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Study of phytochemical changes, enzymatic and antioxidant activity of two halophyte plants: *Salsola dendroides* Pall and *Limonium reniforme* (Girard) Lincz in different seasons

S. Bakhshi[1], H. Abbaspour*[1], S. Saeidisar[2]

[1] *Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran.*

[2] *Dr.Shariaty Technical College, Technical and Vocational University, Tehran, Iran.*

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In order to study phytochemical changes, enzymatic and antioxidant activity of *Salsola dendroides* Pall. and *Limonium reniforme* (Girard) Lincz halophytes, leaf and root samples of these plants were collected from Incheh Borun region in northern Gorgan, Iran, in three seasons (spring in May, summer in August, and autumn in November) in four iterations. The results showed that soil salinity level increased in autumn and significantly increased enzyme activities and antioxidant activities in shoots and roots of both species in most cases. Phytochemical studies showed that phenol content significantly increased in shoot and root of *Salsola dendroides* in summer and autumn and in *Limonium reniforme* in summer, compared to theother two seasons. Flavonoid content also significantly changed with seasonal variation. The highest flavonoid accumulation was observed in shoots and roots of the plants in summer; most of these changes were related to activation of physiological and biochemical processes which allow the plants to adapt to salinity conditions.

Keywords: Phenol, Flavonoid, Antioxidant enzymes, Salinity

INTRODUCTION

Variability of environmental conditions, such as fluctuations in temperature, humidity, salinity, and UV radiation, results in increased production of reactive oxygen species (ROS) inducing oxidative stress in plant cells [1,2]. Antioxidants are compounds which inhibit oxidative reactions of plants by free radicals. Also called reactive oxygen species (ROS), free radicals are frequently produced as by-products of various metabolic mechanisms in plants and are accumulated in different cellular compartments such as chloroplasts, mitochondria, and peroxisomes [3,4]. Accumulation of free radicals such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radical (HO^-), and singlet oxygen causes oxidative damage to proteins, lipids, nucleic acid, and photosynthetic enzymes; therefore, it disrupts normal plant metabolism [5].

Halophytes are natural herbs in saline habitats and can be a good candidate for having unsaturated fatty acids, beta-carotene, sterols, as well as active enzymatic and phytochemical compounds, for example, phenolic compounds, and more specifically fluorutanins, terpenes and alkaloids that have antioxidant and anti-microbial properties [6].

In the past decade, there has been a new interest in the search for phytochemical compounds from natural native plants to provide pharmaceutical and nutritional materials [7] recognizing that the products derived from them can be useful for pharmaceutical purposes [8]. This is mainly due to their high biological activity and antioxidant compounds [9]. Among herbal antioxidant compounds, phenolic compounds such as phenol and flavonoids are widely distributed in many plants which synthesize and accumulate them in response to biological and non-biological stresses such as salinity [10].

Antioxidant properties of phenolic compounds are deliberately due to regenerative capacity of their chemical structure, which enables them to neutralize free radicals, form complexes with metal ions, silence single and triple oxygen molecules and reduce oxidative damages caused by ions. By inhibiting lipoxygenase, they inhibit peroxidation of lipids [11]. Since there is a significant interest in natural antioxidants, efforts have been made to find alternative sources of these antioxidants. Therefore, it is logical that plants such as halophytes are identified due to their high antioxidant properties. Thus, this study tends to investigate phytochemical changes and enzymatic and antioxidant activity in *Salsola dendroides* from the *Chenopodiaceae* family and *Limonium reniforme* from the *Pallumbazinasea* family under naturally applied stress in different seasons.

MATERIALS AND METHODS

Samples of two species, *Salsola dendroides* Pall. (chenopodiaceae family) and *Limonium reniforme* (Girard) Lincz (plumbaginaceae family) were

* To whom all correspondence should be sent:
E-mail: h.abbaspour75@hotmail.com

randomly obtained from their natural habitats in Inchehboron region, Gorgan, North Eastern Iran (37° 27' N, 54° 43 E) in 4 iterations during spring (May), summer (August), and autumn (November), 2015. The samples were then transferred to a laboratory for testing peroxidase, ascorbate peroxidase, polyphenol oxidase, and catalase enzyme activities, antioxidant activity (DPPH test) as well as content of phytochemical compounds. Samples were rinsed by distilled water before the tests.

