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Salinity Induced Changes in Photosynthetic Pigment and Antioxidant Responses in *Sesuvium portulacastrum*

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Abstract: The production of leaf and root antioxidant changes when exposed to saline conditions were investigated in the perennial halophyte *Sesuvium portulacastrum* L. Plants were grown with a nonsterilized soil and sterilized soil with 50 and 100% of sterilized seawater on 25, 55 and 85 Days After Planting (DAP). The plants were harvested on 30th, 60th and 90th DAP and used for analyzing the photosynthetic pigments, antioxidant enzyme activities viz., Superoxide dismutase (SOD; EC1.15.1.1) Ascorbate peroxidase (APX, EC 1.11.1.11) and non enzymatic antioxidant contents like ascorbic acid, α-tocopherol, reduced glutathione were determined. Plants exposed to salinity, either alone (SSW) sterilized seawater/unsterilized soil (USS) along with higher pigments, antioxidative enzymes and Na^+ ions response. This tendency was generally more marked in SSW/USS plants when compared to SSW/SS plants. The concentration of SSW/SS was negatively correlated with the antioxidative capacity of the plant, either enzymatic or non enzymatic and K^+ ions. These data suggest that the enhancement of the antioxidative response is of crucial significance for *S. portulacastrum* plants growing under saline conditions.

Key words: Arid and semiarid region, photosynthetic pigments, antioxidants, halophytes, *Sesuvium portulacastrum*

INTRODUCTION

Salinity is a major environmental factor that affects plant growth and crop yields (Munns, 2002). This is a low osmotic potential of the soil solution (osmotic stress), specific ionic effects (salt stress), imbalance in nutrient acquisition (nutritional stress) or a combined effect of the three factors (Ashraf, 2004; Koyro *et al*., 2008). All these factors induce detrimental effect on plant physiology and biochemistry (Munns, 2002). Some halophytes show optimal growth under saline conditions whereas others grow optimally under non-saline conditions (Flowers and Colmer, 2008). Among these salt tolerant plants, there are some fast growing species which can cover barren soil in short time, especially some succulent genera like *Sesuvium, Batis* and *Mesembryanthemum* (Menzel and Lieth, 1999).

Sesuvium portulacastrum belongs to the family Aizoaceae, facultative halophyte, naturally growing in the subtropical, mediterranean coastal and warmer zones of the world (Lokhande *et al*., 2009). It is also used in ornamentation landscaping, desert greening and sand dune fixation (Messedi *et al*., 2001). This plant has food and medicinal values and also utilized as a wild vegetable crop in India and south East Asia (Kathiresan *et al*., 1997; Burits *et al*., 2001). *S. portulacastrum* is able to express high growth potentialities under severe salinity and low nutrient availability. This species produces decorative branches with pink purplish and occasionally white flowers (Pasternak and Nerd, 1995). Environmentally, the plant has remarkable ability to survive under stress condition of salinity, drought and heavy metal accumulation (Lokhande *et al*., 2009).

Reactive Oxygen Species (ROS) are highly reactive and can damage lipids, proteins, nucleic acids and photosynthetic components (Alscher *et al*., 1997; Meloni *et al*., 2003). In plant cells (ROS) salinity is controlled by enzymatic and non enzymatic defense systems. Non-enzymatic compounds include lipid-soluble antioxidants (such as α-tocopherol and ß- carotene) and water-soluble resultants (such as ascorbate and glutathione) (Kim *et al*., 2004). ROS scavenging by antioxidative enzymes is achieved through series of complex reactions including O_2^- dismutation to H_2O_2 by superoxide dismutase (Asada, 1999; Meloni *et al*., 2003). A strong antioxidant response mechanism is of crucial significance for plants coping with low K^+ availability and salinity (Jithesh *et al*., 2006).

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Shoot-Succulent halophytes meet these requirements since they accumulate enormous Na^+ quantities in the aerial parts. They form the representatives in the study of desalination of salt-affected soils in non-leaching conditions. Selection of suitable species is the first step for affordable soil desalination at a wider scale in arid and semi arid regions (Rabhi *et al*., 2009). Hence, the present research was aimed to study the changes on antioxidants, pigments, sodium and potassium uptake of the plant in responses to salinity.

