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Growth and Mineral constituents' variations in halophytic species under salinity

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ABSTRACT

The present investigation was made to study the effect of different concentrations of sodium chloride on the growth and mineral constituents in different parts of *Clerodendron inerme*. The plant and could survive a wide range of 100-1000 mM of NaCl. The upper limit for the survival of *Clerodendron inerme* was 500 mM NaCl. However, favourable growth response by seedlings was confined to 200 mM NaCl. The morphological parameters such as shoot and length, number of leaves, total leaf area, fresh and dry mass and mineral constituents such as sodium, potassium and calcium were assessed.

Keywords: Halophyte; Mineral content; Salinity

1. INTRODUCTION

World wide, more than 800 millon hectares of land are salt affected, and tolerance to this salinity differs greatly among plant species (Munns and Tester 2008). In India alone, about 30 millon hectares of coastal land is lying barren and uncultivable because of soil affected by salinity.

Stresses associated with temperature, salinity and drought single or in combination are likely to enhance the severity of problems in the coming decades (Claussen *et al.,* 1985). Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity (Allakhverdier *et al*., 2000 and Koca *et al*., 2007).

Plant water stress is often the most prominent physiological response associated with increase in soil and / or water salinity (Munns 2002). Salt tolerance has been attributed as the primary factor in shaping vegetative structures, including biomass and species composition of coastal and estuarine wetlands (Bertness1991; Pennings *et al*., 2001).

Controversial data exist regarding the question of whether halophytes require saline conditions for their existence and vigorous growth or merely tolerate them. One of the major factors in the salt tolerance is believed to be the existence of succulence. Halophytes survive salt concentration equal to or greater than that of seawater and possess physiological mechanisms that maintain lower water potential inside the cell than that in the soil (Ungar 1991).

Salt tolerance in halophytes is brought about by a variety of physiological mechanisms and morphological adaptations. Adaptations of halophytes to the saline environment include high tolerance for the negative effect of salinity as well as positive reaction towards it. The compartmentation of ions in the vacuoles and accumulation of compatible solutes in the cytoplasm and presence of genes for salt tolerance confer salt resistance to halophytes (Gorham 1995).

The present study was made to investigate the salt tolerance of *Clerodendron inerme*.

2. MATERIALS AND METHODS

2. 1. Plant material

The mature stem cuttings were collected from salt marshes in the mangrove area of Pichavaram, on the east coast of Tamil Nadu, India about 10km east of Annamalai University Campus.

2. 2. Growth conditions

The stem cuttings of *Clerodendron inerme* (3cm long with one node and 2 opposite leaves) were planted individually in polythene bags (7”× 5”) filled with homogenous mixture of garden soil containing red earth, sand and farm yard manure (1:2:1). The cuttings were irrigated with tap water and maintained in the Botanical garden, Annamalai University.

2. 3. Salt treatment and Experimental design

One month old and well established cuttings were selected and treated with varying concentrations of NaCl (100-1000 mM). Above 500 mM NaCl concentrations the cuttings were not survived. The experimental plants treated with NaCl up to 500 mM were alone maintained in the experimental site. The experimental yard was roofed with transparent polythene sheet at a height of 3m from the ground in order to protect the plants from rain.

2. 4. Determination of growth and mineral constituents

Samples were collected randomly on 60th day after treatment. The seedlings were separated into leaves, stem and root and used for analysis. The morphological parameters such as shoot and root length, number of leaves, leaf area, fresh and dry mass were analyzed.

The minerals such as sodium, potassium were determined by the method of Willams and Twine (1960). The dried and ground tissues of 0.5g were digested in 100 ml Kjeldahl flasks using 10 ml of concentrated nitric acid, 0.5 ml of 60 per cent perchloric acid and 0.5 ml of concentrated sulphuric acid. Digestion was continued until the nitric acid and perchloric acid were driven off. The inorganic residue was cooled and diluted with 15ml of distilled water and filtered through Whatmann No. 42 filter paper. The filtrate was made up to 50ml with distilled water. The filtrates were used for sodium and potassium estimation and a Flame Photometer (Systronics, India) were used for the purpose and standards were prepared with sodium chloride.

