department, Faculty of Science, Tanta University, Tanta, 31527, Egypt
***Corresponding author e-mail: esraa.osama@science.tanta.edu.eg**

ABSTRACT

A pots experiment was conducted in the greenhouse of Botany Department, Faculty of Science, Tanta University, Gharbiya governorate during the winter season 2015-2016 to study the response of salt-stressed safflower to extracts of two types of naturally growing costal halophytes, *Z. album* and *H. strobilaceum*. Safflower seeds were presoaked either in distilled water or 1% halophytic extract for 24 hrs then sown in plastic pots containing 2:1 w/w clay sandy soil according to the Randomized Complete Block Design until reaching the preflowering stage. After the initiation of cotyledonary leaves, seedlings were divided into two main groups: the first was treated with 1% halophytic extracts as presoaking (pre-treatment) or foliar spray application (post-treatment), while the second was specific for control treatments. Each of which was then divided into two subgroups; unstressed and stressed. Results showed that salt stress imposed negative consequences on growth and metabolic properties of safflower, whereas the extracts have helped the plant to adjust and enhance its performance under salt stress through induction of osmoprotectants, redox homeostasis and membrane integrity.

Keywords: Safflower, salinity, halophytes, metabolism, antioxidants, osmoprotectants.

INTRODUCTION

More than 45 million hectares (ha) of irrigated land, accounted to 20% of total land, have been damaged by salt worldwide due to overdose salts in the soil (Munns and Tester, 2008). Therefore, salinity becomes a prominent obstacle for ensuring agricultural sustainability. The detrimental effects of salinity are categorized into osmotic stress, specific-ion toxicity and nutritional imbalance as primary or direct responses, and the generation of reactive oxygen species (ROS) as secondary or indirect ones (Ahmad *et al*., 2012). Salt-stressed plants are vulnerable to suppression of plant growth starting from germination until yield. On the other hand, salinity causes dynamic changes in metabolic consequences represented in membrane leakage, lipid peroxidation (LPO) and deterioration of nucleic acids as a result of ROS (Mittler, 2002), besides enzymatic and non-enzymatic inhibiting reactions and alteration in growth regulator levels (Mahajan and Tuteja, 2005).

A majority of plant species tend to create defense mechanisms under stress conditions which enable them to compensate water deficit and maintain osmotic adjustment by increasing the uptake of inorganic ions as well as synthesizing low molecular weight chemical chaperons known as compatible solutes (Valliyodan and Nguyen, 2006), in addition to synthesizing a cascade of anti-oxidants. Inorganic solutes are sequestered in the vacuoles, while organic ones are compartmentalized in the cytoplasm to counterbalance the low osmotic potential (OP) in the vacuole (Rontein *et al*., 2002).

Safflower (*Carthamus tinctorius* L.) is a biannual member of family Asteraceae grown mainly for the production of high quality edible oil seed rich in polyunsaturated fatty acids. It has two ectotypes; var. *typicus* with spines on leaves and floral heads and the spineless var. *intimis*. Spineless ectotype is lower in oil content than spiny one (Cosge *et al*., 2007). Beside its dietary uses, safflower possesses a pivotal medical importance due to active constituents originated from either flower, oil or seed extract and enable it to develop several systems vulnerable to severe diseases. Such systems including: Central Nervous System (Wei *et al*., 2005), Hematology (Li *et al*., 2009), Oncology (Arpornsuwan *et al*., 2012), Cardio-vascular respiratory systems (Zhou *et al*., 2014), Hepatology, Nephrology, Immunology (Zhou *et al*., 2014) , Rheumatology, Endocrinology (Gautam *et al*., 2014),.

Because salt tolerance remains both complicated and elusive in its genetic and molecular description, searching for another attempt involving the use of naturally occurring salt-thriving species (halophytes) may give some insights to osmotic adjustment of safflower (Jouyban, 2012). These halophytes are characterized by great adjustment to severe degrees of salinity due to acquisition of some anatomical and morphological adaptations that enable them to excrete salts (Wahid, 2003), besides exhibiting mechanisms allowing them to tolerate both hyperosmotic and hyperionic stresses (Munns and Tester, 2008). The present study aimed to valorizing the impact of aqueous extracts of aerial parts of the halophytes *Zygophyllum album* and *Halocnemum strobilaceum* on growth, photosynthetic pigments, primary metabolic profiles and antioxidant capacity of salt-stressed safflower plant at the pre-flowering stage.

MATERIALS AND METHODS

Preparation of halophytic extracts

Fresh vegetative materials of *Z. album* and *H. strobilaceum* were collected during November 2015 from their natural populations of the salt marshes on the Mediterranean Western Coast of Egypt around the Burullus Lake (31.59 N, 31.03 E). The collected materials were washed with tap water thrice to get rid of sand particles and debris, followed with distilled water then treated with liquid nitrogen before dissolving in aqueous ethanol (1:1 v/v). The obtained extract was filtrated through Whtman No 1 filter paper then evaporated under reduced pressure at 50^{o}C using rotary evaporator (Heidolph, Germany). The obtained dry matter was powdered then dissolved in distilled water for phytochemical assays. The phytochemical analysis of halophytic extracts is illustrated in Table 1.