MATERIALS AND METHODS

Study area and experimental plant: *Sesuvium portulacastrum* L., were collected freshly along with their habitat soil (Kathiresan and Ramanathan, 2000) from Pichavaram mangrove forest (11° 29' to 11° 30' N and 79° 45' to 79° 55' E), southeast coast of India. The plant materials were transported in sterile polythene bags to the laboratory.

Experimental groups: The healthy plants of apparently uniform size were selected and maintained in the sterile polythene bag with rhizosphere soil. The entry of atmospheric microbes was blocked by closing the mouth of the bags. The plant stocks were maintained in six different groups of soil treatments as follows:

Group I: Non-sterilized rhizosphere soil watered with sterilized water
Group II: Sterilized rhizosphere soil watered with sterilized water
Group III: Non-sterilized rhizosphere soil watered with sterilized 50% seawater
Group IV: Sterilized rhizosphere soil watered with sterilized 50% seawater
Group V: Non-sterilized rhizosphere soil watered with sterilized 100% seawater
Group VI: Sterilized rhizosphere soil watered with sterilized 100% seawater

Each group consists of six replicates. The experimental plants were maintained under the natural sunlight for a period of 90 days. Sterilized water was poured to plant stocks two times a day. The plants were assessed on 30, 60 and 90 days of experiment.

Biochemical analysis: The total chlorophyll and carotenoids were extracted in 80% acetone from the leaves and estimated (Arnon, 1949). Antioxidants in terms of enzymatic and non-enzymatic antioxidants were analyzed, ascorbic acid (Omaye *et al*., 1979), α-tocopherol (Baker *et al*., 1980), reduced glutothine (Moron *et al*., 1979), superoxide dismutase (Hwang *et al*., 1999) and ascorbate peroxidase (Chen and Asada, 1989). Concentrations of Na^+ and K^+ in leaf and root samples were determined by using flame photometer (Kingston, 1994).

Statistical analysis: Statistical analysis was performed using SPSS version 17.0 to test the statistical significance, two-way Analysis of Variance (ANOVA) was performed using Duncann's Multiple Range Test (DMRT).

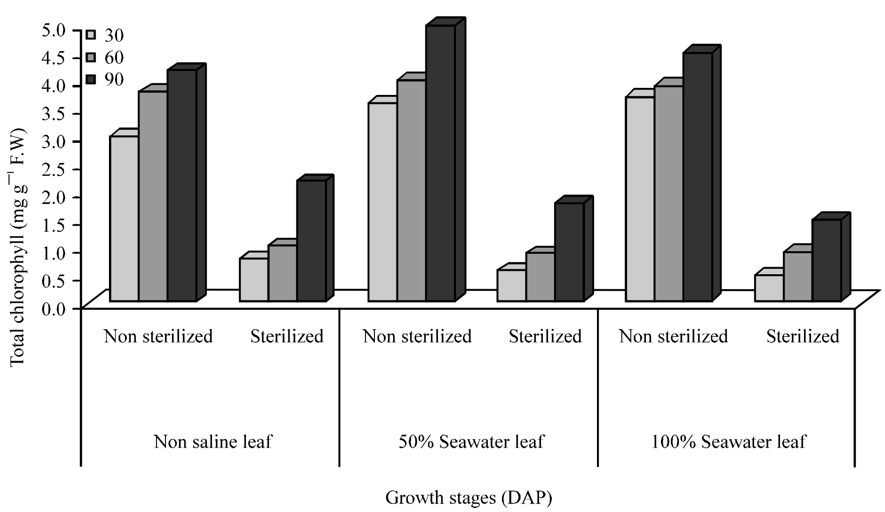

Fig. 1: Effect of salinity induced changes in total chlorophyll content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

RESULTS

Salinity on total chlorophylls: Salinity induced changes in the total chlorophyll contents of the *S. portulacastrum* leaves (Fig. 1). The increase of total chlorophyll increased with salinity. The higher chlorophyll content 5.0 mg g^{-1} Fresh Weight (FW) was recorded in the 50% diluted seawater on 90 DAP sampling and the lowest content (0.80 mg g^{-1} FW) was recorded in the non saline plant of the *S. portulacastrum* leaf on 30 DAP.

