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RESEARCH ARTICLE

Antioxidant defence mechanism operational in a mangrove - *Acanthus ilicifolius* L. subjected to NaCl stress.

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Abstract

Hydroponically grown *Acanthus ilicifolius* were exposed to different concentrations of NaCl (200, 400 and 600 mM) for 15d in order to characterize the potential of antioxidant system. In this study, our focus was to determine the NaCl tolerance potential of *A. ilicifolius*, on the basis of extend of lipid peroxidation, antioxidant enzyme activity and osmoregulation mechanism when subjected to high NaCl concentration. Antioxidant enzymes (SOD, CAT, GPX and APX) showed an enhanced activity in all the treatments and the increased activity was more significant in 400 mM treated plants. In 600 mM treated plants, a decrease in activity of antioxidant enzymes, accumulation of antioxidant molecules and malondialdehyde content was recorded upon increasing the treatment period. However, the PPO activity and ascorbate content showed reduction in all the treatments as compared to the control. These results indicates that *A. ilicifolius* exhibits efficient tolerance to NaCl upto a level of 400 mM, beyond which it turns to be intolerable. It can be concluded that, short term exposure to NaCl could trigger the stress tolerating mechanism of salt excreting mangrove *A. ilicifolius*.

Introduction:-

Mangrove plants form a unique community as they can grow and tolerate high salt concentration and they protect the adjacent land from tidal waves. Mangroves are likely to be one of the first ecosystems to be affected by the global changes because of their location at the interface between land and sea (Parida and Jha, 2010). The degree of salt tolerance among different mangrove species exhibits a wide variation. In experiments performed under controlled conditions, many halophytes show optimal growth in the presence of low or moderate salt concentrations and inhibition of plant growth is always observed at sufficiently high salinity levels (Grigore et al., 2012). According to Ye et al. (2005), the relative growth rate in *Acanthus ilicifolius* seedlings decreased upon increasing the NaCl concentration.

In plants, NaCl stress causes a range of adverse effects, mainly ionic disorders and osmotic stress. A common feature of these effects is the production of reactive oxygen species (ROS) (Ashraf and Foolad, 2007). NaCl stress causes stomatal closure, which reduces the CO_2/O_2 ratio inside leaf tissues and inhibits CO_2 fixation. As a consequence, an over reduction of the photosynthetic electron transport chain occurs, which causes the generation of ROS. Mangrove plant cells are well protected against these detrimental effects of ROS by a complex antioxidant system comprising of non-enzymatic and enzymatic antioxidants (Kathiresan and Bingham, 2001). Of these, the prominent is the enzymatic defence system of mangrove plants, which includes different endogenous enzymes as well as developed network of antioxidants molecules such as ascorbic acid, tocopherols, sugars, amino acids etc. (Naskar and Palit, 2015).

Despite the importance of salinity in controlling vegetation dynamics and primary productivity in coastal marsh

communities, our understanding of how marsh plants respond physiologically to changing salinities still remains limited (Touchette et al., 2009; Baskaran et al., 2016). Halophytes known for their unique ability to tolerate high salinity are widely studied in many species to elucidate the mechanism underlying their capacity to handle high NaCl concentration (Saravanavel et al., 2011; Tabot and Adams, 2012; Wang et al., 2014). Therefore, the objective of the present work is to gain some insight into the NaCl tolerance potential of *A. ilicifolius*, a predominant NaCl excretory mangrove species by studying the antioxidant defence potential under increasing concentrations of NaCl.

Materials and methods:-

Plant material and growth conditions:-

Acanthus ilicifolius L., a halophyte species belonging to the family Acanthaceae is naturally growing abundantly in the mangrove vegetation area of Kadalundi Vallikkunnu Community Reserve (KVCR) of Kerala, India. 20-30 cm long stem cuttings with uniform size and healthy appearance were treated with Indole butyric acid (IBA) 15 µM for 2 h to induce root initiation. Rooted cuttings were then transferred to half strength modified Hoagland solution (Epstein, 1972). Plants were maintained in green house under controlled conditions of temperature (28±2^{o}C), light intensity (100 µmolm^{-2}s^{-1}) and relative humidity (65±5%).

Experimental design

In the initial phase of the study, stem cuttings having a pre growth period of 35d (with two pairs of new leaves) were treated with varying levels of NaCl (0, 100, 200, 300, 400, 500 and 600 mM). The plants growing in half strength Hoagland solution without NaCl were taken as the control plant. Samples (root and leaf) were collected at selected intervals (0, 3, 6, 9, 12 and 15d) for the analyses. The 2nd and 3rd pair of newly formed leaves from the tip of the shoots was harvested for further physiological and biochemical analyses.