Experimental design

Safflower (*Carthamus tinctorius* L. var. Typicus) cultivar Giza-1 seeds were kindly provided by the Desert Research Center (DRC), El-Matarya, Cairo, Egypt. The experiment was conducted from 21st November, 2015 until 6th February, 2016 in the sheltered botanical garden of Botany Department, Faculty of Science, Tanta University, Egypt. The average of maximum air temperature was 25.6 °C while the minimum air temperature was 11.8 °C with 51.4 relative humidity. Safflower seeds were disinfected by soaking in 1% Na-hypochlorite for 3 minutes then rinsed with distilled water. Seeds were divided into three groups; the first one soaked

in distilled water, the second soaked in 1% aqueous *Z. album* extract and the third soaked in 1% *H. strobilaceum* aqueous extract. The soaking period for each group was 24 hrs. Thereafter, they were sown in plastic pots of 45 cm diameter and 40 cm depth, filled with 20 kg clay-sandy soil (2:1 w/w). After emergence, seedlings were thinned to 10 uniform seedlings per pot. After that, the first group was divided into six treatments, control and salinity (150 mM NaCl), in addition to *H. strobilaceum*, *H. strobilaceum* + salinity, *Z. album* and *Z. album* + salinity subjected to spraying method. Extract spraying was made late in the afternoon at regular periods of two weeks starting three days from irrigation. However, both the second and third groups were divided into two subgroups (without and with salinity treatment). The total treatments of the experiment were 10, three replicates for each, with total number of 30 pots. Pots have received the corresponding irrigation solution, water or NaCl, up to 70% field capacity whenever needed until the rosette stage. Therefore, once the soil was irrigated by salt, it was leached next by tap water in order to adjust the soil salinity to the desired level of 150 mM (12.87 dSm-1).

Table 1. Phytochemical analysis of *Halocnemum strobilaceum* and *Zygophyllum album*

Constituents	*H. strobilaceum*	*Z. Album*
Secondary metabolites (mg/g d.wt)		
Phenolics	45.7	81.6
Flavonoids	1.5	1.8
Alkaloids	0.024	0.028
Tannins	2.0	5.5
Osmolytes (mg/g d.wt)		
Total free amino acids (AA)	44.8	146.6
Proline (Pro)	10.0	95.1
Total soluble sugars	2.0	3.4
Total soluble proteins	4.2	7.1
Antioxidant capacity		
Phosphomolybdate assay	7.8	7.3
Reducing power	239.9	273.2
Glycine betaine (GB)	33.0	9.2
Minerals (mg/g d.wt)		
N	0.09	0.71
P	0.18	0.18
K	4.8	2.4
Ca	17.1	3.5
Mg	5.0	1.1
Cu	0.00	0.11
Zn	0.08	0.12
Mn	0.03	0.02
Fe	0.43	0.62

Plant analysis

Determination of oxidative markers

H2O2 content was determined in leaf homogenates using the method given by Velikova *et al.* (2000) and the amount of H2O2 was calculated using the extinction coefficient (0.28 µM–1 cm–1) and expressed as nmol g–1f.wt.

Electrolyte leakage through leaf membranes was measured according to Sairam *et al.* (2005) using EC meter and expressed as µmohs.cm-1.

Estimation of antioxidant enzymes

Safflower fresh leaves were homogenized in a pre-chilled pestle and mortar with 100 mM cold potassium phosphate buffer (pH 6.8) and the obtained supernatant was used for enzyme assay.

Peroxidase (POD) [EC: 1.11.1.7] was assayed by following the method of Egley *et al.* (1983), using guaiacol and H2O2. The increase in absorbance was recorded spectrophotometrically and the specific activity was expressed as µM/ g.f.wt. min-1.

Glutathione S-transferase (GST) [EC: 2.5.1.18] activity was estimated through the formation of the adduct, due to conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) according to the method of Habig *et al.* (1974) and the activity was expressed as nmol/min/mg protein.

Assessment of non-enzymatic antioxidants

Carotenoids (Car) were determined according to the method of Metzner *et al.* (1965); 0.1 g of fresh leaves were homogenized in 85% cold acetone and by using spectrophotometer (JENWAY 6315 UV–Vis Spectro-photometer, Japan) and expressed as mg/g f.wt.

Proline (Pro) was estimated ccording to Bates (1973) where dried tissues were homogenized in 3% aqueous sulfosalicylic acid (w/v) and the supernatant was allowed to react with ninhydrin reagent and acetic acid at 100 °C. The chromophore was extracted with toluene then measured at 520 nm and Pro content was calculated as mg g-1 d.wt. using a prepared calibration curve.

Ascorbic acid (AsA), was estimated according to method of Oser (1965) and the content of AsA content was calculated as mg g-1 d.wt using a prepared calibration curve.

GSH content was determined by the recycling method outlined by Anderson (1985) due to the reaction of tissue homogenate with 5,5' -dithio-bis 2-nitrobenzoic acid (DTNB) and the content was calculated from the standard graph plotted by GSH and expressed as µmol/ml.

Total phenolic content (TPC) was estimated quantitatively using the method described by Jindal and Singh (1975). Ethanolic extract of powdered leaf tissues or standard gallic acid was mixed with Folin reagent and Na2CO3 and the total content of phenolics was expressed as mg g-1 d.wt.