Salinity on carotenoids: The carotenoids content in NS 50% of diluted seawater plants was higher than that of 100% sterilized seawater plants on 30, 60 and 90 DAP (Fig. 2). The highest carotenoids content 137 mg g^{-1} Dry Weight (DW) was recorded on 90 DAP non saline leaf sampling of *Sesuvium portulacastrum*. The lowest carotenoids content (25 mg g^{-1} DW) was recorded in 30 DAP leaf samples of 100% sterilized seawater treated plants.

Salinity on ascorbic acid: Sterilized seawater induced changes in the ascorbic acid contents of the *S. portulacastrum* (Fig. 3). The AA content increased with the age of the plant in both the organs in control and sterilized and unsterilized seawater. Among the plant parts, the root recorded the highest AA content. The

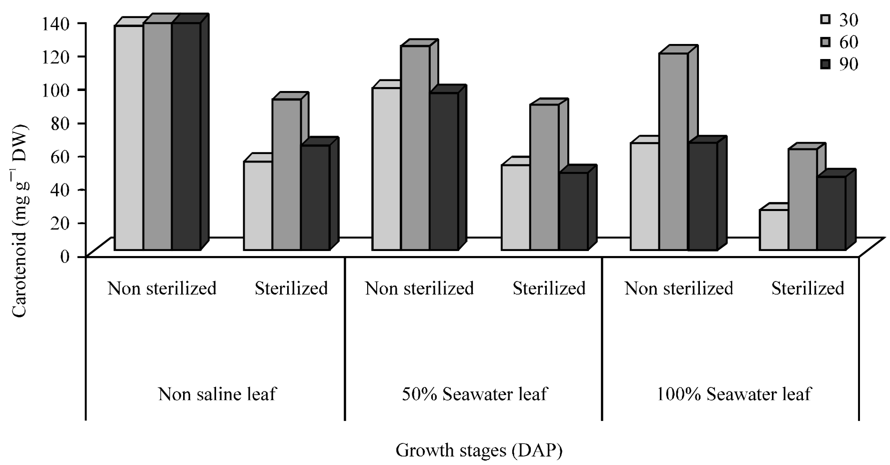

Fig. 2: Effect of salinity induced changes in carotenoid content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

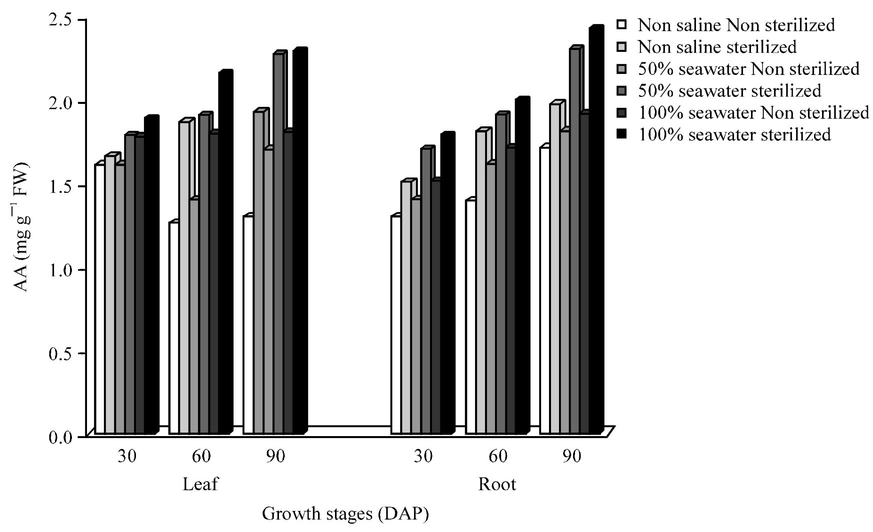

Fig. 3: Effect of salinity induced changes in ascorbic acid content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

higher ascorbic acid content 2.45 mg g^{-1} Fresh Weight (FW) was recorded in sterilized 100% seawater treated roots on 90 DAP sampling and the lowest content (1.28 mg g^{-1} FW) was recorded in the non saline plant of the *S. portulacastrum* leaf on 60 DAP.

