

## PHYTOREMEDIATION CAPABILITIES AND ANTIOXIDANT ENZYMES' ACTIVITIES OF TWO HALOPHYTIC SHRUBS *CAPPARIS DECIDUA* AND *HALOXYLON SALICORNICUM* FROM CHOLISTAN DESERT, PAKISTAN UNDER SALINITY STRESS

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### Abstract

Salt-affected Soils hindering plant biomass production is the burning issue of the world to meet demands for food, fiber and shelter. Recent study was conducted to evaluate two halophytes shrubs species *Capparis decidua* and *Haloxylon salicornicum* towards salinity stress. Antioxidant enzymes activities and phytoremediation capabilities ( $\text{Na}^+$  &  $\text{K}^+$  concentration) were measured. The experiment was conducted in greenhouse at different salinity levels (0, 70, 140 and 210 mM NaCl). One year old seedlings of *Capparis decidua* and *Haloxylon salicornicum* grown through cuttings in the polythene tubes in the nursery area were shifted to hydroponic medium for various parameters. The results revealed that under highly saline conditions *H. salicornicum* was found more salt tolerant and produced maximum number of leaves (27) than *C. decidua* (19) at 210 mM NaCl. Chlorophyll contents in *C. decidua* and *H. salicornicum* increased till 140 mM NaCl (0.24) and (0.48) and decreased (0.06) and (0.33) at 210 mM NaCl respectively. Uptake of  $\text{Na}^+$  concentration was increased with the elevated salinity treatments while  $\text{K}^+$  concentrations were decreased in both species.  $\text{Na}^+$  concentrations were found maximum in *H. salicornicum* (60117ppm) at 210 mM NaCl while it was (23225ppm) at 0 mM NaCl. Antioxidant enzymes activities of superoxide dismutase (SOD) increased with increasing salinity while the Catalase (CAT) activity was at highest value at 0 mM NaCl (37.66) and its value decrease with the increasing salinity in both species. The results concluded that the *H. salicornicum* is more salt tolerant species. Both species can be used for phytoremediation of salinity from saline soils and successful revegetation of deserts.

**Key words:** Halophytes, Salinity, Chlorophyll, Weight,  $\text{Na}^+$ , Antioxidant.

### Introduction

The cost of decontamination of toxic metals from industrial effluents both solid and aqueous substrates using conventional techniques like dredging, ploughing, chemical treatment, sediment/soil washing, soil flushing, pneumatic fracturing, vitrification, thermal desorption and treatment, electrokinetics, incineration, etc. are cost prohibitive (Glass, 1999; Prasad & Freitas, 1999). Abiotic and biotic resources are also tested for remediating or removal of toxic metals from soil or aqueous solutions (Volesky, 1994). Antcliff *et al.*, (1983) stated that plant species which are able to limit the salt accumulation in the shoot are more resistant to salinity. Anon., (2005) reported the occurrence of agriculture saline soils that are 2% of total agriculture lands. Salinity is affecting almost 20% of the land under cultivation and almost half of the land under irrigation respectively (Cheong & Yun, 2007). All processes of plant life from seed germination to fruit setting are badly impacted by high salt concentrations that lead to the reduction in plant productivity (Sairam & Tyagi, 2004). Physically and chemically degraded soils comprising salinity is nearly affecting 15% of the world's total area. Water scarcity as well as saline conditions are becoming principally prevalent in several areas, and are possible by the year 2050 to cause severe increase in salinization of soils of more than 50% of all lands under cultivation (Jamil *et al.*, 2011).

Globally Pakistan is situated in South Asia with the latitude and longitude of 30°N, 70°E respectively.