Aluminum chloride colorimetric technique was used for flavonoids estimation according to Chang *et al.* (2002). Ethanolic extract was mixed with 95% ethanol, 10% aluminum chloride and 1 M potassium acetate. Quercetin was used as standard flavonoid and flavonoid content was expressed as mg/g d.wt.

Data confirmation and statistical analysis

The experimental design was Completely Randomized Blocks (CRB) with 3 replicates. The data were analyzed statistically using the two ways analysis of variance (ANOVA) to determine the degree of significance (P) and least significance difference (LSD) at 0.05 level according to Steel and Torrie. (1980).

RESULTS

Hydrogen peroxide (H_2O_2) content and membrane stability index (MSI)

Exposure of safflower to salt stress caused an elevation of H2O2 level (36.8%) as compared to control. However, H2O2 content decreased in both stressed and unstressed plants by the addition of halophytic extracts as compared to their control (Table 2).

Similarly, membrane leakage increased in safflower leaves due to salt stress application (76.2%) as compared to control. In contrast, application of halophytic extracts, either in presoaking or foliar spray mode, has resulted in a remarkable decline in membrane leakage of unstressed ones, specially foliar spray of Z. album extract which lowered electrolytes leakage by 47.5%. However, the effect was excessive in salt-stressed plants treated by

soaking as compared to those with NaCl alone. In contrast, membrane leakage in salt-stressed plants treated with sprayed *Z. album* extract was recovered; the value was less than those of NaCl by 49%. In general, addition of halophytic extracts showed an ameliorating effect on the unstressed plants (Table 2).

Table 2. Effect of two halophytic extracts on hydrogen peroxide content (nmol/ g f.wt.) and membrane stability index (%) of salt-stressed safflower.

Mode of application	Treatments	Hydrogen peroxide		Membrane stability index	
		Unstressed	Stressed	Unstressed	Stressed
Spray	Control	53.2	72.8	22.3	39.3
	Halo	34.5	47.6	15.7	38.3
	Zygo	34.5	28.9	11.7	20.0
Soak	Halo	39.2	22.4	13.0	45.3
	Zygo	14.0	28.9	12.7	45.3

Halophytic extract	Hydrogen peroxide						Membrane stability index					
	Halo			Zygo			Halo			Zygo		
	F	P	LSD	F	P	LSD	F	P	LSD	F	P	LSD
Effect of salinity	5.6	*	4.9	18.8	**	4.8	524.2	**	2.3	540.6	**	1.8
Effect of application	71.7	**	6.0	126.4	**	5.9	4.5	*	2.8	129.1	**	2.2
Interaction	24.9	**		12.1	**		18.2	**		73.3	**	

*** = Significant, 0.01≤P ≤ 0.05 ** = Highly significant, P ≤ 0.01**

Antioxidant enzymes (peroxidase and glutathione S-transferse)

Salt stress resulted in an appreciable inhibition in peroxidase (POD) activity (87% decline) as compared to control (Table 3). A slight increment in POD activity was observed in plants treated with presoaking in *Z. album* extract, whereas a remarkable decrease was observed in plants presoaked in *H. strobilaceum* extract. Spray application of *Z. album* extract has resulted in a slight decline in POD activity, but no effect was observed with *H. strobilaceum* extract of spray application on POD activity as compared to control. Halophytic extracts exerted variable responses in POD activity in salt stressed plants, where foliar spray of Z. album and presoaking in *H. strobilaceum* extracts caused a significant increase in POD activity of salt-stressed plants. However, foliar spray with *H. strobilaceum* extract has slightly lowered POD activity.

In contrast to POD, salinization has resulted in an enhancement of the activity of glutathione S-transferase (GST) by 50% as compared to control. Application of halophytic extracts to unstressed plants, except presoaking in Z. album extract, has led to a clear decline in GST activity. However, addition of halophytic extracts to salt-stressed plants has inhibited GST activity as compared to NaCl; the effect was remarkable in plants sprayed with *Z. album* extract (Table 3).

Table 3. Effect of two halophytic extracts on leaf peroxidase (µM/g f.wt. min^{-1}) and glutathione S-transferase (nmol/min/mg protein) activities in salt-stressed safflower.

Mode of application	Treatments	Peroxidase		Glutathione S-transferase	
		Unstressed	Stressed	Unstressed	Stressed
Spray	Control	0.23	0.03	0.18	0.27
	Halo	0.23	0.02	0.17	0.21
	Zygo	0.19	0.23	0.05	0.13
Soak	Halo	0.06	0.09	0.13	0.25
	Zygo	0.26	0.03	0.20	0.17

Halophytic extract	Peroxidase						Glutathione S-transferase					
	Halo			Zygo			Halo			Zygo		
	F	P	LSD	F	P	LSD	F	P	LSD	F	P	LSD
Effect of salinity	789.0	**	0.01	219.0	**	0.02	43.2	**	0.03	11.8	**	0.03
Effect of application	73.7	**	0.01	30.2	**	0.03	2.9	NS		26.1	**	0.04
Interaction	311.4	**		93.6	**		4.4	*		5.8	*	

NS= not significant, P > 0.05

Non-enzymatic antioxidants

1- Carotenoids and proline

Exposure of safflower plant to salt stress enhanced the production of carotenoids (Car). They decreased by adding halophytic extracts to salt-stressed plants as compared to NaCl alone, except with *Z. album* extract by soaking application and *H. strobilaceum* extract with spray one (Table 4). Regarding proline (Pro), All halophytic extract applications to either unstressed or salt-stressed plants attained an ameliorating effect on Pro content as compared to NaCl (Table 4).