The calcium content was estimated by the method of Yoshida *et al* (1972). Two ml of the filtrate was mixed with two ml of 5 % lanthanum oxide solution and diluted with ten ml of 1 N hydrochloric acid. The solution was fed into an Atomic Absorption Spectrophotometer (Perkin Elmer – 2280) at 211.9nm. Standard curve was prepared by using calcium chloride.

2. 5. Statistical analyses

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (*P* values ≤ 0.05) with the help of SPPS 20 software package. Means and standard deviation were calculated from 5 replication.

3. RESULT

3. 1. Shoot and Root length

The observations on the effect of different concentrations of NaCl salinity on the shoot and root length on the 60^{th} day after saline treatment is presented in Figure 1. The shoot and root length increased with increasing 200 mM NaCl in *Clerodendron inerme* and thereafter it gradually declined. The maximum increase shoot length was 17.6000 ±0.2000 and root length was 19.4200 ±0.8602 *Clerodendron inerme* over that of control.

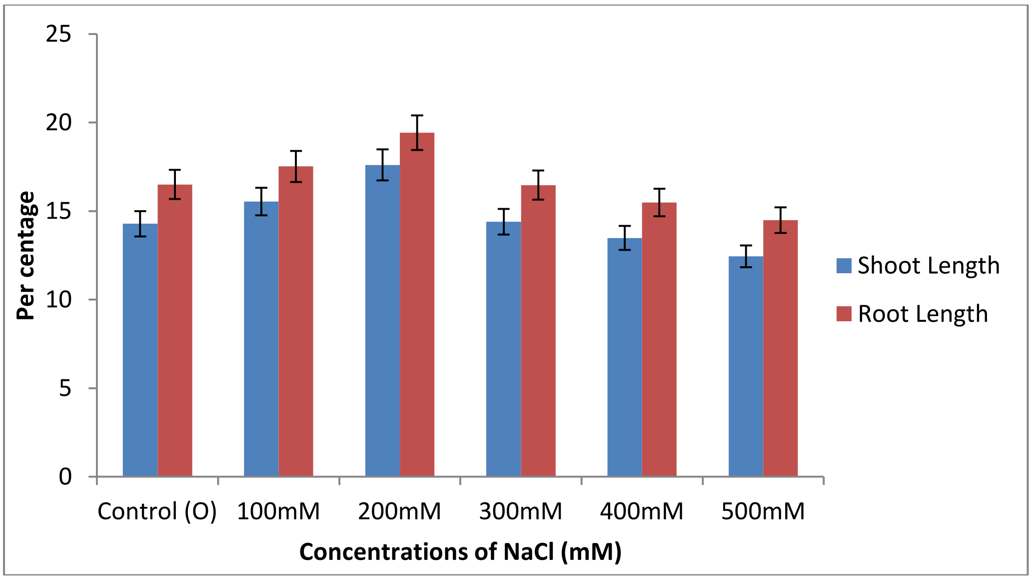

Fig. 1. Effect of NaCl on shoot length (cm $plant^{-1}$) and root length (cm $plant^{-1}$) of *Clerodendron inerme* on 60^{th} day after salt treatment.

3. 2. Leaf area and Number of leaves

The result on the effect of NaCl on the number of leaves per plant is given in Figure 2. The maximum increase in number of leaves and leaf area was observed on the 60^{th} day at 200 mM *Clerodendron inerme*. In the optimum concentration, the calculated number of leaves was 26.2000 ±0.80000 and leaf area was 31.6700 ±0.04506 when compared to that of control. Beyond this optimum concentration, there was a gradual decrease in the number of leaves and leaf area.

