

## Increasing Salt Tolerance and Antioxidant Activity in *Artemisia aucheri* by H<sub>2</sub>O<sub>2</sub>-Priming

Forouzan Azimian and Parto Roshandel\*

Received: December 13 2015 Accepted: December 15 2016

Biology Department, Faculty of Sciences, Shahrekord University, Shahrekord, Iran

\*Corresponding author; Email: roshandelparto@gmail.com

### Abstract

Recent studies have introduced seed priming with H<sub>2</sub>O<sub>2</sub> as an effective technique to alleviate abiotic stresses in plants. In the current study, accomplished at Faculty of Sciences, Shahrekord University, seeds of medicinal plant *Artemisia aucheri* were primed with H<sub>2</sub>O<sub>2</sub> (0, 10, 50, 90 and 140 μM) and grown under salt stress (0 and 150 mM NaCl) for one month. Results showed a decrease in H<sub>2</sub>O<sub>2</sub> and malonyldialdehyde concentrations by H<sub>2</sub>O<sub>2</sub> priming leading to diminish lipid peroxidation at the cellular level. Moreover, seed priming with H<sub>2</sub>O<sub>2</sub> (particularly at 90 μM) increased biomass, total water content, chlorophyll (a+b) and carotenoids concentrations, total phenolic content and antioxidant capacity in the plants from primed seeds under both normal and saline conditions. Higher activities of superoxide dismutase and catalase were observed in the primed *A. aucheri* with 90 μM H<sub>2</sub>O<sub>2</sub>, while the activity of ascorbate peroxidase was at the maximum level at 140 μM H<sub>2</sub>O<sub>2</sub> priming condition. Additionally, hydroxyl and super oxide radicals scavenging activities were at the maximum level in the plants from primed seeds with 90 μM H<sub>2</sub>O<sub>2</sub>. Data revealed that H<sub>2</sub>O<sub>2</sub> priming can induce salt tolerance in *A. aucheri* plants by adjusting physiological and metabolic processes such as photosynthesis, ROS scavenging and detoxification and brings about an improved growth and development in this species. Furthermore, H<sub>2</sub>O<sub>2</sub> priming at 90 μM augmented antioxidant activity and reducing power in *A. aucheri* suggesting an increase in its medicinal properties.

**Keywords:** Antioxidant capacity; *Artemisia aucheri*; Radical scavenging; Salinity; Seed priming

### Introduction

*Artemisia* plants, from Asteraceae, are perennial species distributed in arid and semi-arid lands of Iran. *Artemisia aucheri* Boiss. -locally named 'Dermaneh-Koochi' - is an aromatic species that is used in traditional medicine for treatment of various diseases such as astringent, antipoisoning, antiseptic, antiparasitic, stimulants and reduces the rheumatic pains (Pellicer *et al.* 2011; Asghari *et al.* 2012). This species is relatively resistant to severe environmental conditions and also has ecological benefits in soil stabilization and great forage value. Babaahmadi *et al.* (2013) reported that the phosphate-buffered saline extract of *A. aucheri* flowering tops have an allergenic effect. Moreover, it contains santonian, coumarin and flavonoids that have antioxidant activity (Bahrami Karkondi *et al.*

2010; Dinani *et al.* 2010) and is recommended for human health (Siahpoosh *et al.* 2011). Khajehzadeh *et al.* (2014) reported that alternation in antioxidant enzymes activity and polyphenols production was resulted by the adaptation of *A. aucheri* to the related factors of altitude such as soil moisture percentage, temperature and radiation. In another study, Mousaei Sanjerehei *et al.* (2013) stated ecological characters of *A. aucheri* such as tolerance to increasing elevation.

Salinity increasingly limits plant growth and development. Under stressful conditions such as salinity, reactive oxygen species (ROS) consisting of superoxide radicals, hydroxyl radicals and hydrogen peroxide are commonly generated and accumulated, resulting in oxidative stress (Ozgun *et al.* 2013). The ROS are strong oxidizing agents that

cause oxidative damage to biomolecules such as lipids, proteins, DNA and eventually lead to cell death. On the contrary, plants produce antioxidants (Gill and Tuteja 2010) including enzymatic (such as superoxide dismutase, catalase and peroxidases) and non-enzymatic (e.g. phenolic compounds and flavonoids) antioxidants to alleviate oxidative stress (Gill and Tuteja 2010; Khajehzadeh *et al.* 2014). On the other hand, it is well-documented that plants with strong antioxidant properties (containing high levels of polyphenols and radical scavenging activity) are valuable for human health (Krishnaiah *et al.* 2010). Thus, introducing plant materials rich in phenolics or applying treatments to elevate antioxidant capacity in plants could be of importance to the food industry. These treatments would be more appropriate as enable plants to increase their tolerance to environmental stresses such as salinity.

Seed priming is an easy, low cost and effective approach to augment plant tolerance to stressful environments (Tanou *et al.* 2012). In priming techniques, prior exposure of seeds to a natural or synthetic compound, as an eliciting factor, can hinder the deleterious effects of abiotic stress and make plants more tolerant to future stressful conditions.

Hydrogen peroxide ( $H_2O_2$ ), as a final product of ROS (Quan *et al.* 2008; Karuppanapandian *et al.* 2011), is considered as a toxic chemical at high levels resulting in oxidative damage, but it could also act as a signal molecule at low concentrations leading to develop adaptive mechanisms in plants under stress (Hosseini *et al.* 2015). It is claimed that the effect of  $H_2O_2$  priming on plants is not stress-specific since its ameliorative effects was observed

in rice seedlings stressed by NaCl or by high temperatures (Uchida *et al.* 2002). Also, the benefit of  $H_2O_2$  priming to alleviate other abiotic stresses such as drought, chilling and heat stress is already reported in wheat (He *et al.* 2009), *Brassica juncea* (Kumar *et al.* 2010) and cucumber (Gao *et al.* 2010). Wahid *et al.* (2007) indicated that seed pretreatment of wheat with  $H_2O_2$  caused an improvement in salt tolerance via mitigation of oxidative damage and expression of stress proteins. Azevedo-Neto *et al.* (2005) illustrated the involvement of  $H_2O_2$  metabolism in increasing of salt acclimation in maize. In addition, a number of studies pointed out that  $H_2O_2$  priming could also be useful in Al and Cd stress alleviation (Chao and Kao 2010; Xu *et al.* 2011). Apart from priming technique, the role of  $H_2O_2$  treatment to reduce harmful effects of abiotic stresses has been also reported. For instance, Gondim *et al.* (2012) stated that  $H_2O_2$  foliar spraying improved maize seedling growth under normal and saline conditions. Tanou *et al.* (2009) indicated that pre-exposure to  $H_2O_2$  resulted in long-term antioxidant activity in citrus plants at presence or absence of NaCl. Alternatively, Lin and Block (2010) claimed that the positive effect of exogenous  $H_2O_2$  utilization is not obvious under all experimental conditions tested.

In the current study we investigated the effect of  $H_2O_2$  priming of seeds of *A. aucheri* on the physiological and biochemical changes in the seedlings arising from  $H_2O_2$  primed seeds under saline condition. Here, we hypothesize that seed priming with  $H_2O_2$  might enhance antioxidant capacity and salt tolerance in *A. aucheri*.

## Materials and Methods

### Seed priming

Seeds of *Artemisia aucheri* Boiss. were purchased from Pakan-Bazr (Isfahan, Iran) and sterilized with 70% ethanol for 2 min. Then, seeds were washed with sterilized distilled water for several times. Seed priming with H<sub>2</sub>O<sub>2</sub> was accomplished according to Wahid *et al.* (2007) with some modifications. Seeds were soaked in 0, 10, 50, 90 and 140 μM H<sub>2</sub>O<sub>2</sub> solution for 2, 3, 5, 7 and 9 hours. Seeds were washed with distilled water and blot dried to assess the absorbed H<sub>2</sub>O<sub>2</sub> as described below.