Ascorbic acid and Reduced glutathione

Salinity has increased ascorbic acid (AsA) in safflower leaves, but treatment with halophytic extracts resulted in a significant decline in AsA content as compared to unstressed control (Table 5). Also, salt-stressed plants showed a decline in AsA content which was more pronounced when sprayed with halophytic extracts.

The total amount of reduced glutathione (GSH) decreased by 11.3% as a result of NaCl stress as compared to control (Table 5). Spray application of either halophytic extracts caused a slight elevation in GSH, though priming treatment with extracts showed a slight decline in GSH content relative to unstressed control treatment. *Z. album* extract as a spray treatment has decreased GSH content as compared to NaCl control, but pre-soaking of seeds in such extract relatively raised the level of GSH. In contrast, GSH level was elevated by spraying with *H. strobilaceum*.

Table 4. Effect of two halophytic extracts on Carotenoids (mg/g f.wt.) and proline (mg/g d.wt.) contents in leaves of salt-stressed safflower.

Mode of application	Treatments	Carotenoids		Proline	
		Unstressed	Stressed	Unstressed	Stressed
Spray	Control	46.4	52.6	4.5	8.8
	Halo	42.5	53.4	1.6	5.8
	Zygo	47.7	51.5	4.0	4.0
Soak	Halo	37.7	45.1	1.6	3.2
	Zygo	27.7	61.0	1.4	4.7

Halophytic extract	Carotenoids						Proline					
	Halo			Zygo			Halo			Zygo		
	F	P	LSD	F	P	LSD	F	P	LSD	F	P	LSD
Effect of salinity	63.0	**	0.03	31.9	**	0.03	445.8	**	6.5	461.7	**	5.9
Effect of application	40.1	**	0.04	20.3	**	0.03	4.6	*	8.0	91.7	**	7.2
Interaction	14.3	**		5.4	*		59.0	**		89.6	**	

Table 5. Effect of two halophytic extracts on ascorbic acid content (µM/g f.wt) and reduced glutathione (µmol/ml) in leaves of salt-stressed safflower.

Mode of application	Treatments	Ascorbic acid		Reduced glutathione	
		Unstressed	Stressed	Unstressed	Stressed
Spray	Control	55.5	66.3	20.4	18.1
	Halo	50.9	48.3	21.2	20.0
	Zygo	50.0	41.7	23.8	17.0
Soak	Halo	27.4	64.6	19.1	16.2
	Zygo	42.0	53.4	16.9	21.2

Halophytic extract	Ascorbic acid						Reduced glutathione					
	Halo			Zygo			Halo			Zygo		
	F	P	LSD	F	P	LSD	F	P	LSD	F	P	LSD
Effect of salinity	47.2	**	4.8	5.8	*	4.2	66.7	**	0.57	117.0	**	0.32
Effect of application	16.6	**	5.9	24.0	**	5.2	42.4	**	0.70	31.7	**	0.40
Interaction	28.1	**		11.2	**		3.6	NS		474.7	**	

3-Total phenolics and flavonoids content

Phenolics, as a potent antioxidant compounds, showed an increase by exposure to salt stress with respect to control (Table 6). Results indicated that soaking with *Z. album* extract resulted in a slight increase in total phenolic contents (TPC), whereas other halophytic treatments showed a declining effect on TPC as compared to control, specially plants presoaked with Z. album extract (exerted 40.3% decline). In contrast, spraying with *H. strobilaceum* or presoaking in *Z. album* showed an increase in TPC as compared to other treatments.

Total flavonoids content (TFC) of safflower leaves has aggravated in response to salinity stress, with respect to control (Table 6). However, application of halophytic extracts caused a pronounced decrease in TFC level of unstressed leaves, specially pre-soaking of safflower in *Z. album* extract which exerted 68.9% drop in TFC, with respect to control. Nonetheless, salt-stressed plants treated with halophytic extracts showed still much lower TFC than those with NaCl alone, particularly those sprayed with *Z. album* (54.5%) and soaked with *H. strobilaceum* (63.6%).

Table 6. Effect of two halophytic extracts on total flavonoid and phenolic contents (mg/g d.wt) of salt-stressed safflower.