Enzyme extraction

Proteins were extracted as suggested in[12]. For this purpose, 5 ml 0.05 M Tris buffer-HCl, pH 5.7, was added to fresh leaf tissues in a mortar and kept for 30 min. Then, the tissues were ground by pestle in an ice bath. The solution was centrifuged at 13000 rpm and 4°C. Protein extraction was used for testing peroxidase, catalase, and polyphenol oxidase activities. Ascorbate peroxidase is vulnerable in the absence of ascorbate. Therefore, 0.2 ml 0.5 µM ascorbate was added to test ascorbate peroxidase activity.

Peroxidase measurements

Peroxidase activity was determined as suggested in[13]. For this purpose, 0.1 ml of enzyme extract was added to a mixture containing 2 ml of 0.2 M acetate buffer (pH 5.0), 0.4 ml of 3% H_2O_2 and 0.2 ml of 0.01 M benzidine solution in 50% alcohol. Enzyme activity was determined spectrophotometrically (2800, UV/VIS, UNICO, USA) by recording the absorbance at 530 nm. In order to protect enzyme activity, the procedure was carried out in an ice bath. Enzyme activity was calculated in terms of changes in absorption per min for 1 g fresh weight of the plant tissue.

Ascorbate Peroxidase Measurements

The method reportedin[13] was used to test ascorbate peroxidase activity. For this purpose, 2 ml of 0.05 M phosphate buffer (pH 7), 0.2 ml of 3% (v/v) H_2O_2 and 0.2 ml of 50 mM ascorbate were mixed in an ice bath. Then, 0.1 ml of enzyme extract was immediately added to the indicator and the absorbance changes were recorded at 290 nm (2800UV/VIS, UNICO, USA). Ascorbate peroxidase activity was calculated in terms of changes in absorption per min for 1 g fresh weight of the plant tissue.

Polyphenol oxidase measurements

Polyphenol oxidase was determined as reportedin[15]. Reaction solution contained 1.5 ml of Tris buffer (pH 7.6), 0.4 ml of 0.02 mM pyrogallol, and 0.1 ml of enzyme extract. Incubating at 25°C for 5 min, the reaction was stopped by adding 1 ml of 10% H_2SO_4. The absorbance was then measured at 420 nm using a spectrophotometer (2800 UV/VIS, UNICO, USA). Polyphenol oxidase activity was calculated in terms of changes in absorption per min for 1 g fresh weight of the plant tissue.

Catalase measurements

Catalase activity was measured as suggestedin[16]. Reaction mixture containing 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 0.3 ml of 3% (v/v)H_2O_2 was mixed in an ice bath. Then, 0.2 ml of enzyme extract was added to the mixture. Absorbance changes were read at 240 nm (2800UV/VIS, UNICO, USA). Catalase enzyme activity was calculated in terms of changes in absorption per min for 1 g fresh weight of the plant tissue.

Extract preparation

Fresh roots and shoots of the plants were rinsed by distilled water and dried in an oven at 40°C. One gram of the dried plant material was soaked in 10 ml of methanol at room temperature for 24 h and filtered through a Whatman filter paper. The extract was then stored in dark at 4°C for analysis.

Antioxidant activity by DPPH measurements

The reagent 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical was used for measuring free radical scavenging activity of the extracts as described in[17]. Different concentrations of methanol extract were first prepared and then 1 ml of various concentrations of the solvent extracts was added to 0.5 ml of a 0.2 mM methanol solution of DPPH. The reaction mixture was then vigorously shaken and kept at room temperature in dark for 30 min. Absorbance of the resulting solution was then measured using a spectrophotometer at 517 nm after 30 min. The ability to scavenge DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (%) =
= [(Abs control – Abs sample)/ (Abs control)] × 100.