### Treatments and plant growth conditions

Our preliminary experiments showed that among the 50, 100, 150, 200 and 250 mM NaCl treatments, application of 150 mM NaCl reduced germination percentage by 50%. Also, it was revealed that among the designed exposure times (2, 3, 4, 7 and 9h) for seed priming with H<sub>2</sub>O<sub>2</sub>, seed exposure for 7 hours was the most effective time to enhance ROS scavenging activity (data not shown) as the concentration of H<sub>2</sub>O<sub>2</sub> was at the minimum level in the seed tissues. Accordingly, treatments for the main experiment were: 1) control (with no H<sub>2</sub>O<sub>2</sub> or NaCl treatment), 2) seed priming with in 0, 10, 50, 90 and 140 μM H<sub>2</sub>O<sub>2</sub> for 7h, 3) irrigation of 14-day-old seedlings with Hoagland solution (pH 6.8) containing 150 mM NaCl (no priming with H<sub>2</sub>O<sub>2</sub>), and 4) seed pretreatment with H<sub>2</sub>O<sub>2</sub> (as in 2) along with irrigation of 14-day-old seedlings with 150 mM NaCl. Treated or untreated seeds were sown in polystyrene boxes, filled with a potting mixture composed of 50% perlite and 50% fine sand. The plants were raised in a greenhouse

under controlled conditions (16/8 h light/dark period, 32/25°C temperature, 60-70% RH and 1000-1200 μmol m<sup>-2</sup> s<sup>-1</sup>PAR). The experiment lasted for one month, and at the end, 45-day-old plants were sampled to determine dry weight production. Samples were weighed and dried by analytical balance (±0.0001) (model: JB1603-C/FACT) and oven (model: SHIMAZCO). Spectrophotometric analysis was accomplished by UV-VIS spectrophotometer (model: 2500, ShimadzuCorp).

### Estimation of H<sub>2</sub>O<sub>2</sub> content

Hydrogen peroxide content was evaluated by measuring the absorbance of titanium-hydroperoxide complex (Nag *et al.* 2000). Fresh leaf samples (1 g) were homogenized in 12 mL of cold acetone. Then, 4 mL of titanium reagent was added to the extract followed by 5 mL of concentrated ammonium solution to precipitate hydroperoxide-titanium complex. The mixture was centrifuged in the refrigerated centrifuge for 5 min at 8500/g. The pellet was washed twice with 5 mL acetone followed by dissolving in 1 M sulfuric acid. The absorbance of orange-yellow H<sub>2</sub>O<sub>2</sub>-Ti complex was recorded at 410 nm against blank. Concentration of H<sub>2</sub>O<sub>2</sub> was determined using standard curve plotted with known concentration of H<sub>2</sub>O<sub>2</sub>.

### Measurement of total water content

The water content was determined by drying the leaves at 80°C for 48 h and calculated as:

$$\text{Water content (\%)} = \frac{(\text{fresh weight} - \text{dry weight})}{\text{fresh weight}} \times 100.$$

### ***Estimation of photosynthetic pigment content***

The content of total chlorophyll (Ch-a + Ch-b) and carotenoids were determined according to the method of Strain and Svec (1966) with 80% acetone as the solvent.

$$\text{Chl a (mgmL}^{-1}\text{)} = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chl b (mgmL}^{-1}\text{)} = 21.5A_{646.8} - 5.1A_{663.2}$$

$$\text{Chl Total (mgmL}^{-1}\text{)} = \text{Chl a} + \text{Chl b}$$

$$\text{Car} = (1000A_{470} - 1.82 \text{Chl a} - 85.02 \text{Chl b}) / 198$$

where,  $A_{663}$ ,  $A_{645}$ , and  $A_{470}$  represent absorbance values read at 663, 645 and 470 nm wavelengths, respectively.

### ***Estimation of lipid peroxidation***

Lipid peroxidation was evaluated in terms of malonyldialdehyde (MDA) content (Ksouri *et al.* 2007). Fresh samples of shoots (250 mg fresh weight) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000g for 10 min at 4°C, and a 1 mL supernatant was mixed with 5 mL of 0.5% thiobarbituric acid (TBA) prepared in TCA 20% and incubated at 95°C for 30 min. Reaction was stopped by placing the tubes in an ice bath and samples were centrifuged at 10000g for 5 min. The absorbance of supernatant was measured at 532 nm and after subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient of 155 mM<sup>-1</sup>cm<sup>-1</sup>.

### ***Enzyme extraction and assay***

Enzyme extraction procedure was accomplished according to Chen *et al.* (2000) with some modification. All of the following operations were performed at 4°C. Fresh leaf samples (1g) were ground in a mortar with liquid nitrogen and

extracted in 100 mM Na-phosphate buffer (pH 6), containing 0.1 mM EDTA. The homogenate was centrifuged at 12000 g for 20 min. The supernatant was transferred to Eppendorf tubes and kept in the -20°C freezer. Superoxide dismutase (SOD) activity was assayed in 100 mM potassium phosphate buffer, pH 7.5, 150 mM methionine L-methionine, 840 mM Nitroblue tetrazolium (NBT) and 24 μM riboflavin by using the photochemical NBT method in terms of superoxide dismutase's ability to inhibit reduction of NBT to form formazan by superoxide (Sairam *et al.* 2002). The photoreduction of NBT was measured at 560 nm. Catalase (CAT) activity was evaluated by determining the consumption of H<sub>2</sub>O<sub>2</sub> ( $\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ ) at 240 nm in 50 mM phosphate buffer, pH 7.5 and 200 mM H<sub>2</sub>O<sub>2</sub> (Nemat-Ala and Hassan 2006). Total ascorbate peroxidase (APX) activity was evaluated spectrophotometrically according to Kato & Shimizu (1985) at 280 nm in 0.2 mM potassium phosphate buffer, pH 7.5, 15mM ascorbic acid and 50 mM H<sub>2</sub>O<sub>2</sub>, as ascorbate ( $\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ) was oxidized. Guaiacol peroxidase activity was assayed in 44 mM H<sub>2</sub>O<sub>2</sub> and 45 mM guaiacol. The absorption at 470 nm was recorded and the activity was calculated using the extinction coefficient of 26.6 mM<sup>-1</sup> cm<sup>-1</sup> (Buchanan and Balm 2005). All enzyme activities were expressed as units per mg of protein. Protein content in all enzyme extracts was determined according to Bradford (1976).

### ***Polyphenol extraction and estimation***

Fresh shoots of *A. aucheri* plants were shade dried for one week and ground to fine powder. A sample (1 g) of this dry powder was extracted with 80%

methanol with stirring for 30 min. The extracts were then kept for 24h at 4°C, filtered through a Whatman No. 4 filter paper and evaporated under vacuum. Phenolic compounds were assayed using the Folin-Ciocalteu reagent, following Singleton's method with slight modification (Ksouri *et al.* 2007). A sample of extract (0.125 mL, diluted 10-fold) was added to 0.5 mL of deionized water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 mL of 7% Na<sub>2</sub>CO<sub>3</sub> solution. The solution was then diluted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 80 min at 23°C, the absorbance of sample reaction was read at 760 nm. Total phenolic concentration of plants (three replicates per treatment) was expressed as mg gallic acid equivalents (GAE) g<sup>-1</sup>dry weight through a calibration curve with gallic acid.

#### **Assay of DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical-scavenging activity**

The antioxidant activity of extracts was assayed based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrozyl (DPPH) free radical (Ksouri *et al.* 2007). Methanolic extracts of shoots (2 mL) were mixed with 0.5 mL of 0.2 mM methanolic DPPH; the mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of resulting solution was measured at 517 nm. The scavenging activity was expressed as IC<sub>50</sub> (µg g<sup>-1</sup>dry wt.). The ability to scavenge the DPPH radical was calculated as:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> absorbance of extract or standard.