Mode of application	Treatments	Total phenolic contents		Total flavonoid contents	
		Unstressed	Stressed	Unstressed	Stressed
Spray	Control	46.4	52.6	4.5	8.8
	Halo	42.5	53.4	1.6	5.8
	Zygo	47.7	51.5	4.0	4.0
Soak	Halo	37.7	45.1	1.6	3.2
	Zygo	27.7	61.0	1.4	4.7

Halophytic extract	Total phenolic contents						Total flavonoid contents					
	Halo			Zygo			Halo			Zygo		
	F	P	LSD	F	P	LSD	F	P	LSD	F	P	LSD
Effect of salinity	196.3	**	1.3	283.0	**	1.9	279.9	**	0.49	92.3	**	0.64
Effect of application	73.0	**	1.6	16.3	**	2.3	158.7	**	0.60	68.3	**	0.79
Interaction	5.7	*		121.7	**		18.8	**		23.0	**	

DISCUSSION

Soil salinity is an environmental issue facing many parts of the world and causing a clear reduction in plant growth and productivity. Abiotic stress tolerance is associated with osmotic adjustment, ion exclusion, maintenance of water status, higher nutrient acquisition ability, higher hormone concentrations, higher photosynthetic capacity or antioxidant capacity (Athar and Ashraf, 2009). Foliar application, as well as priming in growth stimulants may represent a meandering way for stimulating plants tolerance or adjustment to stress conditions. Under salinization, overproduction of ROS represented here by hydrogen peroxide causes oxidative stress through loss of plasma membrane integrity and enzyme inhibition, leading to cell death (Mishra *et al*., 2013). Mittler (2002) pointed out that ROS produced by the photosynthetic electron transport chain in chloroplast being due to salt-induced ionic toxicity and osmotic stress. However, in the present work, halophytic extracts applications have diminished salt-induced oxidative damage

by decreasing ROS production Addition of extracts to salt-stressed safflower showed ability to offset the destructive effects caused by salinity through hampering H_2O_2 over-production. It may be due to the presence of some biochemical compounds in the extract such as proline (Pro), glycine betaine (GB) and phenolics that might be required for the induction of antioxidant enzymes responsible for decreasing ROS levels in the salinity stressed plants in halophytic extracts.

Electrical leakage as one of the oxidative markers, enables cell membrane injury to be assessed when plants are subjected to different kinds of stresses, because maintaining the integrity of cellular membranes is considered an integral part of salinity tolerance in plants. Halophytic extracts application with either modes has ameliorated membrane deterioration in the unstressed safflower leaves, whilst spray foliar application was effective in the maintenance of membrane functions under salinity conditions. The outlined results being similar to those of Zaki and Rady (2015) who demonstrated that salt stress significantly increased leaf electrolyte leakage, while the single or combined (seed soaking + foliar spray) applications of MLE significantly decreased it in *Phaseolus vulgaris* L. plants. Such facilitation can be attributed to the antioxidant phytochemicals present in the extracts, thus protect plant from oxidative damage. In contrast, presoaking in such extracts resulted in excessive leakage of cellular electrolytes from safflower leaves.

Antioxidative enzymes have been considered the first response mechanism against salinity stress. The present study showed a significant diminish in POD activity in salt-stressed safflower. However, application of halophytic extracts to unstressed or salt-stressed plants have an inconsistent effect on POD activity. Such decrease or increase in POD activity can be attributed to the presence of antioxidant compounds such as AsA, Pro, betaine and glutathione in halophytic extracts which have the potential to reduce cell damage caused by ROS. These results being in harmony with those reported by Ibrahim *et al*. (2014), they observed a decrease in the activities of antioxidant enzymes in salinity stressed wheat seedlings, while presoaking of grains with *Ulva lactuca* extract has increased them.

Detoxification of ROS generated under stress conditions can be carried out through ascorbate-glutathione cycle with the impacts of ascorbate peroxidase (APX) and glutathione reductase (GR) and glutathione S-transferase (GST) enzymes with oxidation- reduction potentials (Shim *et al*., 2003). Also, GST together with reduced glutathione (GSH) produce less toxic and water-soluble conjugates by catalyzing the binding of different xenobiotics (Edwards *et al*., 2000). In the present work, salt stress has increased GST activity as compared to control, confirming the findings of El-Omari and Nhiri (2015), they detected a steady increase in GST activity of sorghum parallel with the increase in stress factors. The increase in the activity of scavenging enzymes could be a sign of either severe oxidative stress or an efficient stress response mechanism (Zlatev *et al*., 2006). However, application of halophytic extracts under normal or stress conditions has decreased GST activity, specially for plants sprayed with *Z. album* extract. Such results support the findings of El-Baky and El-Baroty (2012), they reported that algal extract rich in antioxidants was able to scavenge hydroxyl radicals and other ROS in plants exposed to salt stress. The non-enzymatic antioxidant components including AsA, GSH, GB, Pro, anthocyanins, phenolic compounds as well as flavonoids have been considered the second line of defense against free radical damage (Gill and Tuteja, 2010). As halophytic extracts contain such bioactive phytochemicals, they can supply scavenging power for free radicals, thus lowering the need for GST activity.

Salt stress has induced a significant increase in carotenoids (Car) levels in safflower leaves. These results are in accordance with those obtained by Misra *et al*. (1997), they reported a significant increase in Car contents in rice seedlings with the increase in salt concentrations. Car are among non-enzymatic components of antioxidative defense system in plants as they have a role in the activation of enzymes regulating photosynthetic carbon reduction and protecting chloroplast from oxidative damage (Nenova *et al*., 2009), besides they function as energy carriers. Salguero *et al*. (2003) reported that Car play an important role as a precursor in signaling during the plant development under abiotic/biotic stress and act as potential quenchers of ROS by the dissipation of excess excitation energy through xanthophyll cycle. Addition of halophytic extracts, *Z. album* and *H. strobilaceum* of soaking and foliar spray modes, respectively to salt-stressed safflower has been effective in view of its synergistic effect, which was compatible with results pointed out by El-Baky *et al*. (2008), they noticed a significant increase in photosynthetic pigments with the application of algal *Chlorella ellipoida* and *Spirulina maxima* extracts to wheat plants irrigated with sea water. Therefore, halophytic extracts can improve salt tolerance of safflower plants by restoring Car. This restoration may be attributed to lower production of ROS, lower Na uptake, and the efficiency of salt to reduce solute potential as the metabolites of halophytic extracts could reach cells.