Phenolic compounds measurements

For extraction of phenolic compounds, the procedure was: (i) the samples weredriedat a temperature of 40-45℃; (ii) 10 ml of 80% methanol was added per one gram of sample; it was placed on a shaker for 24 h, after which it was laid on a filter paper; (iii) total phenolic compounds were measured as follows: 20 µl of the extract was mixed with 1.16 ml of distilled water and 100 µl of folicin reagent was added to the solution. After 5 min, 300

µl of 20% sodium carbonate solution was added to the solution and the samples were kept at 40°C in a water bath for 30 min. Then, the absorbance of the samples was read on a spectrophotometer at a wavelength of 760 nm and the phenol content was calculated in mg of gallic acid per gram of dry weight of the sample [18].

Flavonoid content measurement

For extraction, 10 ml of 80% methanol was added to 1 g of the dried plant powder sample, and the mixture was placed on a shaker for 24 h and filtered with a filter paper. The extract was then used to measure the flavonoid content. Flavonoid measurement was based on the aluminum chloride colorimetric method. According to this method, 0.5 ml of methanolic extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water, and was placed in darkness for half an hour. Then,, the absorption was read on a spectrophotometer at 415 nm. Flavonoid content was calculated and set at 1 mg quercetin per gram of dried powder [19].

Statistical analysis

The obtained data were analysed by SPSS Ver. 21 by comparing the means by Duncan's multiple-range test (P≤0.05) in 4 replications for each study.

RESULTS

The highest peroxidase activities were recorded in shoots of *Salsola dendroides* and *Limonium reniforme* in summer and spring, respectively, while the lowest peroxidase activities were observed in shoots of both species in spring and autumn, respectively. Peroxidase activity increased in roots of both species in summer, while it decreased in spring (Table 1). There is a significant relationship between different seasons and enzyme activities in both species (P≤0.05).

The highest and lowest ascorbate peroxidase activities were observed in shoots of *S. Dendroides* and *L. reniforme* in summer and autumn, respectively. There are significant differences in enzyme activities in the three seasons (P≤0.05). Ascorbate peroxidase activity significantly increased and decreased in roots of both plants in summer and autumn, respectively (Table 2).

Polyphenol oxidase activities significantly increased in summer in shoots of *S. dendroides* and *L. reniforme*. On the other hand, enzyme activities significantly decreased in spring and autumn, respectively (P≤0.05). Polyphenol oxidase activities significantly increased in roots of *S.dendroides* and *L. reniforme* in summer and autumn, while they decreased in autumn and summer (Table 3).

The highest and lowest catalase activities of shoots of *S .dendroides* were recorded in autumn and spring, respectively, while maximum and minimum catalase activities of shoots of *L. reniforme* were recorded in spring and autumn, respectively. There are significant differences between enzyme activities of the plants in different seasons. Catalyse activities increased in the roots of both species in summer, while it decreased in spring; these differences are significant, as shown in Table 4 (P≤0.05).

Methanol extracts of shoots showed the highest level of antioxidant activities in both plants in summer, whereas the lowest antioxidant activities were observed in the methanol extracts of shoots of *S. dendroides* and *L. reniforme*in spring and autumn, respectively, suggesting a significant difference (P≤0.05). Antioxidant activities (DPPH) significantly increased in root extracts of *S. dendroides* in autumn and decreased in summer; in root extracts of *L. reniforme*, these activities significantly increased in summer, while they decreased in autumn; these differences are significant, as shown in Table 5 (P≤0.05).

Phenol content of shoots significantly increased in both *S. dendroides* and *L. reniforme* plants in summer and decreased in *S. dendroides* in spring and in *L. reniforme* in autumn. In *S. dendroides*, the highest phenol content of roots was observed in autumn and the lowest phenol content was observed in summer. In *L. reniforme*, the highest phenol content of roots was observed in summer and the lowest content was observed in spring; a significant difference was found between different seasons in the phenol content of roots (Table 6).