Mostly lands of Pakistan are dry holding 80% of land area comprising of arid and semi-arid regions where largest irrigation system of world lies. Almost 6 million hectares are salt affected in irrigated and non-irrigated areas of Pakistan (Ansari *et al.*, 2007). The saline soils cost totally is equal to the 0.6% of domestic production (GDP) in Pakistan in the year 2004 (Corbishley & Pearce, 2007). Cholistan rangeland comprising xerophytic flora is situated in 27°42'N and 29°45'N latitude and 69°52'E and 75°24'E longitude (Arshad, 2006) with altitude of approximately 112 m from sea level (Ali *et al.*, 2009). In the hot Cholistan rangeland highly saline patches are the key characteristics features particular to these areas. The native natural vegetation surviving in so much stressful conditions can be effective for probing the adaptation mechanism associated with halophytes growth and survival to fight in the way of high salinity levels. The difference between annual precipitation and evapotranspiration causes rapid salts accumulation on the surface soil during the dry seasons. Annual rainfall is not yet enough for leaching down the salts. As a result, in these areas planting of the native halophyte species is the effective tool to combat salinity problems. Recent research by many scientists has shown the ability of plants to eradicate salts from saline soils have been explored and acknowledged (Ashraf & Akram, 2005, Rabhi, 2009). In views of (Flowers & Colmer, 2008), plants able to persist and reproduce in saline soils in which salt concentration goes beyond 200mM of sodium chloride ions are characterized as halophytes. Thus the

halophytes are the plant species that can complete their life cycles under extreme salinity circumstances (Stuart *et al.*, 2012). Several methods have been used for reclamation and appropriate use of salinity affected soils comprising agronomic practices and using halophytes. Among these, use of halophytes (phytoremediation) can be most operative, inexpensive and ecologically sound technique for remediation of salinity affected soils (Hasanuzzaman *et al.*, 2014). Phytoextracton is the novel approach towards dealing the problems of soil contaminations. Many crops such as alfalfa, balm, cotton, menthe, pea, tomato are reported by (Ozturk *et al.*, 2004) for negative effects of salinity stress on water contents (Khan & Ungar, 2000).

Present work concerns about the assessment of salinity stress to the two promising halophyte shrub species of Cholistan rangeland; Karir (*Capparis decidua*) and Lana (*Haloxylon salicornicum*). The objective of study was to assess salt tolerance of two shrubs, phytoremediation potential, antioxidant activity and Suggesting halophyte shrub species to enhance vegetation cover in the saline environment.

## Material and Methods

Hydroponic based experimental research was accompanied in the enclosure at the Experimental Area of The Department of Forestry, Range & Wildlife Management, Faculty of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. The two shrub species plant material one year old *Capparis decidua* and *Haloxylon salicornicum* grown through cuttings in the polythene tubes in the nursery area were selected for various parameters. Hydroponic experiment was conducted in three months from September to October, 2018 in the green shed environment GPS location (29° 22.33'N, 71° 45.91' E) 364 elevation feet above the sea level. The required plant material was collected and propagated from single parent. It was because of the fact to avoid uncertainty in results and to ensure reliable results from the experiment. Uniformed sized plants were used in research. The sprouted cuttings were then shifted to hydroponic system containing Hoglands nutrient solution. The nutrient solutions were altered on weekly basis to minimize nutrient accumulation. pH of solution was maintained at 5.5 to 7.0. Following parameters were studied during the whole research.

**Number of leaves:** Three plants were selected from each replication for every species to count the number of leaves.

**Shoot/Root length:** Root and Shoot were separated from whole plant. Root/Shoots were washed with running water followed by the distill water and lengths were measured by using measuring tape.

**Determination of chlorophyll contents:** Chlorophyll contents were measured by using chlorophyll measuring spade.

**Antioxidant enzymes activities:** For sampling 0.5g plant extract from each treatment for every species was taken after rinsing the plant with distilled water. Place the extracted material on cooled plaster on ice. 2-3ml buffer solution of phosphate was added with pH of 7.8 Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O (16.385g) + NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O (0.663g) and make volume of 1000ml by using distill water. Samples were homogenized on ice and 5ml buffer solution was added. At 4°C samples were centrifuged at 8000-13000 rpm for 20 minutes. Samples were preserved at 4°C.