Metabolites:-

Free proline content was extracted from leaf using 3% sulphosalicylic acid and estimated following the method of Bates et al. (1973) using L-proline as standard. Total soluble sugar content was extracted using 80% ethanol and estimated following the method of Dubois et al. (1956). Standard curve was plotted against D-glucose as standard. Free amino acids were extracted using 70% ethanol and estimation was carried out following the method of Moore and Stein (1948), using ninhydrin reagent. Total free amino acids were calculated from a standard curve prepared against glycine (0–100mg).

MDA estimation:-

Malondialdehyde (MDA) was estimated according to the method of Heath and Packer (1968), and was calculated using its molar extinction coefficient of 155 mM L^{-1} cm^{-1}.

Enzymatic antioxidants:-

Preparation of enzyme extract and assay of enzyme activity:-

Fresh leaf tissues (0.5g) were homogenized in 5mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0) using a prechilled mortar and pestle. The homogenized extract was filtered through 2 layers of muslin cloth and the filtrate was centrifuged at 10,000g for 15 min at 4°C. The supernatants were collected and used for the enzyme assay (Yin et al., 2009).

Superoxide dismutase (SOD, EC 1.15.1.1): Activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to the method of Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT.

Catalase (CAT, EC 1.11.1.6): Activity was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Kar and Mishra, 1976). One unit of the enzyme was defined as µmoles H_2O_2 decomposed per minute per mg protein.

Guaiacol peroxidase (GPX, EC 1.11.1.7): Activity was measured by following the change of absorption at 420 nm due to guaiacol oxidation (Polle et al., 1994). One unit of the enzyme was defined as µmoles of guaiacol oxidized per minute per mg protein.

Ascorbate peroxidase (APX, EC 1.11.1.11): Activity was assayed as described by Nakano and Asada (1981). One unit of the enzyme was defined as μmoles of ascorbate oxidized per minute per mg protein.

Polyphenol oxidase (PPO, EC 1.10.3.1): Activity was assayed by the method of Kumar and Khan (1982). One unit of the enzyme was defined as the amount of enzyme that is required for an increase in absorbance of 0.001 per minute per mg protein.

Non-enzymatic antioxidants:-
Ascorbic acid content was measured after the method of Gillespie and Ainsworth (2007). A standard curve was prepared using commercial L-ascorbic acid. Total polyphenols were extracted by 80% ethanol and estimated according to the method of Folin and Denis (1915). A standard curve was prepared using different concentration of catechol (10–100mg).

Statistical analysis:-
Statistical analysis of the results was carried out according to Duncan's multiple range tests at 5% probability level. Data were subjected to one-way ANOVA using the SPSS software 16.0. The data is an average recordings from three independent experiments each with three replicates (i.e., n=9). The data represent mean ± standard error.

Results:-

In the initial phase of the study, total chlorophyll content and chlorophyll *a* fluorescence analysis were carried out in *A. ilicifolius* treated with different concentrations of NaCl (0, 100, 200, 300, 400, 500 and 600 mM). Total chlorophyll content was showing a decreasing pattern upon increasing the NaCl concentration, i.e., 0.4, 2, 39, 61, 76 and 91% reduction in the treatments of 100, 200, 300, 400, 500 and 600 mM NaCl, respectively as compared to the control on 15d of the treatment (supplementary table). Likewise, the fluorescence parameters Fv/Fm, which is a measure of the quantum yield of primary photochemistry of PSII and performance index (PI_{Total}), which is a parameter to quantify the effects of environmental factors on photosynthesis (Tsimilli-Michael and Strasser, 2008) also showed a decreasing trend with increase in concentration of the treatments as compared to the control plants. The decline in Fv/Fm and PI after 400 mM NaCl was found to be significant as compared to the control plants, i.e., in the case of Fv/Fm, the values reduced from 0.825 (control) to 0.228 (500 mM) and 0.116 (600 mM) and in the case of PI, the values reduced from 2.188 (control) to 0.005 and 0.002 in 500 and 600 mM NaCl, respectively (supplementary fig.). The results of the above parameters gave an indication that the photosynthesis process in *A. ilicifolius* subjected to NaCl above 400 mM was highly impaired. Therefore, detailed analysis of osmoregulation mechanism, lipid peroxidation, antioxidant enzyme activity and accumulation of nonenzymatic antioxidants in *A. ilicifolius* was analysed by subjecting the plants to 0, 200, 400 and 600 mM NaCl.