Salinity on α-tocopherol: The α-tocopherol content in the 50% diluted sterilized seawater was higher than that of control on 30, 60 and 90 DAP (Fig. 4). The highest alpha tocopherol content (9.22 mg g^{-1} FW) was recorded on 60 DAP leaf sampling of 50% sterilized seawater treated *S. portulacastrum*. The lowest α-tocopherol content (2.6 mg g^{-1} FW) was recorded in 30 DAP root samples of untreated plants.

Salinity on reduced glutathione: The variation found in the reduced glutathione content under sterilized and non sterilized seawater treated plants (Fig. 5). It increased with the age of the plants in both the parts of leaves and roots. On 90 DAP, root samples from 100% sterilized seawater showed highest content of GSH (2.72 μg g^{-1} FW) and the lowest content (0.66 μg g^{-1} FW) was recorded in control of non saline leaf samples on 30 DAP.

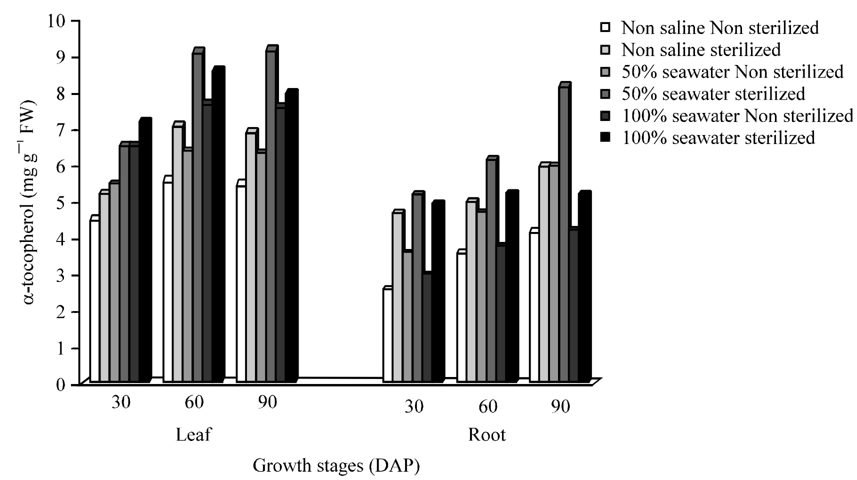

Fig. 4: Effect of salinity induced changes in α-tocopherol content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

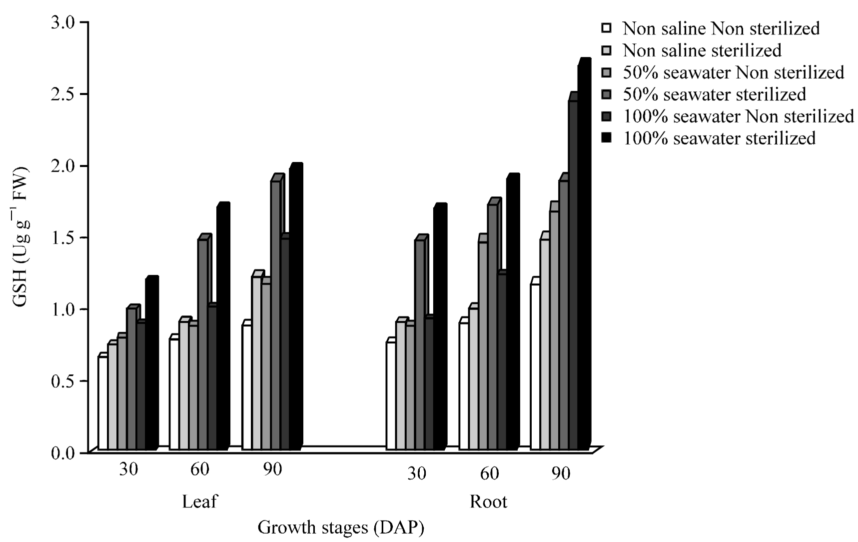

Fig. 5: Effect of salinity induced changes in reduced glutathione content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

Salinity on superoxide dismutase: The activity of SOD was increased under 100% sterilized seawater conditions (Fig. 6). The highest activity (0.96 μg g^{-1} proteins) was recorded in 90 DAP under sterilized seawater treated leaf sampling and lowest activity (0.55 μg g^{-1} proteins) was recorded in the 30 DAP root sampling of *S. portulacastrum*.