**Enzyme assay for superoxide dismutase (SOD):** Take 2.725mL of RS + 0.25ml H<sub>2</sub>O<sub>2</sub> + 0.025 ml Enzyme in 25 ml glass beaker. Control in 100% light-containing 2.75 ml of RS + 0.25ml H<sub>2</sub>O (CK reading). For zero reading, control the samples in 100% Dark, containing 2.75 ml of RS + 0.25ml H<sub>2</sub>O.

Place the control light and all other samples beakers under light conditions at 4000 lux for 20 minutes while control dark sample in 100% dark condition.

Read the samples by using Photo-spectrometer at 560nm.

SOD activity (U/g FW):  $\{(AcK-Ae) \times V\} \div \{0.5 \times AcK \times W \times Vt\}$

**Enzyme assay for catalase (CAT):** Take 2.8ml BPS, 0.1 ml of enzyme and 0.1 ml of H<sub>2</sub>O<sub>2</sub>. Gently shake and take reading at 240 nm through photo-spectrometer. For Zero reading 2.8ml of BPS, 0.1ml of H<sub>2</sub>O and 0.1 ml of H<sub>2</sub>O<sub>2</sub> was taken. Measure the rate by using the formula:

CAT (mM/g FW) activity = (activity\*A\*V/a) / (E\*FW)

## Determination of sodium and potassium in plants

**Reagent solutions:** A. 1:2 Perchloric acid and Nitric Acid mixture solution was made. For this 500 ml (70%) perchloric acid in 1000 ml HNO<sub>3</sub> (69-71 %) was mixed well. The solution was kept to cool and then store in glass bottle and kept in dark.

B. Standard solutions of potassium and sodium were made for flame photometer.

## Digestion

Weigh 1.0 g, ground, sieved and free of contamination plant sample in digestion tube. Add 20 ml acid mixture. Heat at 150-1750C on digestion block till white to slight yellowish plant material color in the digestion tube. Make volume 100 ml with distilled water or as desired for analysis. Make dilution as desired of the digested material. Determine K (ppm) by flame photometer using standard and graph reading.

## Calculations

% K and Na = Na or K ppm x d.f./10000

K and Na ppm = (ppm in extract – blank) x A/Wt  
A is a volume of extract (ml)

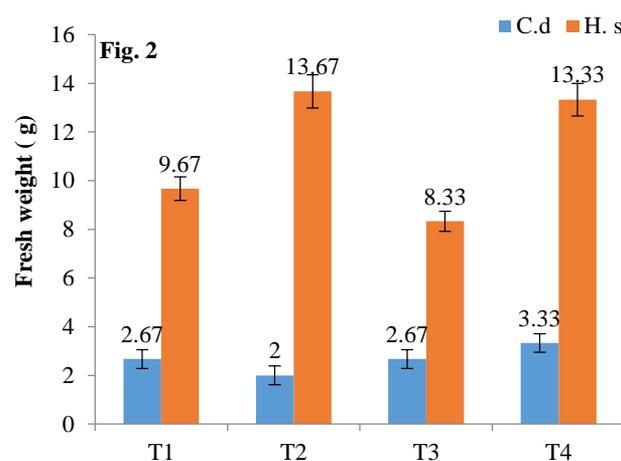
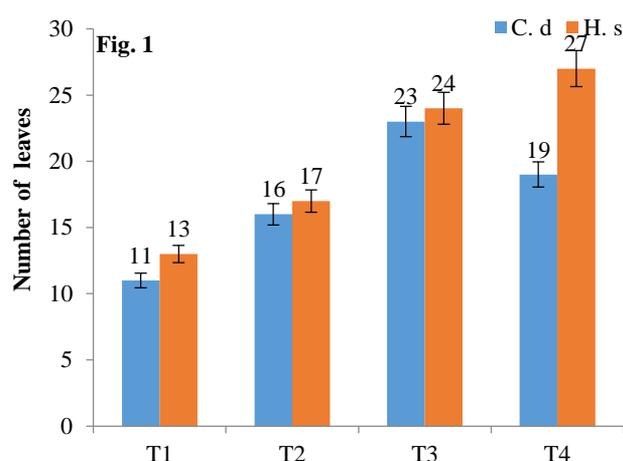
Wt is a weight of sample use (Jones Jr *et al.*, 1991)