Metabolites:-
Maximum proline accumulation was observed on 15d in leaves of plants subjected to 400 mM NaCl and in roots of plants subjected to 600 mM NaCl. About 296 and 127% increase was recorded over the control in the leaf and root tissue respectively in 400 mM NaCl conditions as compared to the 8 and 14% increase in the 200 mM NaCl treated plants. However, it showed a decreased profile in both the leaf and root tissues after prolonged treatment (after 6d) with high NaCl concentration of 600 mM (Fig. 1a, b). Similar trend of increase was also observed in the case of soluble sugars in both the leaf and root tissues of *A. ilicifolius*. In both the leaf and root tissues, maximum accumulation of soluble sugar was recorded on 15d of 400 mM NaCl treatment i.e., about 126 and 201% increase over the control plants. The minimum sugar accumulation was observed in the 600 mM NaCl treated plants, i.e., about 82 and 30% decrease in the leaf and root respectively over the control plants (Fig. 2a, b). The highest content of aminoacids was recorded in leaves on the 12d of 200 mM NaCl treatment (145.32 mg/g DW) and in roots on 9d of 600 mM NaCl treatment (183.17 mg/g DW). The least aminoacid content was recorded on 15d when the plants were treated with 600 mM NaCl i.e., about 2 and 15 fold decrease in both the leaf and root tissue respectively over the control plants (Fig. 3a, b).

MDA content:-
Upon treatment with different NaCl concentrations, the MDA content was found to be increased in both leaf and root tissues. In leaves, the highest MDA content was recorded on 9d in plants treated with 400 mM NaCl i.e., about 149% increase over the control plants. Similarly, the maximum MDA content in root tissue was observed in plants treated with 600 mM NaCl on 12d i.e., about 405% increase over the control plants. Where as

in 200 mM NaCl treated plants the increase was found only 36 and 56% in the leaf and root tissues respectively over the control plants on 15d (Table 1).

Enzymatic antioxidants:-
In plants subjected to NaCl of 400 mM, there was significant increase in the activity of SOD, in both the leaf and root tissues on 9d of treatment (107 and 127% respectively). However in 200 mM NaCl, less increase was observed upto 6d of treatment and beyond the period no significant increase was observed in both the leaf and root tissues. Likewise in 600 mM NaCl, a significant increase was observed upto 9d of treatment followed by a sharp decrease in SOD activity both in leaf and root tissues (Fig. 4a, b).

A gradual increase in CAT activity was observed upto the 15d of treatment period in the leaf and root tissues of 200 mM NaCl treated plants. But, in 400 mM NaCl treated plants the increase was much more significant i.e., 20 and 3 fold increase on 9d of treatment in the leaf and root tissues respectively. Even though an initial increase was observed in the activity of CAT in the 600 mM NaCl treated plants it was found to exhibit a sharp decline thereafter in both the tissues (Fig. 4c, d).

Like CAT, GPX activity was found to increase gradually in 200 mM NaCl treated plants and the increase was found to be maximum in the 400 mM NaCl treated plants i.e., a 10 and 6 fold increase in activity as compared to the control plants in the leaf and root tissues respectively. However, in the extreme saline conditions of 600 mM NaCl, both the leaf and root tissues exhibited an initial enhanced profile of activity followed by a decreased activity (Fig. 4e, f).

Likewise, activity of APX was found to be enhanced upon NaCl treatment as that of GPX, CAT and SOD. In both the leaf and root tissues maximum APX activity was recorded when plants were treated with 400 mM NaCl (1457 and 305% respectively) followed by the 200 mM NaCl (131 and 480% respectively) treatment. The activity was found to be least in the 600 mM NaCl treated plants, i.e., 66 and 36% in the leaf and root tissues respectively (Fig. 4g, h).

In contrast with the above mentioned antioxidant enzymes, the activity of PPO was found to be decreased upon increasing concentrations of NaCl, i.e., 600 mM NaCl stressed plants showed maximum decrease in PPO activity and the values reduced from 5.44 to 0.94 Umg^{-1} protein in leaves and from 1.38 to 0.26 Umg^{-1} protein in roots (Fig. 4i, j).

Non-enzymatic antioxidants:-
About 1.6 fold decrease in ascorbate content was recorded in the 200 mM NaCl treated plants as compared to the control plants. In the case of 400 mM NaCl treatment, the reduction of ascorbate content recorded was 3 and 4 fold in the leaf and root tissues respectively as compared to the control plants. The observed value of acsorbate for the control plants was 12.17 µmol/g DW, and it reduced upto 0.96 µmol/g DW in the leaves of 600 mM NaCl treated plants. Similarly, in roots the ascorbate level reduced from 13.33 µmol/g DW in control to 0.99 µmol/g DW in 600 mM NaCl treated plants (Fig. 5a, b).