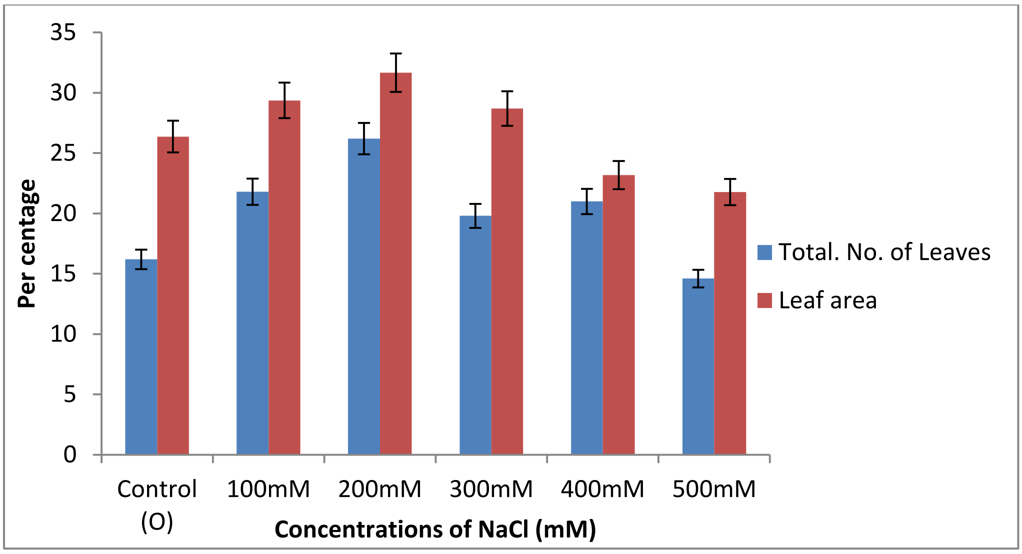

Fig. 2. Effect of NaCl on total no. of leaves (plant^{-1}) and leaf area (cm^{2} plant^{-1}) of *Clerodendron inerme* on 60th day after salt treatment.

3. 3. Fresh Mass

The results on the effect of NaCl on the fresh mass of leaf stem and root on the 60th day after saline treatment are given in Figure 3. An increase in the fresh mass of tissues was observed up to 200 mM NaCl in *Clerodendron inerme* on the 60th sampling days. Concentrations of NaCl beyond 200 mM had reduced the fresh mass in all the three tissues. The maximum increase in the fresh mass of leaf, stem and root was 4.7160 ±0.06615, 3.2029 ±0.02311 and 2.0940 ±0.2502 over that of control.

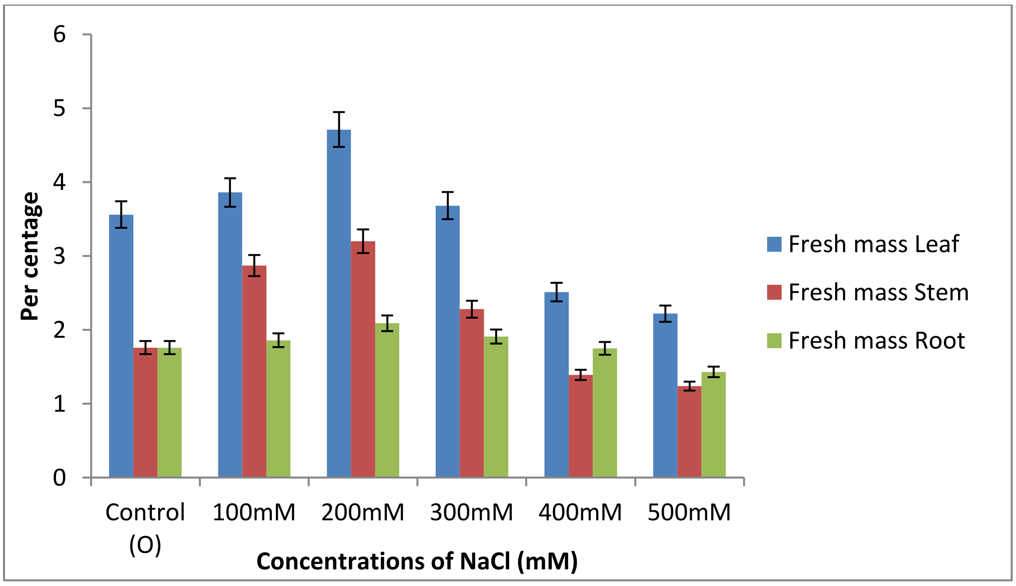

Fig. 3. Effect of NaCl on fresh mass of leaf, stem and root (g plant^{-1}) of *Clerodendron inerme* on 60th day after salt treatment.