#### **Assay of superoxide anion radical (<sup>•</sup>O<sub>2</sub><sup>-</sup>)-scavenging activity**

Measurement of superoxide anion scavenging activity was based on the method of Kumaran and Joel Karunakaran (2006). The reaction mixture consisted of 50 mM phosphate buffer, pH 7.6, 20 µg riboflavin, 12 mM EDTA and NBT 0.1 mg 3mL<sup>-1</sup>, added in that sequence. Reaction was started by illuminating (fluorescent lamp) the reaction mixture with different concentrations of the extract for 80 seconds. Immediately after illumination, the absorbance was read at 580 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes, with reaction mixture were kept in the dark and served as blanks. The antioxidant activity of the extracts was based on IC<sub>50</sub> (µg g<sup>-1</sup>dry wt.). The superoxide anion radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> was the absorbance of the extract/standard.

#### **Assay of hydroxyl-radical (<sup>•</sup>OH) scavenging activity**

Hydroxyl radical scavenging activity of methanolic extracts was measured according to Yuan *et al.* (2005). The final reaction solution (1 mL) consisted of aliquots (500 µl) of various concentrations of the methanolic extracts of shoots of *A. aucheri*, 1mM FeCl<sub>3</sub>, 1mM Na<sub>2</sub>EDTA, 10 mM H<sub>2</sub>O<sub>2</sub>, 1 mM L-ascorbic acid, 36 mM 2-deoxy-D-ribose in 25 mM phosphate buffer (pH7.4). The reaction mixture was incubated for 1 h at 37 °C and further heated in a boiling water bath for 15 min after addition of 1 mL of 2.8% TCA and 1 mL of

1% TBA. The color development was measured at 523 nm. Evaluating the antioxidant activity of the extracts was based on IC<sub>50</sub> (μg g<sup>-1</sup> dry wt.). The antiradical activity was expressed as IC<sub>50</sub> (μg g<sup>-1</sup> dry wt.). The ability to scavenge the hydroxyl radical was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> was the absorbance of extract/standard.

### **Reducing power determination**

The reducing power of methanolic extracts of shoots of *A. aucheri* was determined according to the method of Kumaran and Joel Karunakaran (2006). Different amounts of the extract (50-1500 μg mL<sup>-1</sup>) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>]. Mixture was incubated at 50°C for 20 min, followed by addition of 2.5 mL of 10% TCA, and then centrifuged for 10 min. The upper layer of solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% FeCl<sub>3</sub>, and the absorbance measured at 700 nm. Increase in absorbance of the reaction mixture indicated increased reducing power.

### **Statistical analysis**

The experiment was laid as factorial based on completely randomized design. The data was analyzed using the SAS (V. 9.0) software and the least significant difference (LSD) among treatment means for each trait was calculated. All the measurements were carried out in triplicate and were expressed as means of three analyses ± standard error. P values less than 0.05 were considered to be statistically significant. The

relationships between dependent and independent variables were carried out using the polynomial regression function in the Excel program 2007.

### **Results**

Results showed that salinity (150 mM) significantly decreased the biomass of *A. aucheri* by 54% as compared to control (p≤0.05) (Figure 1A). H<sub>2</sub>O<sub>2</sub> priming, at all applied levels, brought about a large increase in the biomass of salinized plants of *A. aucheri* (between 1.7 to 2.2 folds compared to the saline condition alone). H<sub>2</sub>O<sub>2</sub> priming also caused a significant increase in the biomass of non-salinized plants (+1.7 folds) compared to control. H<sub>2</sub>O<sub>2</sub> priming at 90 μM was the most effective level to increase the biomass under both control and saline conditions.

Total water content significantly decreased by salt stress (p≤0.05) (Figure 1B). H<sub>2</sub>O<sub>2</sub> priming at 90 μM, increased this variable significantly (p≤0.05). Under non-saline condition, total water content increased by H<sub>2</sub>O<sub>2</sub> priming, but it didn't significantly differ from control (Figure 1B).

Data analysis revealed that salt stress (150mM) decreased total chlorophyll content by 58% as compared to control (Figure 2A). H<sub>2</sub>O<sub>2</sub> priming significantly increased chlorophyll (a+b) concentration under salinity (p≤0.05). H<sub>2</sub>O<sub>2</sub> priming, particularly at 50 and 90 μM, augmented total chlorophyll content in the aerial parts of *A. aucheri* under both normal and saline conditions (Figure 2A). The percentage increase ranged from 9 to 16% and 38 to 41% under normal and saline conditions, respectively.

Carotenoids content decreased (75%) by 150 mM of NaCl (Figure 2B). Priming with H<sub>2</sub>O<sub>2</sub>

significantly increased carotenoids level in *A. aucheri*, but this positive effect was dominant under normal condition. At the absence of NaCl, applied concentrations of H<sub>2</sub>O<sub>2</sub> increased carotenoids content from 1.4 to 2.2 folds, as 10 μM

H<sub>2</sub>O<sub>2</sub> was the best priming level. Under salt stress, carotenoids concentration augmented up to 47% by H<sub>2</sub>O<sub>2</sub> priming, but there was no significant difference between different levels of H<sub>2</sub>O<sub>2</sub> priming (Figure 2B).

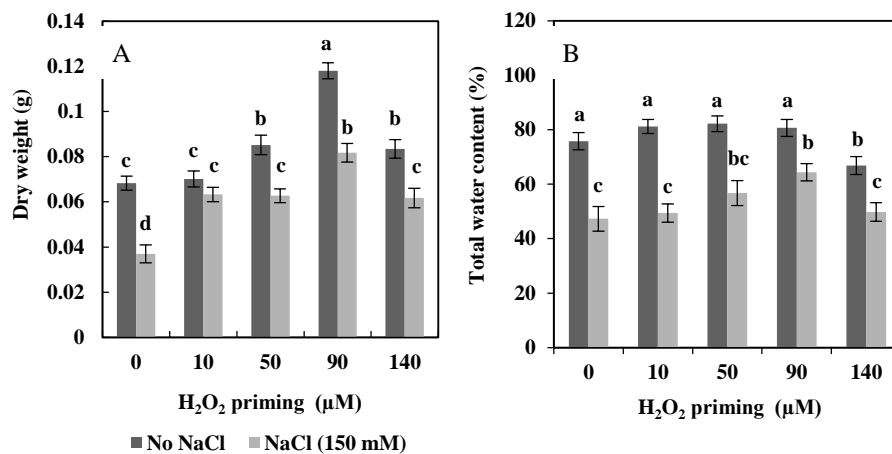


Figure 1. Plants of 45-day-old *Artemisia aucheri* primed with H<sub>2</sub>O<sub>2</sub> and/or irrigated for 31 days with NaCl (150 mM). A) Dry weight, B) Total water content. Means (three replicates) with the same letter are not significantly different at  $p \leq 0.05$ . Bars represent standard errors.

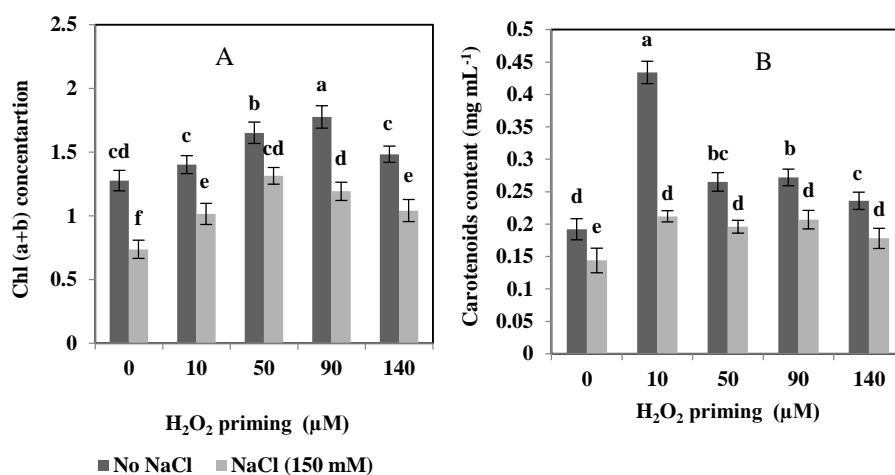


Figure 2. Plants of 45-day-old *Artemisia aucheri* primed with H<sub>2</sub>O<sub>2</sub> and/or irrigated for 31 days with NaCl (150 mM). A) Chlorophyll (a+b) concentration, B) Carotenoids content. Means (three replicates) with the same letter are not significantly different at  $p \leq 0.05$ . Bars represent standard errors.