Table 1. Effect of seasonal variation in peroxidase activity in shoots and roots of *Salsola dendroides* and *Limonium reniforme* (min g^{-1}FW)

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	0.246 ± 0.005c	0.491 ± 0.028a	0.403 ± 0.012b	0.129 ± 0.004c	0.203 ± 0.012a	0.153 ± 0.003b
Limonium reniforme	0.202 ± 0.006a	0.172 ± 0.002b	0.141 ± 0.004c	0.116 ± 0.005c	0.158 ± 0.005a	0.124 ± 0.003b

Different letters in each row indicate significant difference at P≤0.05 by Duncan test; FW: fresh weight

Table 2. Effect of seasonal variation in ascorbate peroxidase activity in shoots and roots of *Salsola dendroides* and *Limonium reniforme* (min g^{-1}FW)

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	0.063 ± 0.004b	0.092 ± 0.004a	0.045 ± 0.003c	0.036 ± 0.001b	0.057 ± 0.003a	0.028 ± 0.001c
Limonium reniforme	0.049 ± 0.002b	0.071 ± 0.004a	0.036 ± 0.002c	0.035 ± 0.002b	0.047 ± 0.004a	0.026 ± 0.003c

Different letters in each row indicate significant difference at P≤0.05 by Duncan test; FW: fresh weight

Table 3. Effect of seasonal variations in polyphenol oxidase activities in shoots and roots of *Salsola dendroides* and *Limonium reniforme* (min g^{-1}FW)

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	0.181 ± 0.009c	0.511 ± 0.023a	0.249 ± 0.013b	0.431 ± 0.041b	0.631 ± 0.033a	0.325 ± 0.031c
Limonium reniforme	0.271 ± 0.007b	0.349 ± 0.018a	0.236 ± 0.004c	0.117 ± 0.003b	0.091 ± 0.005c	0.152 ± 0.005a

Different letters in each row indicate significant difference at P≤0.05 by Duncan test; FW: fresh weight

Table 4. Effect of seasonal variations in catalase activities in shoots and roots of *Salsola dendroides* and *Limoniumreniforme* (min g^{-1} FW)

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	0.045 ± 0.003c	0.058 ± 0.005b	0.105 ± 0.005a	0.041 ± 0.004c	0.073 ± 0.004a	0.054 ± 0.005b
Limonium reniforme	0.151 ± 0.005a	0.126 ± 0.002b	0.118 ± 0.006c	0.043 ± 0.004c	0.086 ± 0.004a	0.052 ± 0.004b

Different letters in each row indicate significant difference at P<0.05 levels by Duncan test; FW: fresh weight

Table 5. Effect of seasonal variations in activities of antioxidant (DPPH) in shoots and roots of *Salsola dendroides* and *Limonium reniforme*

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	60.32 ± 0.8c	83.08 ± 3.7 a	65.58 ± 0.9b	84.35 ± 1.2b	66.08 ± 1.3c	91.23 ± 2.1a
Limonium reniforme	59.07 ± 0.3b	69.91 ± 0.6a	57.06 ± 0.8c	64.62 ± 0.4b	70.04 ± 1.4a	59.98 ± 0.4c

Different letters in each row indicate significant difference at P≤0.05 levels by Duncan test.

Table 6. Effect of seasonal variations in activities of phenol shoots and roots of *Salsola dendroides* and *Limonium reniforme* (mg GAEg^{-1}DW)

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	0.896 ± 0.048 a	1.398± 0. 066 b	1.184±0.066 c	0.455 ± 0.044b	0. 223± 0.008 c	0.837 ± 0.066a
Limonium reniforme	1.626 ±0.068b	1.887 ±0.110 a	1.144 ±0.060 c	0. 265± 0.010a	0.695 ±0.017 b	0.476 ± 0.027 c

Different letters in each row indicate significant difference at P≤0.05 levels by Duncan test. GAE: Gallic acid equivalent; DW: dry weight

Table 7. Effect of seasonal variations in activities of flavonoid shoots and roots of *Salsola dendroides* and *Limonium reniforme* (mg QUEg^{-1}DW)

	Shoots			Roots		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Salsola dendroides	5.47± 0.450 c	9± 0.309 a	6.46 ± 0.467 b	5.02 ±0.46 b	6.01 ±0.73a	3.9 1 ±0.31 c
Limonium reniforme	2.9 7 ± 0.409 c	7. 44± 0.384 a	5.40 ± 0.616 b	2. 75± 0.31 c	5.61 ±0.33 a	3. 82 ± 0.37 b

Different letters in each row indicate significant difference at P≤0.05 levels by Duncan test. QUE: Quercetin equivalent; DW: dry weight

The highest flavonoid content was observed in shoots of *S. dendroides* and *L. reniforme* in summer and the lowest content was observed in spring. This difference was statistically significant between seasons. In roots of both *S.dendroides* and *L.reniforme*, the highest flavonoid content was observed in summer; the lowest root flavonoid content was observed in S. *dendroides* in autumn and in L. *reniforme* in spring. The results showed statistically significant difference between seasons (Table 7).