Pro can accumulate preferentially in leaves to maintain Chl level and cell turgor to protect photosynthetic activity under salt stress (Silva-Ortega *et al.*, 2008). Besides its role as an osmolyte, (Sharma *et al*., 2011) stated that Pro contributing for scavenging ROS, stabilizing subcellular structures, modulating cell redox homeostasis, supplying energy and functioning as a signal. This supports the assumption that Pro accumulation being a part of a physiological response in plants to intense stress. Generally, addition of extracts to unstressed leaves brought about an antagonistic effect with Pro. However, results highlighted that Pro accumulation was very small with *Z. album* extract by either applications indicating that *Z. album* has a limited capability to synthesize Pro as a compatible compound and safflower posses different strategy for tolerance or mitigating salt stress. These results were confirmed by the higher level of Pro accumulation in rice resulting from GB application under salinity as reported by Demiral and Türkan (2006).

It is well known that Halliwell–Asada cycle can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via APX reaction. Throughout the present study, AsA has increased under salinity as compared to control which agreed with Arshi *et al*. (2010), they reported an increase of AsA level in wheat with NaCl. However, utilization of halophytic extracts with either salt-stressed or unstressed safflower has decreased AsA level. Previous report by Afzal *et al*. (2012) elucidated that treatment with exogenous protectants as ascorbate, salicylic acid and kinetin effectively relieved salinity deteriorations in wheat through decreasing Na^+ and Cl^- uptake and enhancing K^+ uptake.

Glutathione (GSH) plays a potential role in salt-stress tolerance by reacting with ROS and helping AsA regeneration (Foyer and Noctor, 2003). Salt stress resulted in a slight decrease in GSH level of safflower leaves. Under salinization, Mittler (2002) reported an overproduction of ROS due to ionic toxicity and osmotic stress resulting in loss of plasma membrane integrity and enzyme inhibition. Variability of halophytic extracts effect on GSH content has been expressed in reduction of presoaking mode and enhancement of spray mode. Salt-stressed plants sprayed with *H. strobilaceum* and soaked with *Z. album* possessed higher GSH than NaCl alone. These results are in accordance with those obtained by Chen *et al*. (2012), they elucidated the role of algal extract in modifying salt stress induced changes of wheat through the biosynthesis of glutathione. On the other hand, salt-stressed plants sprayed with *Z. album* or soaked with *H. strobilaceum* possessed lower GSH than NaCl.

Phenolic compounds are secondary metabolites serving as non-enzymatic antioxidants increasing or decreasing under abiotic stress (Amarowicz *et al*., 2004). In the present work, the level of phenolic compounds has stimulated after exposure to salinity. Analogous results have been reported on the enhancement in total phenolic contents (TPC) by salt stress in radish seedlings by Kasim *et al*. (2016). Induction of phenol accumulation under salt stress would initiate secondary metabolism as one of the defense techniques tailored by plants to counteract salinity stress (Radi *et al*., 2013). Controversially, TPC decreased after the treatment of unstressed plants with halophytic extracts, except for spraying with *Z. album* extract. This result may reflect the protective role provided by halophytic extracts under normal or saline conditions, due to its high antioxidant capacity. A comparable pattern to total phenols in safflower leaves was recorded for flavonoids under saline and non-saline conditions. Addition of halophytic extracts has decreased flavonoid levels, which can be attributed to the effect of extracts in reducing H_2O_2 levels in salinized plants and consequently reducing the need for flavonoid accumulation.

CONCLUSION

Halophytes represent a group of plants that can thrive salinity environments and synthesize certain metabolites during their adjustment to cope with such conditions. The present study aimed at introducing the extracts of certain common species represented by *H. strobilaceum* and *Z. album* inhabiting the salt marshes of the north coastal zone of Egypt to safflower which is relatively salt-tolerant as an attempt to improve salt tolerance through induction of osmoprotectants and antioxidants.

REFERENCES

Afzal, I.; Hussain, B.; Basra, S.M.A. and Rehman, H. (2012). Priming with MLE reduces imbibitional chilling injury in spring maize. Seed Sci. Technol., 40(2): 271–276.

Ahmad, P.; Hakeem, K.U.R., Kumar, A.; Ashraf, M. and Akram, N.A. (2012). Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica Juncea* L .) African J. Biotechnol., 11(11): 2694-2703.

Amarowicz, R.; Pegg, R.B.; Rahimi-Moghaddam, P.; Barl, B. and Weil, J.A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem., 84(4): 551–562.

Anderson, M.E. (1985). Determination of glutathione and glutathione disulphide in biological samples. Methods Enzymol., 113(1983): 548–555.