Results revealed that salinity (150 mM) increased the concentration of H<sub>2</sub>O<sub>2</sub> (over 2 folds) in the aerial parts of *A. aucheri* compared to control (Figure 3A). H<sub>2</sub>O<sub>2</sub> priming significantly reduced the level of H<sub>2</sub>O<sub>2</sub> (from 20 to 43%) in the shoots of salinized plants ( $p \leq 0.05$ ). H<sub>2</sub>O<sub>2</sub> priming at 90  $\mu$ M showed the highest effect to diminish H<sub>2</sub>O<sub>2</sub> level in the shoot. At the absence of NaCl, H<sub>2</sub>O<sub>2</sub> priming resulted in reducing of H<sub>2</sub>O<sub>2</sub> level in the tissues by 35%, particularly at 50 and 90  $\mu$ M.

As is shown in Figure 3B, NaCl at 150 mM significantly increased the MDA concentration by 2 folds as compared to control, indicating an increase of lipid peroxidation in the salinized shoots of *A. aucheri*. H<sub>2</sub>O<sub>2</sub> priming, however, decreased MDA concentration in the plants grown in the saline culture solution ( $p \leq 0.05$ ). This decrease ranged from 20 to 42% by different levels of H<sub>2</sub>O<sub>2</sub> priming. At the absence of NaCl, the lowest amount of MDA was recorded in plants of pretreated seeds with 90  $\mu$ M H<sub>2</sub>O<sub>2</sub> (29% compared to control). Also, the most effective level of H<sub>2</sub>O<sub>2</sub> priming to reduce MDA (by 19% compared to control) in the salinized plants was 90  $\mu$ M (Figure. 3B).

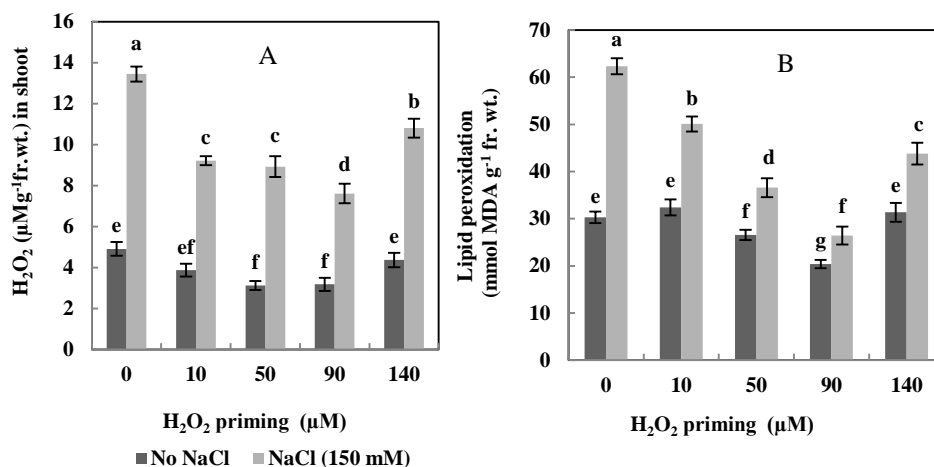
Salt stress alone, significantly decreased the level of polyphenols by 25% as compared to control ( $p \leq 0.05$ ) (Figure 4A). H<sub>2</sub>O<sub>2</sub> priming significantly increased the total phenolic concentration in both normal and saline conditions ( $p \leq 0.05$ ). At the absence of NaCl, H<sub>2</sub>O<sub>2</sub> priming augmented the level of polyphenols from 1.2 to 2 folds. In this case, H<sub>2</sub>O<sub>2</sub> priming at 50 and 90  $\mu$ M displayed the best outcome, with no significant difference between them. At the presence of NaCl

(150 mM), H<sub>2</sub>O<sub>2</sub> priming brought about an increase in phenolic concentration from 1.6 to 2.7 folds. The highest value was obtained from the H<sub>2</sub>O<sub>2</sub> priming at 90  $\mu$ M.

Results showed that DPPH-radical scavenging activity of the methanolic extract of *A. aucheri* (IC<sub>50</sub>= 245.1  $\mu$ g g<sup>-1</sup>dry wt.) was significantly affected by H<sub>2</sub>O<sub>2</sub> priming and salinity ( $p \leq 0.05$ ) (Figure 4B). Salt stress reduced DPPH-radical scavenging activity (IC<sub>50</sub>= 301.7  $\mu$ g g<sup>-1</sup>dry wt.), while priming with H<sub>2</sub>O<sub>2</sub> increased antioxidant properties in *A. aucheri* under both saline and non-saline conditions. The most efficient H<sub>2</sub>O<sub>2</sub> priming was observed at 90  $\mu$ M which caused a decrease in the IC<sub>50</sub> values by 55 and 60% in the saline and control samples, respectively. Apart from the effect of H<sub>2</sub>O<sub>2</sub> at 90  $\mu$ M, hydrogen peroxide at 50 and 140  $\mu$ M were more effective to increase this variable compared to the primed plants with 10  $\mu$ M.

IC<sub>50</sub> for superoxide anion radical scavenging activity in the aerial parts of *A. aucheri* was 116.7  $\mu$ g g<sup>-1</sup>dry wt. in the non-saline condition, which increased to 175.3  $\mu$ g g<sup>-1</sup>dry wt. under salt stress (near to +1.5 folds) (Figure 5A). Exposure of seeds to different concentrations of H<sub>2</sub>O<sub>2</sub> significantly increased superoxide anion radical scavenging activity and the IC<sub>50</sub> values ranged from 53.3 to 86.5  $\mu$ g g<sup>-1</sup>dry wt. under control and saline conditions. H<sub>2</sub>O<sub>2</sub> priming at 90  $\mu$ M was the most effective level in decreasing IC<sub>50</sub> for superoxide radical scavenging activity under saline condition (40.5% as compared with the treated plants only by NaCl).

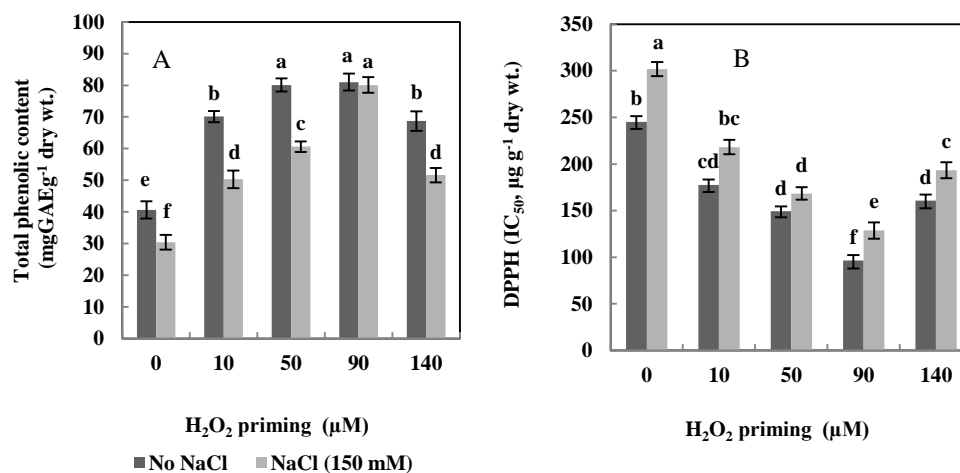




**Figure 3.** Plants of 45-day-old *Artemisia aucheri* primed with H<sub>2</sub>O<sub>2</sub> and/or irrigated for 31 days with NaCl (150 mM). A) H<sub>2</sub>O<sub>2</sub> concentration, B) MDA concentration in the shoots. Means (three replicates) with the same letter are not significantly different at  $p \leq 0.05$ . Bars represent standard errors.