DISCUSSION

Halophytes are a group of plants with strong antioxidant systems including enzymatic and non-enzymatic components. Non-enzymatic components include antioxidants such as tocopherol, carotenoids, ascorbate and glutathione; enzymatic components include enzymes such as superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase, which protect their important biomolecules including lipoproteins and DNA from destruction by ROS [20,21]. It has been suggested that high antioxidant capacity of halophytes compared with glycophites is one of the important reasons for increasing tolerance and adaptation of these plants to salinity stress [22,23]. Since the severity of damages caused by salinity varies in different plant species, plant organs, or at different stages of growth, the performance of various plant species can be different in synthesis and accumulation of organic compounds, change in capacity and activity of antioxidant enzymes in response to salinity [24].

Studies have shown that enzyme activities increased in plants as an antioxidant defense mechanism against oxidative stress induced by various abiotic stresses [25].

Peroxidases (POXs) are a family of isoenzymes primarily found in apoplastic species of plants which can scavenge H_2O_2 [26]. Peroxidase is considered as the first line of plant defense against abiotic stresses such as salinity; increased activity is reported in plants exposed to salt stress [27].

In organic plants, it has been shown that peroxidase enzyme participates in a number of cellular processes such as host defense mechanism, lignin and suberin wall, resistance to pathogens, tolerance to salinity and aging [28].

In the present study, peroxidase activity significantly increased in shoots of *S.dendroides* and *L.reniforme* in summer compared to autumn. In fact, the highest and lowest levels of peroxidase were recorded in roots of both species in summer and spring, respectively. Significant increase was also observed in peroxidase activities of species such as *Suaeda arcuata, Cressacretica* and *Salsola turcomanic* as abiotic stresses, particularly salinity stress, increased in spring to summer [29]. These results support the findings of the present study. Increased peroxidase activity under salinity stress has been reported in many plants, e.g. genotypes of wheat [30], *Suaeda salsa*[31], *Cakile maritime* [32], *Atriplex halimus*[33], and *Hordeum marinum*[34]. Increased peroxidase activity was also reported in callus cultures of *Suaeda nudiflora* where higher concentrations of NaCl increased peroxidase activity in salinized cells of *S.nudiflora* improving their capacity for decomposing H_2O_2[35]. These findings are consistent with the present study, where salinity significantly increased peroxidase activity of both species in dry seasons.

Ascorbate peroxidase is an antioxidant with generally the same function as peroxidase and catalase. However, this antioxidant enzyme catalyzes scavenging H_2O_2 by using ascorbate as a reductant [36,37]. In this study, ascorbate peroxidase activities increased in shoots and roots of both species in summer compared with spring. This is consistent with the study of Ghorbanli*et al.*[38] who studied *Flavoparmelia caperata* and *Physcia dubia* and reported higher activity of ascorbate peroxidase in both species during summer compared to spring for protection of plants. Moreover, some scientists have reported increased ascorbate peroxidase activity under saline conditions in halophyte plants, e.g. *Atriplex portulacoides*[39], and *Bruguiera parviflora*[36]. Ascorbate peroxidase induction clearly shows that this enzyme plays a critical role in controlling H_2O_2 level in plant cells under initial salt-induced oxidative stress [40].

Polyphenol oxidase is part of an enzymatic mechanism for eliminating reactive oxygen species (ROS) under UV radiation, heat, and stress conditions [41]. This study showed that polyphenol oxidase activity significantly increased in shoots and roots of both species in summer and autumn, respectively. This is consistent with the study of Szecsko*et al.*[42] who found maximum level of polyphenol oxidase activities in rootstocks of plum cultivars during autumn (November) and Sen and Mukherji [43] who studied *Lycopersicon esculentum* and reported an increase in polyphenol oxidase activities during summer. Increased activity of polyphenol oxidase in summer and rainy seasons could be attributed to protective function of plants in response to abiotic stress.