3. 4. Dry Mass

The results on the effect of NaCl on the dry mass of leaf stem and root on the 60th day of saline treatment are presented in Figure 4. The dry mass of the three tissues increased with increasing NaCl up to 200 mM *Clerodendron inerme* and thereafter it decreased. The maximum increase in dry mass of leaf, stem and root was 0.56600 ±0.0241, 0.4400 ±0.02236 and 0.3520 ±0.0960 over that of control.

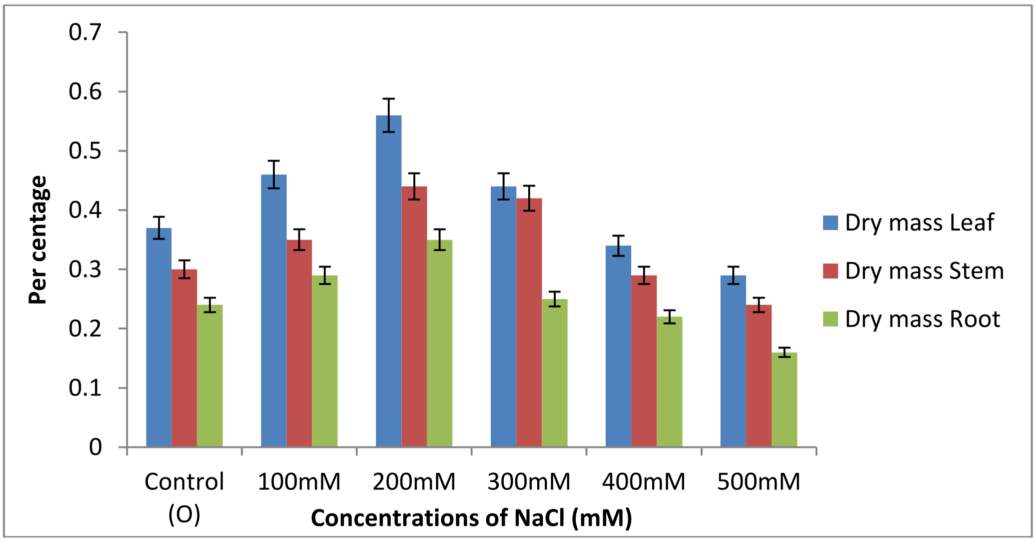

Fig. 4. Effect of NaCl on dry mass of leaf, stem and root (g plant^{-1}) of *Clerodendron inerme* on 60th day after salt treatment.