Salinity on ascorbate peroxidase: Ascorbate peroxidase was higher in *S. portulacastrum* when compared to SOD activities (Fig. 7). It increases under seawater conditions in both the organs when compared to control on all the experiment showed higher in the APX activity. The highest APX activity (1.89 μg g^{-1} proteins) was recorded in 100% sterilized seawater treated leaves on 90 DAP while the lowest activity (0.72 μg g^{-1} proteins) was recorded in 60 DAP samplings from root of non saline *S. portulacastrum*.

Salinity on sodium content: Na^+ was more abundant in leaves than in roots (Fig. 8). There was a significant accumulation of Na^+ in leaves of plants under non sterilized condition when compared to control. The amount of Na^+ is various in different parts of the plant. The highest amount (465 mM) was recorded in 90 DAP sampling from leaf of 100% unsterilized seawater

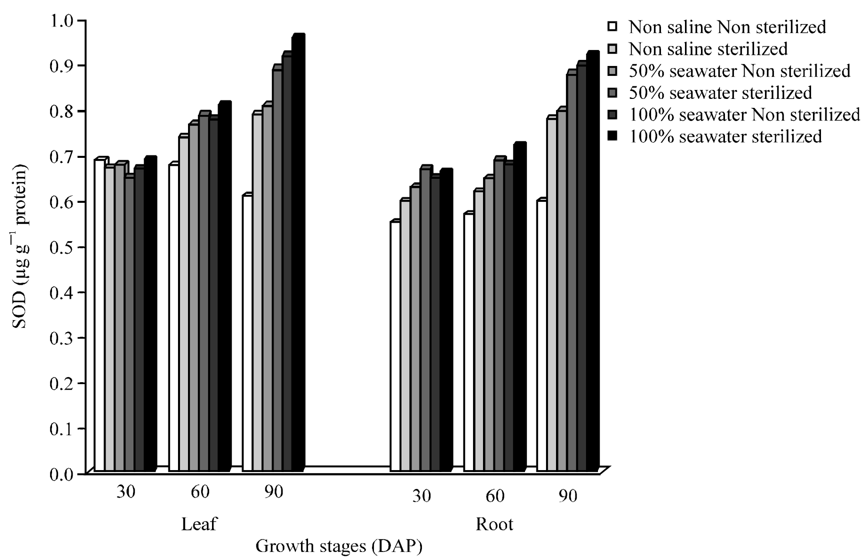

Fig. 6: Effect of salinity induced changes in SOD activity content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

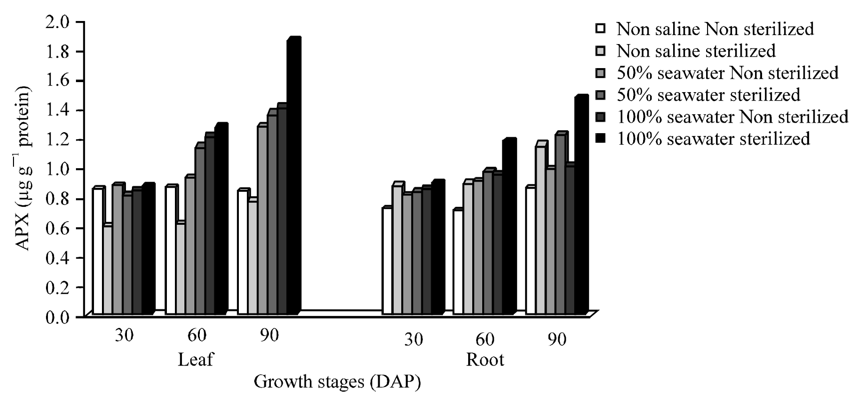

Fig. 7: Effect of salinity induced changes in APX activity content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