Arpornsuwan, T.; Petvises, S.; Thim-uam, A.; Boondech, A. and Roytrakul, S. (2012). Effects of *Carthamus tinctorius* L. solvent extracts on anti-proliferation of human colon cancer (SW 620 cell line) via apoptosis and the growth promotion of lymphocytes. Songklanakarin J. Sci. Technol., 34(1): 45-51.

Arshi, A.; Ahmad, A.; Aref, I.M. and Iqbal, M. (2010). Calcium interaction with salinity-induced effects on growth and metabolism of soybean (*Glycine max* L.) cultivars. J. Environ. Biol., 31: 795-801.

Athar, H. and Ashraf, M. (2009). Strategies for crop improvement against salinity and drought stress: An Overview. In: Salinity and water stress: improving crop efficiency. (Eds.): Ashraf, M., Ozturk, M. and Athar, HR. Springer-Verlag, The Netherlands, pp: (1–16).

Bates, L.E. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Chang, C.; Yang, M.; Wen, H. and Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.

Chen, J.H.; Jiang, H.W.; Hsieh, E.J.; Chen, H.Y.; Chien, C.T.; Hsieh, H.L. and Lin, T.P. (2012). Drought and salt stress tolerance of an *Arabidopsis* glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. Plant physiol., 158(1): 340-351.

Cosge, B.; Gurbuz, B. and Kiralan, M. (2007). Oil content and fatty acid composition of some (*Carthamus tinctorius* L.) varieties sown in spring and winter. Int. J. Nat. Eng. Sci., 1(3): 11-15.

Demiral, T. and Türkan, I. (2006). Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. Environ. Exp. Bot., 56(1): 72-79.

Edwards, R.; Dixon, D.P. and Walbot, V. (2000). Plant glutathione S- transferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci., 5: 193–198.

Egley, G.; Paul, R.; Vaughn, K. and Duke, S. (1983). Role of peroxidase in the development of water-impermeable seed coats in *Sida spinosa* L. Planta, 157(3): 224-232.

El-Baky, H.H.A. and El-Baroty, G.S. (2012). Characterization and bioactivity of phycocyanin isolated from *Spirulina maxima* grown under salt stress. Food Funct., 3(4), 381-388.

El-Omari, R.E.L. and Nhiri, M. (2015). Adaptive response to salt stress in sorghum (*Sorghum bicolor*). Am-Euras. J. Agric. Environ. Sci., 15(7): 1351-1360.

Foyer, C.H. and Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol. Plant, 119(3): 355-364.

Gautam, S.; Bhagyawant, S.S. and Srivastava, N. (2014). Detailed study on therapeutic properies, uses and pharmacological applications of safflower (*Carthamus tinctorius* L.). Int. J. Ayur. Pharm. Res., 2(3): 5(-16.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem., 48(12): 909-930.

Habig, W.H.; Pabst, M.J. and Jacoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Ibrahim, W. M.; Ali, R. M.; Hemida, K. A. and Sayed, M. A. (2014). Role of *Ulva lactuca* extract in alleviation of salinity stress on wheat seedlings. Scientific World J., 2014: 1-11.

Jindal, K.K. and Singh, R.N. (1975). Phenolic content in male and female *Carica papaya*: A possible physiological marker for sex identification of vegetable seedlings. J. Plant Physiol., 33: 104-107.

Jouyban, Z. (2012). The effects of salt stress on plant growth. Technol. J. Eng. Appl. Sci. 2(1): 7-10.

Kasim, W.A.; Saad-Allah, K.M. and Hamouda, M. (2016). Seed priming with extracts of two seaweeds alleviates the physiological and molecular impacts of salinity stress on radish (*Raphanus Sativus*). Int. J. Agric. Biol., 18(3): 653–660.

Li, H.X.; Han, S.Y.; Wang, X.W.; Ma, X.; Zhang, K.; Wang, L.; Ma, Z.Z. and Tu, P.F. (2009). Effect of the carthamins yellow from *Carthamus tinctorius* L. on hemorheological disorders of blood stasis in rats. Food Chem. Toxicol., 47: 1797-1802.

Mahajan, S. and Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. Arch. Biochem. Biophys., 444: 139–158.

Metzner, H.; Rau, H. and Senger, H. (1965). Studies on Synchronization of Some Pigment-Deficient Chlorella Mutants. Planta, 65(2): 186-194.

Mishra, P.; Bhoomika, K. and Dubey, R.S. (2013). Differential responses of antioxidative defense system to prolonged salinity stressin salt-tolerant and salt-sensitive indica rice (*Oryza sativa* L.) seedlings. Protoplasma, 250(1): 3–19.

Misra, A.; Sahu, A.N.; Misra, M.; Singh, P.; Meera, I.; Das, N.; Kar, M. and Sahu, P. (1997). Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biol. Plant., 39(2): 257–262.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance: Review. Trends Plant Sci., 7 (9): 405-410.

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. Ann. Rev. Plant Biol., 59: 651-681.

Nenova,V.; Merakchiy, M.; Geneva, G.; Zozikova, E. and Landjeva, S. (2009). Physiological response of wheat (*Triticum aestivum* L.)– *Aegilops Sharonensis* introgression lines to excess copper. Agron. Crop Sci., 195: 197-203.