3. 5. Sodium

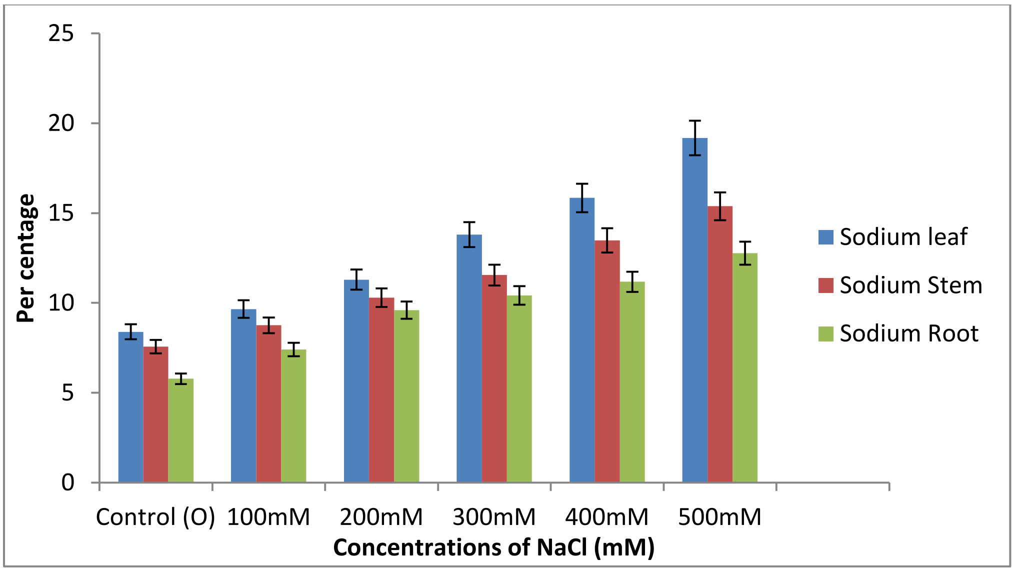

Fig. 5. Effect of NaCl on sodium content (mg/g dr. wt.) of the leaf, stem and root of *Clerodendron inerme* on 60th day after salt treatment.

The results on the effect of NaCl salinity on the sodium content of leaf, stem and root of *Clerodendron inerme* are presented in Figure 5. There was a considerable increase in sodium content with increasing concentration of NaCl salinity up to extreme levels in *Clerodendron inerme*. Of the three tissues, the leaf accumulated more sodium than the stem and root.

3. 6. Potassium

The results on the effect of NaCl salinity on the potassium content of leaf, stem and root of *Clerodendron inerme* are presented in Figure 6. There was a steady increase in the potassium content with increasing concentrations of NaCl in the leaf, stem and root tissues on 60^{th} the sampling days. The accumulation of potassium ions increased up to the extreme level 500 mM NaCl in *Clerodendron inerme* than that of control.

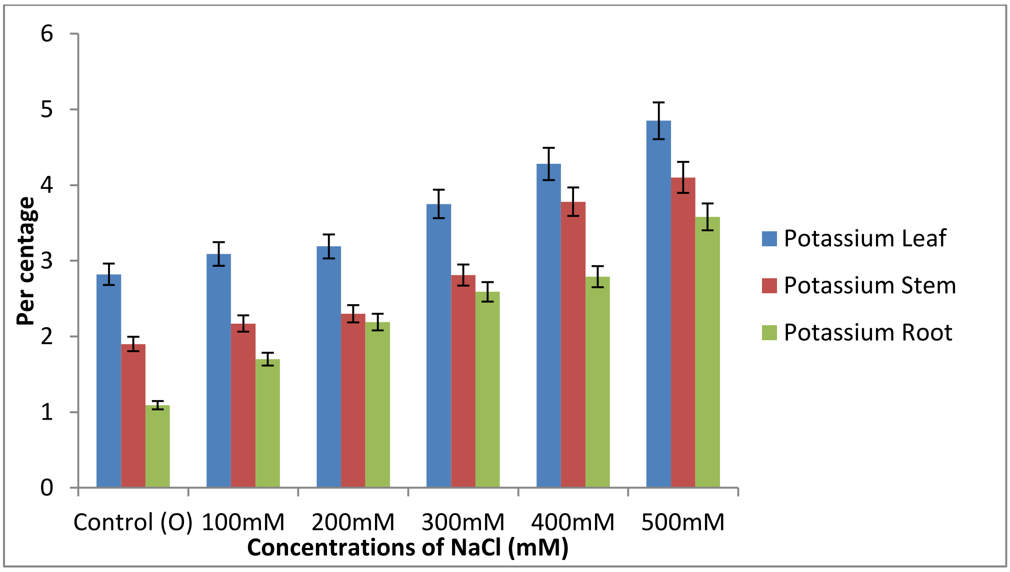

Fig. 6. Effect of NaCl on potassium content (mg/g dr. wt.) of the leaf, stem and root of *Clerodendron inerme* on 60^{th} day after salt treatment.

3. 7. Calcium

The results on the effect of different concentrations of NaCl on calcium content in the leaf, stem and root are given in Figure 7. The calcium content of all the three tissues increased with increasing salinity the optimum concentration in *Clerodendron inerme* and at higher concentrations the calcium content had decreased gradually. The leaf calcium was always higher than that of stem and root.