## Results and Discussions

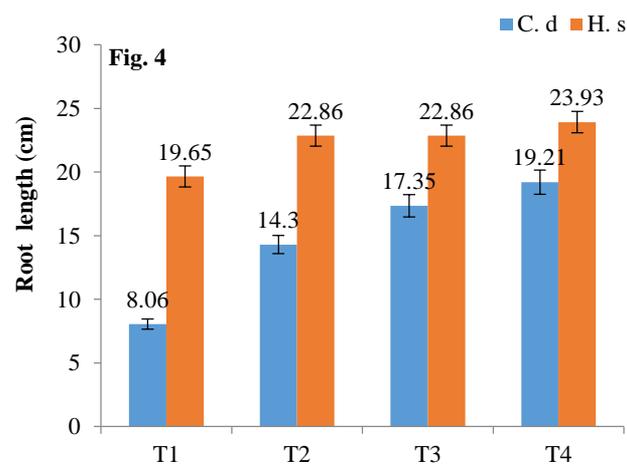
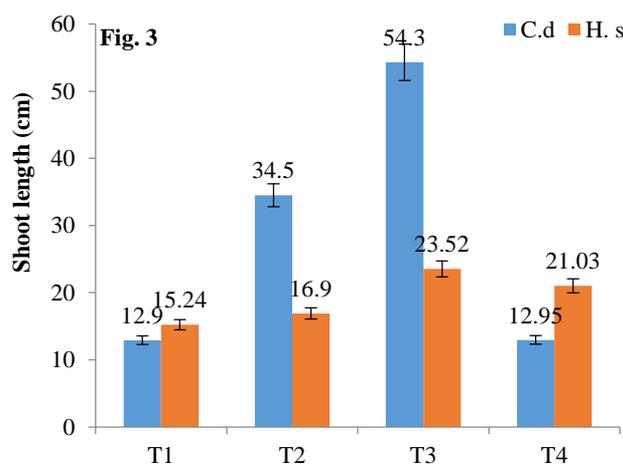
**Biomass production:** Numbers of leaves are plant growth indicator. More numbers of leaves mean more biomass production. Salinity adversely impacts numbers of leaves resulting reduction in biomass production (Hameed *et al.*, 2008). Number of leaves for *Haloxylon salicornicum* increased from T1 (13) to maximum at T4 (27) while *Capparis decidua* reduced its leaves at T4 (Fig. 1). Shoot length initially increased for *Capparis decidua*, and found maximum at T3 (140 Mm NaCl) noted 54.3 cm and then declined. Shoot length for *Haloxylon salicornicum* however showed increased indicating not affected by salinity (Fig. 3). While root lengths in both species increased with increasing salinity in all treatments shown in (Fig. 4). Salinity has a significant impact on total weight (root and shoot). Biomass production which is the main goal is drastically reduced by salinity (Khan *et al.*, 2000). Weight is the indicator of plant health and vigour. Results showed that for *Capparis decidua* minimum weight mean was at T1 (1.99g) and maximum at T3 (3.35g) but declined at T4 (2.1g). This shows weight is declined at higher salinity levels. The comparisons of both species are shown in (Fig. 2). Any abiotic stress to the plant life leads to the reduction in the growth. Salinity stress also induces changes in the chlorophyll contents. Growth is an indispensable event in the life cycle of plants. In *Capparis decidua* chlorophyll contents increased at initial treatment levels maximum at T3 (0.48) and then declined at T4 (0.06)