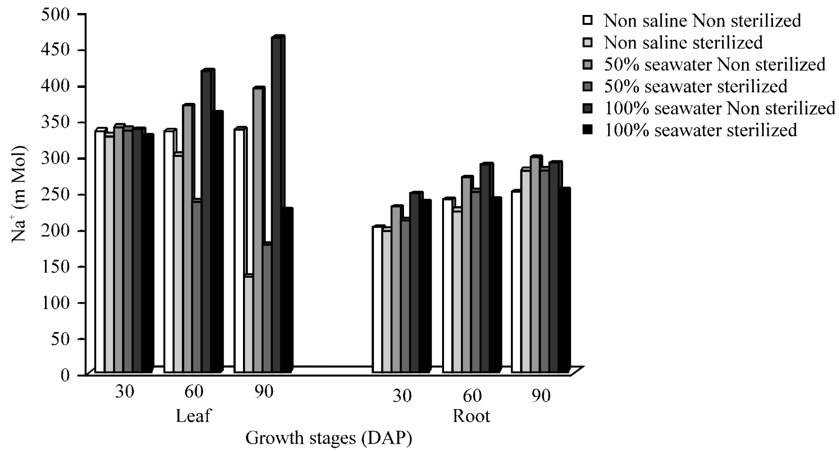

Fig. 8: Effect of salinity induced changes in Na^+ content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

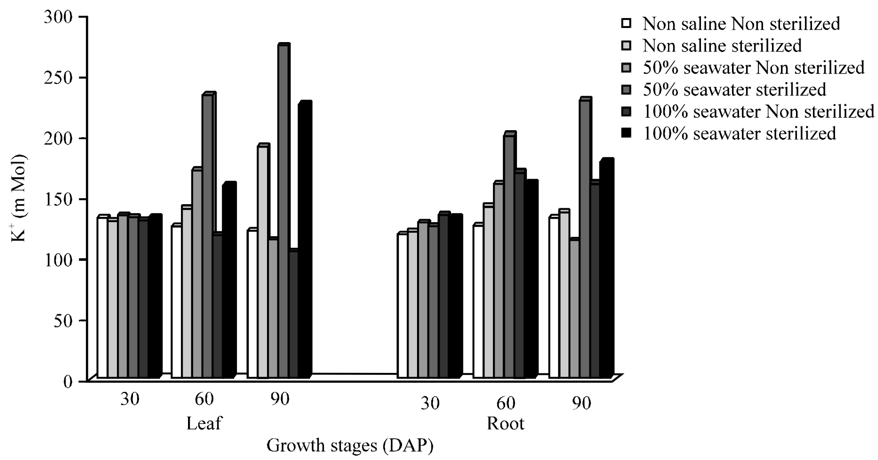

Fig. 9: Effect of salinity induced changes in K^+ content of *Sesuvium portulacasrtrum* L. Value are Mean±SD of six samples

conditions, while the lowest amount (200 mM) was recorded in 30 DAP sampling from root of sterilized non saline plant (Fig. 9).

Salinity on potassium content: The highest amount of K^+ (229 mM) was recorded in 90 DAP sampling from leaf of 100% sterilized seawater while lowest amount (120 mM) was recorded in 30 DAP sampling from root of sterilized control plants (Fig. 9).

DISCUSSION

Soil salinity limits the plant growth and crop yield in many parts of the world, particularly in the arid and semi arid areas. The only plants that can survive in this condition are halophytes. Lonard and Judd (1997) described *Sesuvium portulacastrum* as an important pioneer species on sandy beaches in the tropics and subtropics. They attributed its success as a colonizing species, in its ability to be propagated through salt-tolerant vegetative fragments. *S. portulacastrum* is used as food and fodder (Lokhande *et al*., 2009). It was earlier proved that *S. portulacastrum* is able to express high growth potential even under severe salinity (Messedi *et al*., 2004). Maas (1987) reported that in most halophytic species, growth decreases gradually with the increase of salt in the culture medium above a critical threshold specific to each species. Least NaCl concentration stimulates the growth of the halophytic species but an excess salt decreases growth and biomass production (Slama *et al*., 2007). In present study, the plants raised in NS soil and sea water shared significant biochemical changes when compared with sterilized soil.