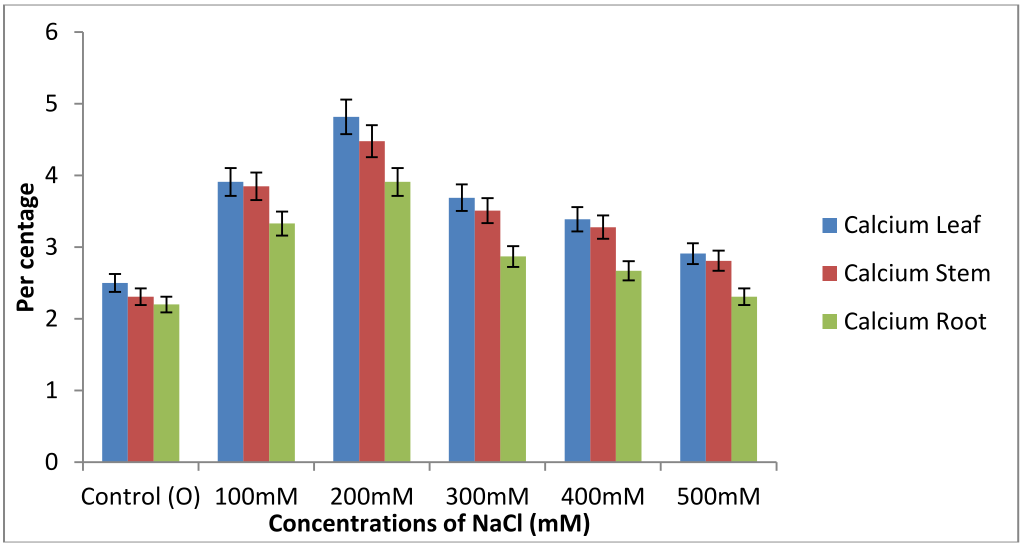

Fig. 7. Effect of NaCl on calcium content (mg/g dr. wt.) of the leaf, stem and root of *Clerodendron inerme* on 60th day after salt treatment.

4. DISCUSSION

In the present study, NaCl had favorably affected the growth of the seedlings of *Clerodendron inerme* by increasing shoot length and root length with increasing salinity. The upper limit for survival of seedlings to NaCl salinity was 500 mM. At the optimum concentration, NaCl had approximately similar effect on the growth of the seedlings. The data on growth response to revealed a depressed growth both in the absence of salt and at high salinity of NaCl. A stimulation of growth in response to moderate levels of NaCl salinity has been reported in several halophytes. *Sesuvium portulacastrum* survived up to 900 mM NaCl, but produced favorable growth at 600 mM NaCl (Venkatesalu *et al*., 1994*). Salicornia europaea* and *Arthocnemum australiasicum* survived double the strength of seawater (McMillan, 1974). A positive growth response to moderate salinities has been reported in the mangrove species *Avicennia marina* (Downton, 1982; Clough, 1984) and *Aegiceras corniculatum* (Ball and Forquhar, 1984).

The decline in leaf number at high concentrations was due to the leaf fall because of ageing. Salinity has been shown to be one of the external factors that influence the process of senescence and the consequent shedding of leaves (Pool *et al*., 1975). The number of leaves reduced only at the highest salt concentration but the dead leaves were increased with salinity as a mean of protecting the young growing leaves to toxic levels of the salts as well as off-loading the plants of excess salts (Wahome, 2001). The increase in the leaf area could be due to the increase in the volume of mesophyll cells due to increase in water content of the leaves and increase in succulence. The external NaCl stimulated the leaf area at optimum level of seawater in *Rhizophora mangle* (Hwang and Chen, 1995). Salinity has been reported to promote succulence in several plant species viz., *Plantago maritima* (Flanagan and Jefferies, 1988) and *Sesuvium portulacastrum* (Venkatesalu and Chellappan, 1993).