Oser, B. (1965). Determination of serum phosphatase activity: In: Hawk's Physiological Chemistry 14th ed.(BL Oser, Ed.): McGraw-Hill, New York.

Radi, A.A.; Farghaly, F.A. and Hamada, A.M. (2013). Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. J. Biol. Earth Sci., 3(1): 72–88.

Rontein, D.; Basset, G. and Hanson, A.D. (2002). Metabolic engineering of osmoprotectants accumulation in plants. Metab. Eng., 4: 49-56.

Sairam, R.; Srivastava, G.; Agarwal, S. and Meena, R. (2005). Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biol. Plant., 49(1): 85-91.

Salguero, A.; de la Morena, B.; Vigara, J.; Vega, J.M.; Vilchez, C. and León, R. (2003). Carotenoids as protective response against oxidative damage in *Dunaliella bardawil*. Biomol. Eng., 20(4), 249-253.

Sharma, S.; Villamor, J.G. and Verslues, P.E. (2011). Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. J. Plant Physiol., 157: 292-304.

Shim, I.S.; Momose, Y.; Yamamoto, A.; Kim, D.W. and Usui, K. (2003). Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. Plant Growth Regul., 39 : 285–292.

Silva-Ortega, C.O.; Ochoa-Alfaro, A.E.; Reyes-Agüero, J.A.; Aguado-Santacruz, G.A. and Jiménez-Bremont, J.F. (2008). Salt stress increases the expression of p5cs gene and induces proline accumulation in cactus pear. Plant Physiol. Biochem., 46(1): 82-92.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedures of Statistics. 2 nd Ed. New York: McGraw-Hill Book Co.

Valliyodan, B. and Nguyen, H.T. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr. Opin. Plant Biol., 9: 1–7.

Velikova, V.; Yordanov, I. and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci., 151: 59-66.

Wahid, A. (2003). Physiological significance of morpho-anatomical features of halophytes with particular reference to Cholistan flora. Int. J.Agric. Biol., 5(2): 207-212.

Wei, X.B.; Liu, H.Q.; Sun, X.; Fu, F.; Zhang, X.; Wang, J.; An, J. and Ding, H. (2005). Hydroxysafflor yellow A protects rat brains against ischemia-reperfusion injury by antioxidant action. Neurosci. Lett., 386: 58–62.

Zaki, S.S. and Rady, M.M. (2015). *Moringa oleifera* leaf extract improves growth, physio-chemical attributes, antioxidant defence system and yields of salt-stressed *Phaseolus vulgaris* L. plants.Int. J. ChemTech Res., 8(11): 120-134.

Zhou, X.; Tang, L.; Xu, Y.; Zhou, G. and Wang, Z. (2014). Towards a better understanding of medicinal uses of *Carthamus tinctorius* L. in traditional Chinese medicine: a phytochemical and pharmacological review. J. Ethnopharmacol., 151(1): 27-43.

Zlatev, Z.S.; Lidon, F.C.; Ramalho, J.C. and Yordanov, I.T. (2006). Comparison of resistance to drought of three bean cultivars. Biol. Plant., 50(3): 389-394.

تأثير مستخلصات بعض النباتات الملحية علي النظام المضاد للأكسدة لنبات القرطم المجهد ملحياً

محمد نبيه الشوربجي ، خليل محفوظ سعد الله ، السيد عبد اللطيف فودة و اسراء أسامة الرزاقي*

قسم النبات-كلية العلوم - جامعة طنطا

أجريت تجربة اصيص في الحديقة النباتية بقسم النبات كلية العلوم جامعة طنطا-محافظة الغربية خلال الموسم الشتوي٢٠١٥-٢٠١٦ لدراسة إستجابة نبات القرطم (*Carthamus tinctorus* L.) المجهد ملحياً لمستخلصات نوعين من النباتات الملحية ينموان طبيعياً بالنطاق الساحلي وهما الرطريط (*Z. album*) والحضادي (*H. strobilaceum*) حيث تم نقع البذور إما في الماء المقطر أو في مستخلص النباتات الملحية لمدة ٢٤ ساعة ثم زرعت في أصيص تحتوي علي تربة طينية رملية بنسبة ٢: ١ طبقا لتصميم القطاعات الكاملة العشوائية حتي الوصول الي مرحلة ما قبل التزهير. وبعد ظهور الأوراق الفلقية قسمت البادرات الناتجة الي مجموعتين رئيسيتين: الأولي تم معاملتها بتركيز ١% من مستخلصات النباتات الملحية سواء بطريقة النقع المسبق أو الرش الورقي في حين خصصت الثانية لمعاملات المقارنة وقد قسم كل منهما الي مجموعتين فرعيتين: الغير مجهدة والمجهدة. وقد أوضحت النتائج أن الإجهاد الملحي قد فرض تابعات سلبية علي النمو والخصائص الأيضية لنبات القرطم بينما ساعدت المستخلصات علي إنضباط النبات وتحسين كفاءته تحت ظروف الإجهاد الملحي عن طريق تخليق مواد اسموزية وقائية بالاضافة الي الاتزان الأيوني الريدوكسي وسلامة الأغشية.

كلمات البحث: القرطم ، الملوحة، النباتات الملحية، الأيض، مضادات الأكسدة، المواد الأسموزية الوقائية.