(Fig. 5). These results suggests *Haloxylon salicornicum* more salt tolerant and results are similar to the findings of (Naz, *et al.*, 2010) which shows the species is most dominant in term of percent cover under moderate saline conditions. Plants responses to salinity depend on the salt concentration of the available water for root uptake, on the duration of the stress and on plant salt tolerance (Neumann, 1997) which varies among species and, within the same species, among genotypes. In plants there are differences between the limit values damaged by salt. Reduction in biomass production have been experimented in *Matricaria chamomile* species by Razmjoo *et al.*, (2008). These results matched with Munns (2002) and expressed that salinity cause reduction in root shoot length and retarded the overall production. These results resembled with results discussed by (Khan & Ungar, 2000). Similar results were also described by Naz *et al.*, (2010) that number of leaves decreased with increasing salinity.

Both species has shown declining chlorophyll contents as depicted in (Fig. 5). Salinity stress may cause the death of the plant as well as hinder growth depending on tolerance, may cause chlorosis and necrotic stains and also decrease yield and quality (Hasegawa *et al.*, 1986; Mer *et al.*, 2000). The reactions of plants to salt vary depending upon the time of exposure to salt, the growth period of the plant, salt concentration, climate and soil properties (Greenway & Munns, 1980).



Figs. 1-2. Comparison of number of leaves and fresh weight of two halophyte species under different NaCl treatments.



Figs. 3-4. Comparison of root/shoot length of two halophyte species under different NaCl treatments.

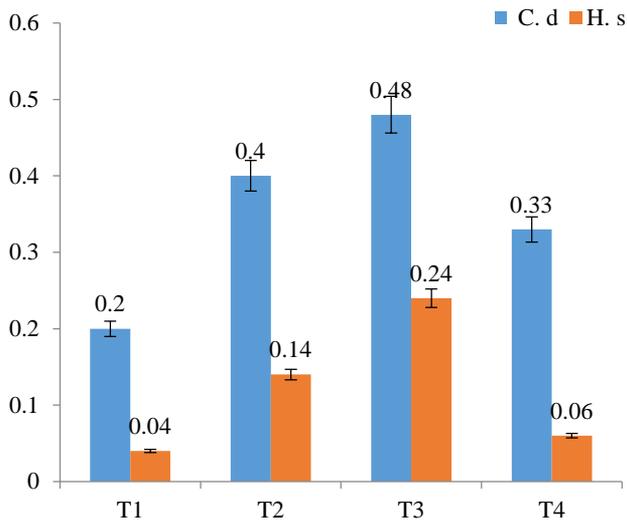


Fig. 5. Comparison of Chlorophyll measurement of two halophyte species under different NaCl treatments.

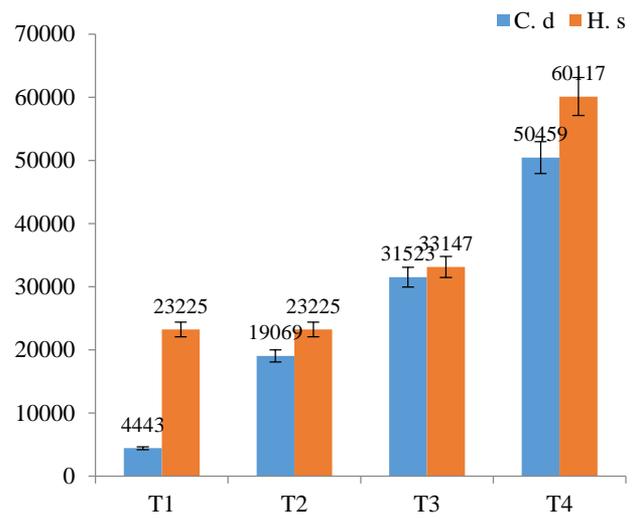


Fig. 6. Comparison of Na<sup>+</sup> uptake (ppm) by *Capparis decidua* and *Haloxylon salicornicum*.