The IC<sub>50</sub> value for hydroxyl radical scavenging activity in the aerial parts of *A. aucheri* was 183.6 μg g<sup>-1</sup> dry wt. in normal condition, which increased to 247.5 μg g<sup>-1</sup> dry wt. under salinity (near to +1.4 folds) (Figure 5B). Introduction of seeds to the utilized levels of H<sub>2</sub>O<sub>2</sub> significantly augmented <sup>•</sup>OH-scavenging activity; the IC<sub>50</sub> values ranged

from 103.7 to 202.7 μg g<sup>-1</sup> dry wt. in both control and saline conditions. Under saline condition, H<sub>2</sub>O<sub>2</sub> priming at 90 μM was most effective to decrease the IC<sub>50</sub> value for <sup>•</sup>OH-scavenging activity (42% decrease compared to the primed plants only by NaCl). Furthermore, H<sub>2</sub>O<sub>2</sub> priming at 50 and 140 μM showed the second ranking with no statistical



**Figure 4.** Plants of 45-day-old *Artemisia aucheri* primed with H<sub>2</sub>O<sub>2</sub> and/or irrigated for 31 days with NaCl (150 mM). A) Total phenolic content, B) DPPH scavenging activity of the shoots. Means (three replicates) with the same letter are not significantly different at  $p \leq 0.05$ . Bars represent standard errors.

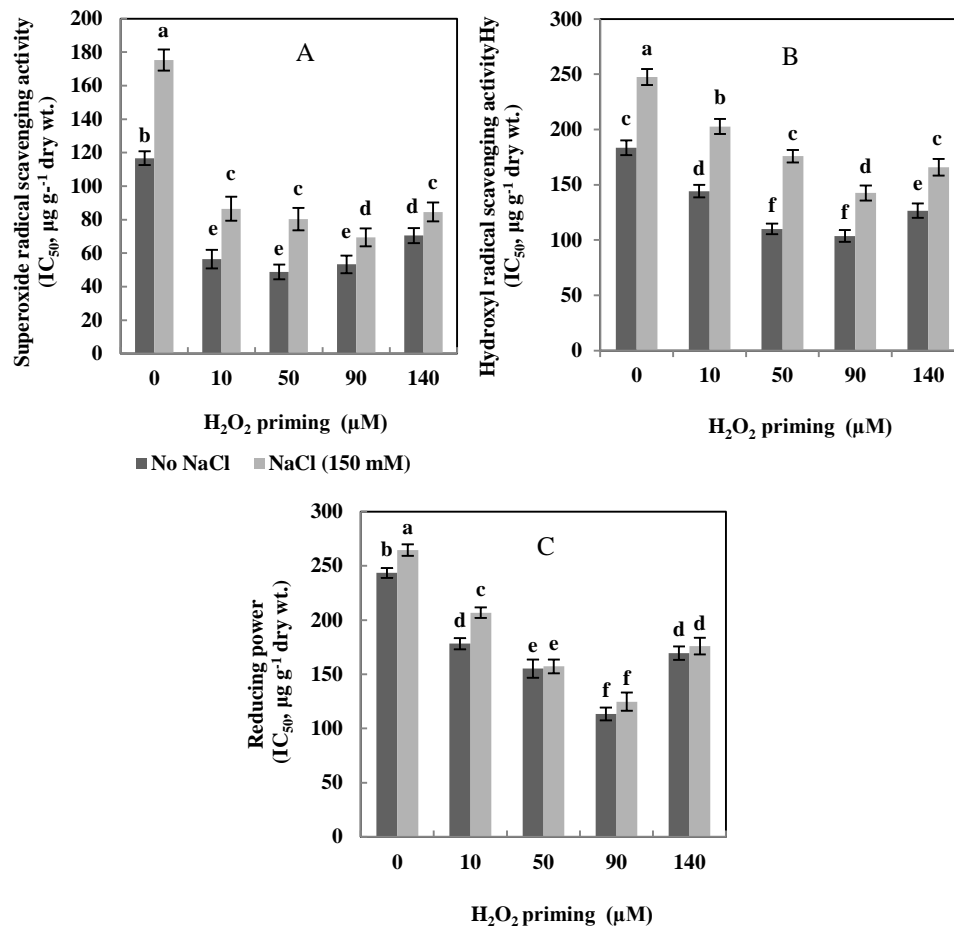
difference. Under non-saline condition OH-scavenging activity of the extracts of primed *A. aucheri* was the highest at 50 and 90  $\mu\text{M}$   $\text{H}_2\text{O}_2$ .

Data analysis indicated that salt stress (150 mM) resulted in a significant increase in  $\text{IC}_{50}$  (264.6  $\mu\text{g g}^{-1}$  dry wt) for reducing power of *A. aucheri* (9% increase compared to control) ( $p \leq 0.05$ ) (Figure 5C).  $\text{H}_2\text{O}_2$  priming significantly increased the reducing power in both non-salinized and salinized plants (ranged from 113.3 to 206.8  $\mu\text{g g}^{-1}$  dry wt). Increasing the level of  $\text{H}_2\text{O}_2$  from 10 to 90  $\mu\text{M}$  enhanced the effects of  $\text{H}_2\text{O}_2$ , but plants from  $\text{H}_2\text{O}_2$ -primed seeds at 90  $\mu\text{M}$  exhibited the highest level of reducing ability in both normal and saline conditions compared to the control values. After 90  $\mu\text{M}$  level and at the second rank,  $\text{H}_2\text{O}_2$  at 50  $\mu\text{M}$  was more efficient to enhance reducing power of *A. aucheri* compared to other  $\text{H}_2\text{O}_2$  priming.

The lowest activity of superoxide dismutase was recorded in the control plants (Figure 6A). Salt stress and  $\text{H}_2\text{O}_2$  priming significantly increased the activity of superoxide dismutase compared to control ( $p \leq 0.05$ ). Almost in all  $\text{H}_2\text{O}_2$ -primed

salinized plants, however, the activity of this enzyme was significantly higher than that in the plants from  $\text{H}_2\text{O}_2$ -primed seeds grown in normal condition. Under saline condition, the highest value of superoxide dismutase activity was found in the plants from primed seeds with 90  $\mu\text{M}$  (+ 1.2 folds more than that in the exclusively salinized plants). At the absence of NaCl, primed *A. aucheri* with 90  $\mu\text{M}$   $\text{H}_2\text{O}_2$  exhibited the highest value for the superoxide dismutase activity (+1.5 folds compared to control).

Catalase activity significantly increased by NaCl and  $\text{H}_2\text{O}_2$  ( $p \leq 0.05$ ) (Figure 6B). At the absence of salt, all plants from  $\text{H}_2\text{O}_2$ -primed seeds showed higher activity of catalase compared to control (ranged from +39% to 1.9 folds associated with 140 and 90  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$ , respectively). Under salt stress, catalase activity increased from 43 to 81% in all primed *A. aucheri* compared to the exclusively salinized plants. Plants from primed seeds with 10, 50 and 90  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  were at the same position, but higher than the salinized plants from primed seeds with 140  $\mu\text{M}$   $\text{H}_2\text{O}_2$ .



**Figure 5.** Plants of 45-day-old *Artemisia aucheri* primed with H<sub>2</sub>O<sub>2</sub> and/or irrigated for 31 days with NaCl (150 mM). A) Superoxide anion radical scavenging activity, B) Hydroxyl radical scavenging activity, C) Reducing power of the shoots. Means (three replicates) with the same letter are not significantly different at  $p \leq 0.05$ . Bars represent standard errors.