Sodium chloride salinity increased the fresh weight of leaf, stem and root with increasing salinity up to the optimum concentrations. The increase in fresh weight of the leaf tissue can be attributed to the increase in leaf thickness (Clipson, 1987) and the accumulation of ions and water in the tissues (Khan *et al*., 2005; Lee *et al*., 2005). Also, the fresh weight increase could be largely attributed to cell enlargement by water absorption, cell vacuolation and turgor driven wall expansion (Ayala and O'Leary, 1995). The increase in water content could be good reason for tissue succulence (Storey and Wyn Jones, 1979) and salinity promoted succulence in a number of halophytes (Ayala and O' Leary, 1995). The dry weight increase could be attributed to the accumulation of inorganic salts and organic matter in the plant tissues. In the dicotyledonous halophytes, it was observed that Na^+ and Cl^- ions were 30-50 % of the dry weight (Flowers *et al*., 1986). The results of the present study also indicated the obligate requirement of optimum concentration of NaCl for cell growth and increase in dry weight. The accumulation of salt has a positive function. Similar observations have been observed in certain other halophytes such as, *Chenopodium quinoa* (Prado *et al*., 2000), *Kandelia candel* (Hwang and Chen, 2001), *Heleochola setulosa* (Joshi *et al*., 2002) and *Aegiceras corniculatum* (Manikandan and Venkatesan, 2004).

The NaCl treatment increased the sodium content more in the leaf tissues than in the stem and root tissues with increasing of NaCl salinity up to extreme levels in *Clerodendron inerme* and *S. portulacstrum* on 60th day the sampling days. Increased availability of sodium in the soil influenced a proportional increase in the uptake of sodium by the seedlings. Many salt tolerant organisms accumulate higher intracellular ion concentration than their non-tolerant counter parts. Different halophytes have been reported to differ in the rate of accumulation of sodium in their cells and differences are mainly due to difference in the mode of salt regulation. Some vascular halophytes accumulate high levels of sodium and other salts in their above ground tissue while others did not (Gorham *et al.,* 1987).

The accumulation of high sodium content in halophytes in the present study are in agreement with those of several other accumulating type of halophytes such as *Suaeda maritima* (Clipson, 1987) *Suaeda nudiflora* (Joshi and Iyengar, 1987), *Sesuvium portulacastrum* (Venkatesalu *et al*., 1994), *Ipomoea pes-caprae* (Venkatesan *et al*., 1997) and *Suaeda fruticosa* (Khan *et al*., 2000). The concentration of Na^+ in the leaves of salinized plants was approximately 40-fold higher than that measured in non-salinized controls (Maggio *et al*., 2003). While sodium is not considered to be a plant nutrient, it is essential for halophytes to accumulate salts to maintain turgor pressure and growth (Borox, 2002), for survival in high salt (Wong *et al*., 2006).

Potassium is a macronutrient that is required in the plant cell in high concentrations. It plays essential roles in growth regulation, osmotic adjustment and regulation of stomata opening (Brownell, 1979). In all plant cells, cytosolic K^+ concentrations must remain relatively high (Leigh and Wyn Jones, 1984). Chloroplasts and mitochondria also contain relatively high levels of potassium. On the other hand, Na^+ concentrations must be maintained at low levels in the cytoplasm and in the organelles (Wang *et al*., 2004). The increasing salinity decreased the potassium contents of some halophytes such as *Suaeda maritima* (Clipson, 1987); *Rhizophora mangle* and *Laguncularia recimosa* (Medina *et al*., 1995). Potassium uptake was adversely affected by NaCl treatment in *Hordeum vulgare* and *Salicornia europea* (Demiral *et al*., 2005; Ushakova *et al*., 2005).

Increase in calcium content can be attributed to the overall performance of metabolic activity of plants treated with the salts up to the optimum concentrations. Calcium also serves to protect membrane damage and it plays a key role in the selective transport of potassium in the presence of excess of sodium, and thereby making a plant more salt tolerant (Epstein, 1980). Increasing external calcium salinity decreased the Ca^{2+} contents in *Aegiceras corniculatum* (Shindle and Bhosale, 1985); *Rhizophora mucronata* and *Avicennia officinalis* (Bhosale and Malik, 1991); *Allenrolfea occidentalis* (Bilquees *et al*., 2000) and *Atriplex* *griffithii* (Khan and Ungar, 2000). In *Suaeda nudiflora*, salinity caused no change in Ca^{2+} content (Joshi and Iyengar, 1987).

5. CONCLUSION

In conclusion, the observation on NaCl treated *Clerodendron inerme* plant suggested that growth and minerals constituent's increased up to optimal level of salinity and at higher salinity declined gradually.

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