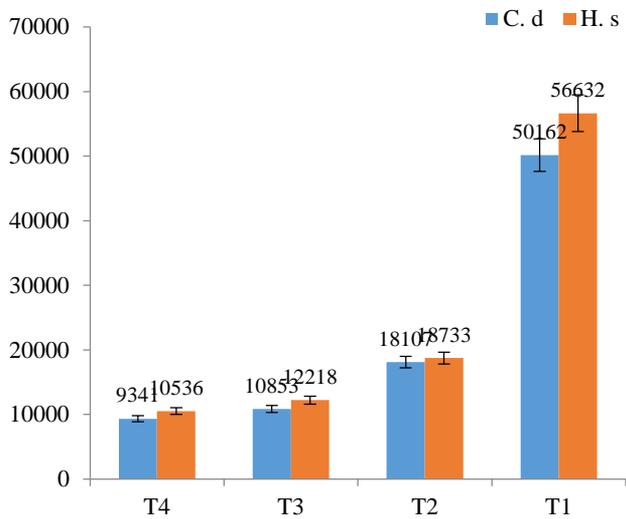


Fig. 7. Comparison of K<sup>+</sup> uptake (ppm) by *Capparis decidua* and *Haloxylon salicornicum*.

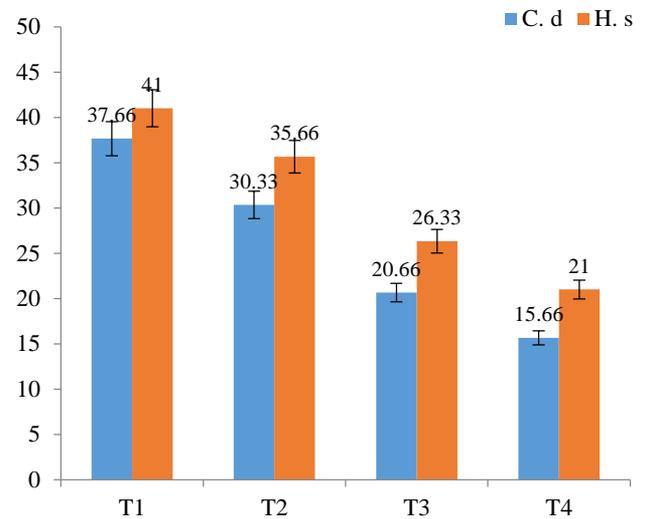


Fig. 9. Comparison of CAT activity in *Capparis decidua* and *Haloxylon salicornicum*.

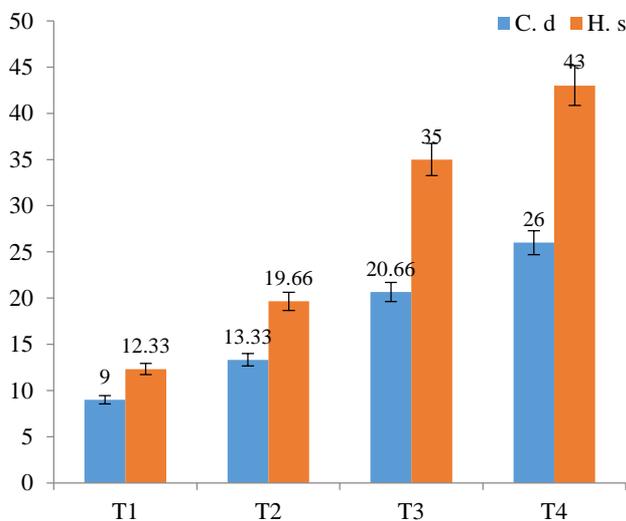


Fig. 8. Comparison of SOD activity in *Capparis decidua* and *Haloxylon salicornicum*.