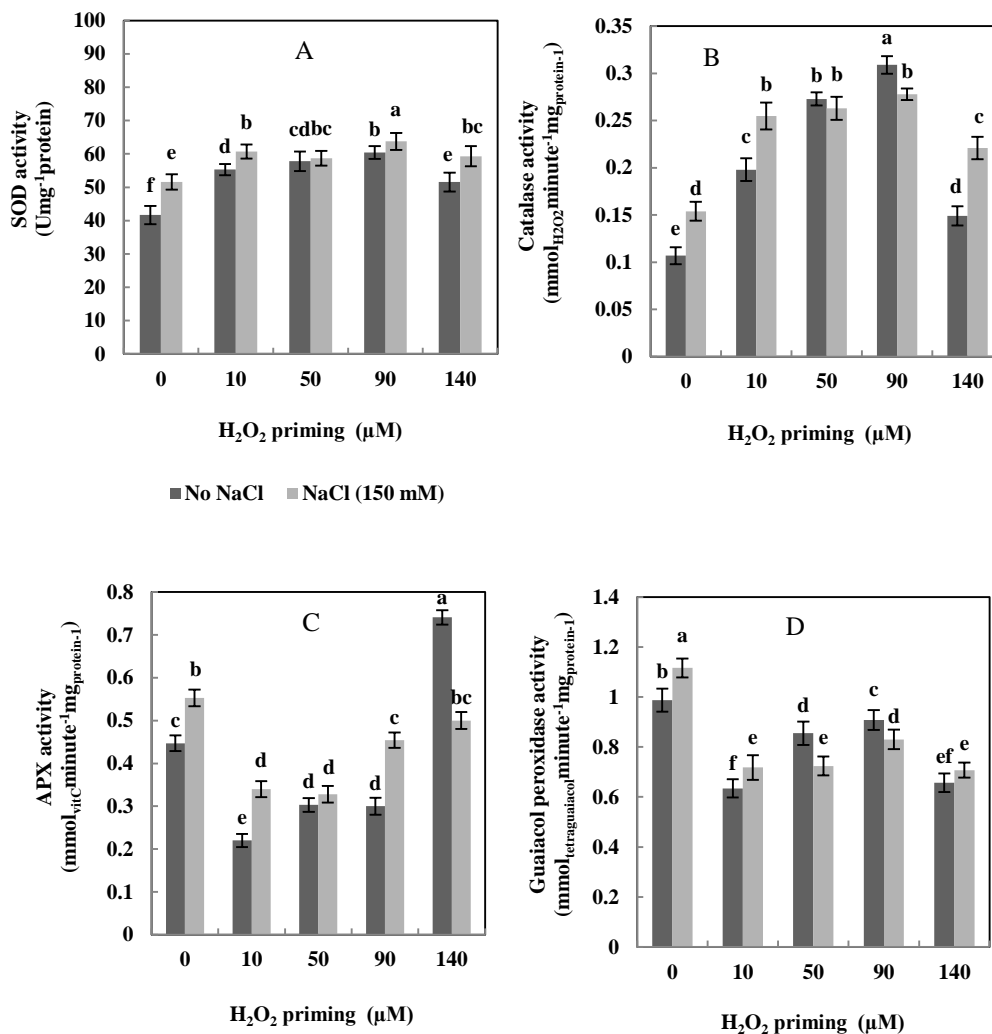
Data analysis showed that salt stress alone at 150 mM caused an increase in ascorbate peroxidase activity by 1.2 folds compared to control ( $p \leq 0.05$ ) (Figure 6C). Under non-saline condition, H<sub>2</sub>O<sub>2</sub> priming at 10, 50 and 90 µM decreased ascorbate peroxidase activity from 32% (at 10 µM H<sub>2</sub>O<sub>2</sub>) to 50% (at 50 and 90 µM H<sub>2</sub>O<sub>2</sub>). Ascorbate peroxidase activity, however, in the plants from primed seeds with 140 µM H<sub>2</sub>O<sub>2</sub> increased by 1.6 folds compared to control ( $p \leq 0.05$ ). Under saline condition, H<sub>2</sub>O<sub>2</sub> priming significantly decreased ascorbate peroxidase

activity from 18 to 39% ( $p \leq 0.05$ ), excluding H<sub>2</sub>O<sub>2</sub> priming at 140 µM. No significant difference was obtained for ascorbate peroxidase activity in the salinized plants from primed seeds with 140 µM H<sub>2</sub>O<sub>2</sub> and control.

Results illustrated that guaiacol peroxidase activity had the highest values in the exclusively salinized and control *A. aucheri* plants (Figure 6D). Under salt stress, the activity of this enzyme was 1.1-fold more than that in the control ( $p \leq 0.05$ ). Reduction of guaiacol peroxidase activity (ranged from -26 to -36%), however, occurred in the shoots

of primed *A. aucheri* under salinity. Under salinity (150 mM NaCl), the higher activity of guaiacol

peroxidase was observed in the plants from primed at 90  $\mu\text{M}$   $\text{H}_2\text{O}_2$ .



**Figure 6.** Antioxidant enzyme activity in the shoots of 45-day-old *Artemisia aucheri* plants primed with  $\text{H}_2\text{O}_2$  and/or irrigated for 31 days with NaCl (150 mM). A) Superoxide dismutase activity, B) Catalase activity, C) Ascorbate peroxidase activity, D) Guaiacol peroxidase activity. Means (three replicates) with the same letter are not significantly different at  $p \leq 0.05$ . Bars represent standard errors.

## Discussion

In the current study, we investigated the effect of  $\text{H}_2\text{O}_2$  priming to reduce oxidative stress due to NaCl in *A. aucheri*. Numerous studies demonstrated that salinity provokes oxidative stress in plants, driving to cell damage or death, and the ability of plants to ROS detoxification through

enhancing endogenous antioxidant system, leading to decrease deleterious effects of salt stress (Gill and Tuteja 2010). Plants employ an internal complex defensive system to eliminate or reduce detrimental effects of oxidative stress. It includes enzymatic and non-enzymatic antioxidant components (Apel and Hirt 2004). Antioxidant

enzymes can directly operate ROS detoxification or cooperate by producing non-enzymatic antioxidants. A concerted action of antioxidant components is required to detoxify ROS such as  $\cdot\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ . Superoxide dismutase converts  $\cdot\text{O}_2^-$  to  $\text{H}_2\text{O}_2$ . Peroxidase has an essential role in scavenging  $\text{H}_2\text{O}_2$  which is produced through dismutation of  $\cdot\text{O}_2^-$  catalyzed by superoxide dismutase. Catalase, as a main enzyme, removes or reduces  $\text{H}_2\text{O}_2$  in the mitochondrion and microbody (Shigeoka *et al.* 2002). Also, non-enzymatic antioxidants associate with radical scavenging activity and the maintenance of photosynthetic membranes integrity over oxidative stress. Thus, all these enzymes help in alleviation of the injurious effects of oxidative stress. Results obtained from evaluation of growth characters including dry weight, total water content and photosynthetic pigments concentration (Figures 1 & 2) suggested that  $\text{H}_2\text{O}_2$  priming could increase salt tolerance in *A. aucheri* plants. This result was consistent with the previous studies on wheat and citrus under salt stress (Wahid *et al.* 2007; Tanou *et al.* 2009). On the other hand, measuring several variables related to oxidative stress i.e. MDA and  $\text{H}_2\text{O}_2$  concentrations, activities of antioxidant enzymes (particularly superoxide dismutase, catalase and ascorbate peroxidase) and radical scavenging activity (Figures 3-6) revealed that positive effects of  $\text{H}_2\text{O}_2$  priming to enhance salt tolerance was associated with the reduction of oxidative damages in *A. aucheri* plants. This finding was in agreement with previous reports about other priming in *Artemisia* species (Azimian & Roshandel 2015; Roshandel & Azimian 2015).