The level of total chlorophylls and carotenoids showed a trend of decrease with increasing salinity in all groups. These results are supported by the previous studies showing changes in physiological process in plants in response to salinity and many other stress factors (Yourris *et al*., 2008). Salinity caused reduction in net photosynthic rate is attributed to reduce the capacity of photosynthetic machinery (Seeman and Sharkey, 1986). The decreased chlorophyll content can also be attributed to the fact that salt stress decreases total chlorophyll content by increasing the activity of cell degrading chlorophyllase (Yourris *et al*., 2008), inducing the destruction of chloroplast structure and instability of pigment protein complex (Singh and Dubey, 1995). The unsterlized group exhibited more amounts of chlorophylls and carotenoids when compared to sterilized group.

The increase in antioxidative metabolism can be correlated with plants with native protective mechanism against oxidative stress raised from the unsterlized group. Being the major antioxidant species in plants, the AA, α-tocopherol and GHS contents vary in different sub-cellular compartments, according to the intensity of stress (Gaspar *et al*., 2002). An increase in AA content was reported in sterilized group. Ascorbic acid is important antioxidant which functions as the terminal antioxidant because the redox potential of ascorbate/ monodehydroascorbate pair is lower than that of bioradicals (Scandalios *et al*., 1997). A number of mechanisms are used by halophytes to achieve osmotic adjustments, including inorganic ion accumulation, synthesis or accumulation of organic compounds prevent water loss (Ungar, 1991). Levels of antioxidant enzymes enhance during salinity stress (Shalata and Tal, 1998; Jaleel *et al*., 2007) and hence antioxidant concentrations are used as indicators of oxidative stress in plants. The sterilized group of plants showed and increased α-tocopherol content in leaves when compared to unsterlized group. Reactive oxygen species causes oxidative damage to biomolecules such as lipids and proteins and eventually leads to cell death (Del Rio *et al*., 2003; Sivakumar *et al*., 2010). GSH content was found increased under pesticide and herbicide application, drought stress, sweet potato, Datura (Davis and Swanson, 2001; Sivakumar *et al*., 2010; Sivakumar and Panneerselvam, 2011). The increase in GSH can be correlated with its ability to scanvenge singlet oxygen, peroxide and hydroxyl radicals also involved in recycling of AA in the ascorbate-glutathione pathway in chloroplast (Pastori *et al*., 2000).

In the present study, increase in antioxidant enzyme activities (SOD, APX) were recorded in two groups of saline treated plants. According to Pastori *et al*. (2000), many stress situation caused an increase in the foliar SOD activity in maize, sweet potato and *Datura* (Fletcher *et al*., 2000; Sivakumar *et al*., 2010; Sivakumar and Panneerselvam, 2011). Stimulation of stress-related antioxidative enzymes in combating stress in *Cassia* seedlings (Sheela and Pandy, 2003) showed an increased SOD activity. Increased APX activity by sterilized group of plants would increase the demand for ascorbate regeneration (Jaleel *et al*., 2007; Sivakumar and Panneerselvam, 2011). Similar results revealed that increasing salinity stress significantly increased antioxidant potential levels.

In our experiments the Na^+ accumulated more with increasing NaCl concentrations in all treatments when compared to control plants. However, K^+ ion did reduce. This response of halophytes reflect the utilization of ions for osmotic adjustment (Flowers, 1985) a process in which Na^+ can be much more suitable than K^+ (Marschner, 1995) since the halophyte accumulates preferentially in the vacuoles (Koyro and Huchzermeyer, 1999). Interestingly, even the 50% saline plants (Group I and Group II) accumulate NaCl to maintain normal growth. The reported accumulation of ions in the leaves of *S. portulacastrum* resembling a true halophyte is in agreement with the experiments using halophytic species, *Suaeda salsa* (Kefu *et al*., 2003). The present study noticed growth reduction at high salinity which may be due to nutritional distruption by salt (Messedi *et al*., 2006). The present study thus experimentally proved the effect of salinity stress induced changes in pigment and antioxidant potentials of *S. portulacastrum*.

CONCLUSION

In conclusion, present results indicate that the sterilized group of plants could increase the photosynthetic pigments and antioxidant defense mechanisms of halophytes.

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