Increased polyphenol oxidase activity under salt stress was already reported in halophytes such as

Sesuvium portalacas[44] and *Excoecaria agallocha* [45]. Subhashini and Reddy [46] argued that increased polyphenol oxidase activity under salinity stress implies that this enzyme is able to oxidize and degrade the toxic compounds which are generally accumulated under saline conditions.

Catalases are tetramer enzymes with four heme groups per tetramer, capable of direct dismutation of H_2O_2 into H_2O and O_2 which are indispensable for ROS detoxification under stress conditions [47]. Findings of this study revealed a significant increase in catalase activity in shoots of *S. dendroides* in summer compared with spring, while the enzyme activity was higher in *L. reniforme* in summer compared with autumn. The highest catalase activity was recorded in roots in summer, while the enzyme activity was at its lowest in spring. This is consistent with Farzamisepehr *et al.* [48] who studied *Populus deltoids* and showed that increase in temperature and drought during summer increases catalase activities in comparison with spring and autumn.

Effects of salt stress on catalase activity were evaluated in different groups of halophytes such as *Hordeum marinum* [34], *Suaaeda maritime* [49], and *Limonium bicolar* [50]. All these studies reported a significant increase in catalase activity under salt stress conditions, which is consistent with the present study. Increased catalase activity seems to be linked to gene expression regulation. Lower oxidative damage has generally been reported in plants with higher catalase activity, suggesting the protective function of this enzyme [51].

Many plant extracts have potential chemo-preventive properties against cancer through antioxidant mechanism and scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation or stimulating cellular antioxidant defense [52]. There are many reports on polyphenol accumulation and enhanced antioxidant ability in halophytes [53,22]. Superior Fe^{-} reduction power and DPPH radical scavenging ability were reported in shoot extracts of halophytes compared with glycophytes [53]. In this study, methanol extracts of shoots and roots of both species had the highest antioxidant activities in summer and autumn, respectively. This variability could be due to meteorological factors such as solar irradiation, photoperiod, temperature, and relative humidity [54] or edaphic effects such as salinity.

Findings of the present study are consistent with those of Zhang*et al.*[55] who studied antioxidant activity of *Sauedasalsain vitro* and reported that extracts had the highest antioxidant activity in summer, as well as of Jallali *et al.*[56] who studied *Crithmum maritimum* and found that the plant extracts had richer phenolic compounds at reproductive stage (summer), suggesting higher antioxidant activities in comparison with extracts at vegetative stage in spring.

Phytochemical studies showed that phenolic compounds significantly increased in shoots of the species in summer and significantly decreased in *S.dendroides* in spring and in *L.reniforme* in autumn. In roots of the studied plants, the highest phenolic compounds were observed in *S.dendroides* in autumn and in *L. reniforme* in summer. The results of this study were consistent with those of Jallali *et al.*[56] who showed that phenolic compounds significantly increased in *Crithmum maritimum* species in summer compared with spring. Medini *et al.* [57] studied the halophytic species *Limonium densiflorum* and found that phenolic compounds increased in summer and decreased in autumn. Variation in production of phenolic compounds in different seasons may be due to various environmental factors such as weather, precipitation, salt increase in soil and sunlight, which may affect biosynthesis and accumulation of phenolic compounds in stressed plants. Many reports confirm the role of phenolic compounds as an important antioxidant in many plants, particularly halophyte plants [20]. Bagal *et al.*[58] claimed that the reason for increase in phenolic compounds in plants exposed to biological and non-biological stresses is rapid induction of expression of phenylalanine ammonilase gene.Phenylalanine ammonilase enzyme plays a key role in biosynthesis of phenolic compounds and regulation of products derived from phenylpropanoid pathway. These compounds play important roles in plants such as protection against alive and non-alive stresses, UV protection, and intercellular signals. Studies show that high levels of phenolic compounds are the main reason for high antioxidant activity of some plant extracts, because evidence suggests a positive relationship between phenolic compounds and antioxidant strength of plants which can be extracted through plant extracts [59,60]. This is consistent with our results that antioxidant activity of the samples is directly related to phenolic and flavonoid compounds, so that methanolic extract of the studied plants with the highest levels of phenolic and flavonoid compounds has the highest antioxidant activity. For example, *Hyoscyamus aureus*[61] and *Solanum guaraniticum* [62] exhibited high antioxidant activity in DPPH-free radical inhibitors due to high polyphenolic compounds. Moreover, studies on *Hypericum brasiliense* showed a positive relationship between