Although salinity significantly increased  $\text{H}_2\text{O}_2$  and MDA concentrations, but  $\text{H}_2\text{O}_2$  priming (particularly at 90  $\mu\text{M}$ ) decreased the value of these variables at both normal and saline conditions. Similar results have already expressed by other researches (Lin *et al.* 2010; Gondim *et al.* 2012). It has been suggested that  $\text{H}_2\text{O}_2$  pretreatment operates a  $\text{H}_2\text{O}_2$  signaling process in seed and subsequently seedlings to activate antioxidant systems (Wahid *et al.* 2007) and at the first stage a decrease in  $\text{H}_2\text{O}_2$  concentration would appear in the tissues of salinized plant. Current data showed that  $\text{H}_2\text{O}_2$  priming was also effective in increasing membrane integrity (subsequently a decrease in MDA concentration) (Figure 3), chlorophylls and carotenoids content in *A. aucheri* plants which were coincided with decreasing  $\text{H}_2\text{O}_2$  in the tissues. At the cellular level, the intensity of lipid peroxidation of the membranes (occurred by ROS) increases in salinized plants. As a result, MDA concentration builds up, which is often used as an indicator of oxidative damage. Wahid *et al.* (2007) believed that improved membrane integrity and decreased ion leakage were a consequence of low level of  $\text{H}_2\text{O}_2$  due to pretreatment of wheat seeds with  $\text{H}_2\text{O}_2$ . This occurrence was also reported by Azevedo-Neto *et al.* (2005), Gao *et al.* (2010) and Gondim *et al.* (2012). Protection of membrane integrity by  $\text{H}_2\text{O}_2$  priming could be the main reason to protect chloroplasts ultrastructure, leading to an increase in total chlorophylls and carotenoids content in the salinized plants.

Data analysis showed that the activity of all evaluated antioxidant enzymes augmented in response to salinity (Figure 6). However, the effect of  $\text{H}_2\text{O}_2$  priming on the activity of superoxide

dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase appeared in different patterns. Superoxide dismutase and catalase showed maximum responses to H<sub>2</sub>O<sub>2</sub> priming, particularly at 90 µM. It seems that the higher activity of these two enzymes would be the main causes for decreasing H<sub>2</sub>O<sub>2</sub> and subsequently enhancing salt tolerance in H<sub>2</sub>O<sub>2</sub> primed *A. aucheri*. Positive effects of H<sub>2</sub>O<sub>2</sub> priming in triggering adaptive responses have been already reported (Wahid *et al.* 2007; Kumar *et al.* 2010; Hossain *et al.* 2015) and proposed that the ability of H<sub>2</sub>O<sub>2</sub>-treated plants to reduce injurious effects of NaCl is highly associated with the increased activity of antioxidant enzymes. Furthermore, Gondim *et al.* (2012) showed that H<sub>2</sub>O<sub>2</sub> treatment highly increased catalase activity which was mostly responsible for alleviation of oxidative damages due to salt stress. Under other abiotic stresses such as Al and Cd stresses, the benefit of H<sub>2</sub>O<sub>2</sub> priming was also correlated with the induction of antioxidant defense capacity to scavenge generated ROS during stressful conditions (Chao and Kao 2010; Xu *et al.* 2011). Previous studies have indicated the improvement of relative water content in plants by H<sub>2</sub>O<sub>2</sub> treatment (Kukerja *et al.* 2005; He *et al.* 2009). In line with these findings, our results showed an increment in water content of primed *A. aucheri* at both normal and saline conditions. Kukerja *et al.* (2005) expressed that augmentation of ABA content and induction of antioxidant system were involved in this phenomenon. Tanou *et al.* (2009) revealed that treatments with low levels of H<sub>2</sub>O<sub>2</sub>, regardless of NaCl presence, altered specific proteins involved in photosynthesis, defense and energy metabolism.

Moreover, a stimulation of protein S-nitrosylation was observed in H<sub>2</sub>O<sub>2</sub>-primed citrus plants under salinity (Tanou *et al.* 2009). In this way, present data revealed highly induced protein level in the aerial parts of primed *A. aucheri*, regardless of salt stress (data not shown).

Total phenolic content increased by different levels of H<sub>2</sub>O<sub>2</sub> priming in all primed *A. aucheri*, regardless of NaCl presence (Figure 4). Seed priming with 90 µM H<sub>2</sub>O<sub>2</sub> increased the values of polyphenols in both salinized and non-salinized plants. Polyphenols which are categorized in secondary metabolites have wide distribution and function in different manners such as electron donors, enzyme co-factors and reducing compounds (Arbona *et al.* 2003). Evidence strongly supports that plants with higher total phenolic content have higher antioxidant activities (Maisuthisakul *et al.* 2005). A higher radical scavenging activity which was observed in all primed *A. aucheri* plants (particularly at 90 µM) indicated the positive effect of H<sub>2</sub>O<sub>2</sub> priming on increasing antioxidant activity. Our data indicated that antioxidant activities of superoxide dismutase and catalase were in coordinate with non-enzymatic antioxidant activity which measured as hydroxyl, superoxide anion and DPPH radical scavenging activities and reducing power of the methanolic extracts of primed salinized *A. aucheri* (particularly at 90 µM). Some reports have suggested the central role of non-enzymatic antioxidants in H<sub>2</sub>O<sub>2</sub>-signaling to ameliorate oxidative damage due to NaCl. For instance, Yu *et al.* (2003) stated that H<sub>2</sub>O<sub>2</sub> treatment increased chilling tolerance by enhancing the glutathione level in mung bean seedlings. In another study,

Chao and Kao (2010) showed the up-regulation of ascorbate production in H<sub>2</sub>O<sub>2</sub>-treated rice seedlings under Cd stress.

### Conclusion

Overall, it could be concluded that H<sub>2</sub>O<sub>2</sub> priming (especially at 90 µM) can increase salt tolerance in *A. aucheri* plants via mitigation of oxidative stress. Apparently, H<sub>2</sub>O<sub>2</sub> acts as a signal molecule to enhance activation of enzymatic and non-enzymatic antioxidant systems in the seeds, which preserves in the seedlings to counteract the ion-induced oxidative damage. Furthermore, H<sub>2</sub>O<sub>2</sub> priming can increase medicinal properties of *A. aucheri* through augmentation of total phenolic content and radical scavenging activity in this

species under both normal and saline conditions. The current results suggest that enhancement of salt tolerance in *A. aucheri* by H<sub>2</sub>O<sub>2</sub> priming is substantially due to decreasing in H<sub>2</sub>O<sub>2</sub> concentration and lipid peroxidation. This reduction of lipid peroxidation results in improvement of cellular membrane integrity helping to maintain the ultrastructure of chloroplasts and vacuoles. Furthermore, increase in the activity of antioxidant enzymes (particularly superoxide dismutase and catalase) could also increase salt tolerance in H<sub>2</sub>O<sub>2</sub> primed *A. aucheri*. Among applied H<sub>2</sub>O<sub>2</sub> concentrations in this study, 90 µM appeared as the most efficient level to obtain *A. aucheri* plants with enhanced salt tolerance as well as elevated medicinal properties.