**Phytoremediation**

**Na<sup>+</sup> and K<sup>+</sup> Uptake:** Na<sup>+</sup> uptake increased in both species at all treatment levels, maximum at T4 (50459 and 60117) respectively as shown in (Fig. 6). Results showed that K<sup>+</sup> uptake decreased in both species in all treatments, maximum K<sup>+</sup> was observed in *C. decidua* at T1 (50162) and in *H. salicornicum* (56632) respectively as shown in (Fig. 7).

*Capparis decidua* low palatable species, availability through the year and can grow under normal to moderate salinity stress as compared to *Haloxylon salicornicum* that is highly palatable by the camels and available year around which grows under highly saline environments (Ali *et al.*, 2009). However, in a study by Rajpurohit *et al.*, (1977), *Haloxylon recurvum* seed germination was inhibited even at 100 mM NaCl. But 5-20% species germinated at 400 mM NaCl in a thermoperiods studied by Khan & Ungar (2000). According to Kleinkopf & Wallace (1974), since much smaller quantities of sodium are present in roots and stems than in leaves, the tolerance mechanism for

*Haloxylon* species is probably located in the leaves. This salt-concentrating mechanism effectively removes the high salt stresses from internal cellular tissue (Kleinkopf & Wallace, 1974). Although salt secreted by the glands of most halophytes is predominantly sodium chloride, *Haloxylon* revealed that there is a great variation in the cation composition (carbonate, potassium, bromine, calcium, nitrate, magnesium and sulphate) (Waisel, 1961; Berry, 1970), which depends on the composition of the root environment (Thomson *et al.*, 1969; Antcliff *et al.*, 1983). Most investigators have studied salt secretion by glands under controlled conditions with treatment solutions differing in salinity (Arisz *et al.*, 1955; Ramoliya *et al.*, 2009) have studied the mineral uptake by *Prosopis cineraria* plants exposed to salt stress and noticed that the seedlings showed greater accumulation of sodium in the leaf tissue as compared to that in root tissue.

### Antioxidant enzymes

**Superoxide dismutase (SOD) and catalase (CAT) activity:** Superoxide Dismutase (SOD) is an important antioxidant enzyme. In plants against oxidative stress; it is the 1<sup>st</sup> line of defense. SOD activity was increased in both species with increasing salt concentrations as shown in (Fig. 8). At treatment T1 (0 mM NaCl) SOD activity was 9 that increased at 26 at 210 mM NaCl salinity level in *C. decidua*. Similar results were recorded for *H. salicornicum* for SOD and its value increased from 12.33 to 43 with the leading salinity. While Catalases (CAT) activities showed different pattern and its value decreased with the increasing salinity toxicity as shown in (Fig. 9). Its activity in *C. decidua* was 37.66 at T1 (0 mM NaCl) that reduced to 15.66 at T4 (15.66). *H. salicornicum* also behaved like *Capparis* species and CAT activity was 41 at T1 and after salinization it was 21 at T4 (210 mM NaCl). CAT activity was decreased in both species with increasing salt concentrations shown in figure 9.

Plants have its own defensive mechanism against abiotic stresses produced by Reactive Oxygen Species (ROS) (Pallavi Sharma *et al.*, 2012). These are involved in the removal of superoxide radicals, H<sub>2</sub>O<sub>2</sub> and singlet oxygen (Guan & Scandalios, 1993). Sobhanian *et al.*, (2010) experienced the same results and reported that Antioxidant enzymes (SOD and CAT) activities were significantly higher at higher (750 mM NaCl) salt stress. CAT activity was lower at low stress while activities of SOD increase with the increasing level of salts (Sobhanian *et al.*, 2010). Activities of antioxidants enzymes SOD and CAT were also studied by (Ivan *et al.*, 2012) in halophytes, and described the similar results.

### Conclusion

The study concluded that both species; *Haloxylon salicornicum* and *Capparis decidua* are halophytic in nature and can be used for phytoremediation of Na<sup>+</sup> and K<sup>+</sup>. These produced well under normal to moderate saline conditions and showed retarded growth under high salinity treatments. These are recommended for the successful re-vegetation of the deserts.

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