phenolic compounds and their antioxidant function; in dry and saline environments, increase in total phenol content added to antioxidant function of plants, that is, chemical and active compounds of the plant, and most importantly, their antioxidant properties vary by various environmental factors [63].

According to the results, flavonoid content was significantly higher in shoots and roots of the studied species in summer than in other seasons. Studying seasonal variations, Fardus *et al*. [64] measured phenolic compounds such as flavonoids in *Oenothera biensis* and showed that flavonoid content varied as seasons changed; the highest level was observed in summer. Kheira *et al*. [65] observed the highest flavonoid content in the *Ballota hirsuta* Benth plant in summer, which is consistent with the findings of this study. Flavonoids are a group of secondary polyphenolic metabolites of the plant derived from phenylpropanoid pathway, which plays a major role in plant responses to environmental conditions, particularly under biological and non-biological stresses. Flavonoids have been reported to have an antioxidant effect and, by eliminating free radicals, can protect cells [66]. Accumulation of secondary metabolites is known as a defense mechanism in plants, and plants can respond and adapt to stress by altering their cellular metabolism by various defense mechanisms [67].

CONCLUSION

Generally, the findings of this study suggest that accumulation of antioxidant compounds and their activities in the studied plants change as the seasons change; in most cases, the highest antioxidant contents and activities were observed in roots and shoots of the studied plants during summer (along with an increase in draught and salinity levels). Therefore, it seems that there is a strong relationship between tolerance against oxidative stresses due to abiotic conditions and increase in antioxidant activities and phytochemical compounds in the studied plants. In their attempt to reduce oxidative damages due to salinity stress, these plants increase activities of their enzymes such as catalase, peroxidase, ascorbate peroxidase, and polyphenol oxidase and phytochemical compounds, responding to various salinity levels in different seasons and adapting to conditions. Further studies are required to understand the nature of adaptation and the defense mechanisms of halophytes and their tolerance, which in turn may result in identification of the genes which play a role in adaptability of these plants to abiotic stresses.

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ИЗСЛЕДВАНЕ НА ФИТОХИМИЧНИТЕ ПРОМЕНИ, ЕНЗИМНАТА И АНТИОКСИДАНТНАТА АКТИВНОСТ НА ДВЕ ХАЛОФИТНИ РАСТЕНИЯ - *Salsola dendroides* PALL. И *Limonium reniforme* (GIRARD) LINCZ ПРЕЗ РАЗЛИЧНИТЕ СЕЗОНИ

С. Бакши[1], Х. Абаспур[1]*, С. Саедисар[2]

[1] *Департамент по биология, клон Дамган на Ислямски Азад университетт, Дамган, Иран*
[2]*Технически колеж „Д-р Шариати", Технически и специализиран университет, Техеран, Иран*

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(Резюме)

С цел да се изследват фитохимичните промени, ензимната и антиоксидантната активност на халофитните растения *Salsola dendroides* Pall. и *Limonium reniforme* (Girard) Lincz, проби от листа и корени на тези растения са събрани от района Инчех Борун в Северен Горган, Иран през три сезона (пролет – май, лято – август и есен - ноември). Установено е, че засолеността на почвата се увеличава през есента и в повечето случаи значително повишава ензимната и антиоксидантната активност в стъблата и корените на двете растения. Фитохимичните изследвания показват, че съдържанието на феноли в стъблата и корените на *Salsola dendroides* значително нараства през лятото и есента, а на *Limonium reniforme* – през лятото. Съдържанието на флавоноиди също се променя значително през сезоните. Най-високо натрупване на флавоноиди в стъблата и корените на растенията се наблюдава през лятото. Повечето от тези промени са свързани с активиране на физиологичните и биохимичните процеси, позволяващи на растението да се адаптира към условията на засоляване.