### References

- Aebi H, 1984. Catalase *in vitro*. *Methods Enzymology* 105: 121-126.
- Apel K and Hirt H, 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annual Review of Plant Biology* 55: 373-399.
- Arbona V, Flors V, Jacas J, García-Agustín P and Gómez-Cadenas A, 2003. Enzymatic and Non-enzymatic antioxidant responses of *Carrizo citrange*, a salt-sensitive citrus rootstock, to different levels of salinity. *Plant Cell Physiology* 44 (4): 388-394.
- Asghar G, Jalili M and Sadooghi E, 2012. Antimicrobial activity and chemical composition of essential oil from the seed of *Artemisia aucheri* Boiss. *Jundishapur Journal of Natural Pharmaceutical Products* (1): 11-15.
- Azevedo-Neto AD, Prisco JT, Enéas-Filho J, Medeiros JV and Gomes-Filho E, 2005. Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *Journal of Plant Physiology* 162 (10): 1114-1122.
- Azimian F and Roshandel P, 2015. Magnetic field effects on total phenolic content and antioxidant activity in *Artemisia sieberi* under salinity. *Indian Journal of Plant Physiology* 20 (3): 264-270.
- Babaahmadi M, Amjad L and Roozbehani SH, 2013. The effect of allergenicity of *Artemisia aucheri* flowering taps in guinea pigs. *International Journal of Agriculture and Crop Sciences* 5 (18): 2079-2083.
- Bahrami Karondi M, Moshtaghyan SJ, Madani SH, Manzoni P, Adibi SH and Kazemi S, 2010. Effect of extract hydro-alcoholi *Artemisia aucheri* on fibrosis induced pulmonary by bleomycin in rats. *Journal of Shahrekord University Medical Science* 3(4): 33-40 (In Persian with English abstract).
- Bradford M, 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Annals of Biochemistry* 72: 248-254.
- Buchanan BB and Balmer Y, 2005. Redox regulation: a broadening horizon. *Annual Review of Plant Biology* 56: 187-220.
- Chao YY and Kao CH, 2010. Heat shock-induced ascorbic acid accumulation in leaves increases cadmium tolerance of rice (*Oryza sativa* L.) seedlings. *Plant and Soil* 336: 39-48.
- Chen DH, Ye HC and Li GF, 2000. Expression of a chimeric farnesyldiphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation. *Plant Science* 155: 179-185.

- Dinani NJ, Asgary A, Madani H, Naderi G and Mahzoni P, 2010. Hypocholesterolemic and antiatherosclerotic effect of *Artemisia aucheri* in hypercholesterolemic rabbits. *Pakistan Journal of Pharmaceutical Science* 23 (3): 321-325.
- Gao Y, Guo YK, Lin SH, Fang YY and Bai JG, 2010. Hydrogen peroxide pretreatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat stressed cucumber leaves. *Scientia Horticulturae* 126: 20–26.
- Gill SS and Tuteja N, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48: 809-930.
- Gondim FA, Gomes-Filho E, Costa JH, Mendes Alencar NL and Prisco JT, 2012. Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize. *Plant Physiology and Biochemistry* 56: 62-71.
- He L, Gao Zh and Li R, 2009. Pretreatment of seed with H<sub>2</sub>O<sub>2</sub> enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. *African Journal of Biotechnology* 8 (22): 6151-6157.
- Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li H, Burritt DJ, Fujita M and Tran LP, 2015. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Frontiers in Plant Science* 6: 1-19.
- Kato M and Shimizu S, 1985. Chlorophyll metabolism in higher plants. *Plant Cell Physiology* 26: 1291-1301.
- Karuppanapandian T, Moon JC, Kim C, Manoharan K and Kim W, 2011. Reactive oxygen species in plants: their generation, signal transduction and scavenging mechanisms. *Australian Journal of Crop Science* 5 (6): 709-725.
- Khajehzadeh MH, Zare-maivan H, Ghanati F and Sharifi M, 2014. Changes of enzymes activity and production of secondary metabolites of *Artemisia aucheri* in different altitudes and its relation to adaptation. *Journal of Chemical Health Risks* 4 (3): 57–66.
- Krishnaiah D, Sarbatly R and Nithyanandam D, 2010. A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing* 24 (4-5): 506-515.
- Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C and Abdelly C, 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of halophyte *Cakile maritima*. *Plant Physiology and Biochemistry* 45: 244-249.
- Kukreja S, Nandwal AS, Sharma SK, Unvi V and Sharma PK, 2005. Plant water status, H<sub>2</sub>O<sub>2</sub> scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biologia Plantarum* 49 (2): 305-308.
- Kumar M, Sirhindi G, Bhardwaj R, Kumar S and Jain G, 2010. Effect of exogenous H<sub>2</sub>O<sub>2</sub> on antioxidant enzymes of *Brassica juncea* L. seedlings in relation to 24-epibrassinolide under chilling stress. *Indian Journal of Biochemistry and Biophysics* 47 (6): 378-382.
- Kumaran A and Joel Karunakaran R, 2006. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry* 97: 109-114.
- Lin WC and Block GS, 2010. Can H<sub>2</sub>O<sub>2</sub> application reduce chilling injury of horticultural crops? *Acta Horticulture* 875: 33-36.
- Maisuthisakul P, Pasuk S and Ritthiruangdej P, 2008. Relationship of antioxidant properties and chemical composition of some Thai plants. *Journal of Food Composition and Analysis* 21: 229-240.
- Mousaei Sanjerehei M, Jafari M, Mataji A, Baghestani Meybodi M and Bihamta MR, 2013. Influence of environmental factors on distribution of plant species in Nodushan rangelands of Yazd Province (Iran). *Desert* 18: 19-26 (In Persian with English abstract).
- Nemat-Ala MM and Hassan NM, 2006. Changes of antioxidants levels in two maize lines following atrazine treatments. *Plant Physiology and Biochemistry* 44: 202–210.
- Nag S, Saha K and Choudhuri MA, 2000. A rapid and sensitive assay method for measuring amine oxidase based on hydrogen peroxide-titanium complex formation. *Plant Science* 157: 157-163.
- Ozgur R, Uzilday B, Sekmen AH and Turkan I, 2013. Reactive oxygen species regulation and antioxidant defence in halophytes. *Functional Plant Biology* 40: 832-847.
- Pellicer J, Garnatje T and Valles J, 2011. *Artemisia* (Asteraceae): understanding its evolution using cytogenetic and molecular systematic tools, with emphasis on subgenus *Dracunculus*. *Records in Advanced Pharmaceutical Sciences* 2: 199-222.
- Quan LJ, Zhang B, Shi WW and Li HY, 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology* 50 (1): 2-18.



- Roshandel P and Azimian F, 2015. Effects of magnetic field on growth and antioxidant capacity of *Artemisia aucheri* in normal or saline conditions. *Biological Forum* 7 (2): 1095-1103.
- Sairam RK, Veerabhadra Rao K and Srivastava GC, 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* 163: 1037-1046.
- Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y and Yoshimura K, 2002. Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany* 53: 1205-1219.
- Siahpoosh A and Amraee F, 2011. Antioxidant capacity of various extract of *Asteragallus murinus* Boiss. *Journal of Shahid Sadoughi University of Medical Sciences* 19 (4): 437-444 (In Persian with English abstract).
- Strain HH and Svec WA, 1966. Extraction, separation, estimation and isolation of chlorophylls. In: Vernon LP and Seely GR (Eds). *The Chlorophylls*. Pp. 21–66. Academic Press, New York.
- Tanou G, Filippou P, Belghazi M, Job D, Diamantidis G, Fotopoulos V and Molassiotis A, 2010. Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. *Plant Journal* 72: 585–599.
- Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A and Job D, 2009. Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. *Plant Journal* 60: 795–804.
- Uchida A, Jagendorf AT, Hibino T, Takabe T and Takabe T, 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Science* 163: 515–523.
- Wahid A, Perveen M, Gelani S and Basra SM, 2007. Pretreatment of seed with H<sub>2</sub>O<sub>2</sub> improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *Journal of Plant Physiology* 164 (3): 283-294.
- Xu FJ, Jin CW, Liu WJ, Zhang YS and Lin XY, 2011. Pretreatment with H<sub>2</sub>O<sub>2</sub> alleviates aluminum induced oxidative stress in wheat seedlings. *Journal of Integrative Plant Biology* 53: 44-53.
- Yu CW, Murphy TM and Lin CH 2003. Hydrogen peroxide- induces chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Functional Plant Biology* 30: 955–963.