The total phenolic contents were showing an increased pattern of accumulation under NaCl treatment in both the root and leaf tissues. However, the increase was found to be more significant in 400 mM NaCl treated plants (80 and 21%) than the 200 and 600 mM NaCl treatments over the control plants. Extreme saline condition (600 mM NaCl) caused a significant decrease in the total phenolic content of the plants i.e., about 9 and 6 fold decrease in the leaf and root tissues respectively over the control plants (Fig. 6a, b).

Discussion:-

Metabolites:-
Generally, proline protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, protect cellular components from dehydration injury and stabilization of enzymes/proteins (Jisha and Puthur, 2014). Our observation is consistent with the previous reports that described a linear relationship of proline accumulation in plant with conductivity and salinity of the growth medium (Tabot and Adams, 2012). Increased proline content in mangrove plant *Suaeda nudiflora* Moq with decreased ROS production under NaCl stress has been observed

(Cherian and Reddy, 2003). Thus, the significant increase in proline accumulation in the 400 mM NaCl treatment over the control plants is a clear indication of NaCl stress stimulated accumulation of it. Moreover, it is proposed that the high levels of proline actually provide the basis of resistance to salt accumulation (Bandaranayake, 2002).

The initial (6d) enhancement in total soluble sugars in roots of plants treated with 600 mM NaCl was an attempt to cope with the external high osmoticum, but later on (9-15d) it was found that the sugar content decreased drastically showing that the process of sugar synthesis was negatively affected. In 400 mM, there was an increase in concentration of total soluble sugars recorded from 3d, which progressed steadily upto 15d. This is a clear indication that, sugar accumulation contributed significantly towards maintaining the osmoticum of the cell sap. The presence of higher amounts of soluble sugars has been reported as main contributors to osmotic adjustment in the *Atriplex halimus* plants exposed to PEG and NaCl stresses and it was correlated with the response of NaCl stress on soluble sugar synthesis (Martinez et al., 2005).

Synthesis and accumulation of free amino acids in plant cells is considered as an adaptive stress response of the plant (Gaspar et al., 2002) and regarded as compatible solute that adjust osmotic potential in cytoplasm (Arshi et al., 2005; Bartels and Sunkar, 2005). Thus, the enhanced aminoacid profile recorded in the 200 and 400 mM NaCl treated plants contribute towards the osmoregulation mechanism under increased concentration of Na^+ and Cl^- ions. Parida et al. (2002) showed that in the cell sap of *Bruguiera parviflora*, the increment of osmotic solutes, like total sugar and total free amino acids occurred under NaCl treatment and these solutes help to restore more negative water potential and might be considered as marker of salt tolerance.

MDA content:-

Parameters of oxidative stress such as malondialdehyde (MDA), a product of lipid peroxidation have shown increasing trend with increased salinity in mangroves (Parida and Jha, 2010). During salt stress as a normal physiological response, plants generates reactive oxygen species, which led to the membrane lipid peroxidation, resulting in the dramatic increase in MDA content, and the emergence of this signal could induce the activation of antioxidant defense system and the expression of related enzymes in plants (Liang et al., 2008). The increased amount of MDA content recorded in *A. ilicifolius* treated with varying concentrations of NaCl (0-600 mM) indicates that the membrane lipid peroxidation was aggravated upon NaCl treatment. Antioxidant enzymes which scavenge the reactive oxygen (CAT, GPX, APX and SOD) also showed significant increase in activity upon NaCl treatment. In consistent with our results, MDA content in the root of *Kandelia candel* had increased upon NaCl treatment with the subsequent increase in the activity of various antioxidant enzymes (Wang et al., 2014).

Enzymatic antioxidants:-

In order to assess the role of the antioxidative defence system against NaCl treatment, the activities of major antioxidative enzymes were monitored in *A. ilicifolius* subjected to varying levels of NaCl under hydroponic culture. In *A. ilicifolius*, NaCl treatment preferentially enhanced the activity of APX, GPX, SOD, CAT, whereas it induced the decrease of PPO activity. Generally, environmental stresses increase the production of superoxide at a rate depending on the species, the stress period, intensity and the age of plants. Recent works and many previous works have showed that NaCl tolerance is closely related to the efficiency of antioxidant enzymes. SOD, CAT and GPX are among the major antioxidant enzymes involved in scavenging reactive oxygen species (Thatoi et al., 2014). Our data support this hypothesis, clearly displaying significantly increased activities of the above enzymes in leaves and roots of the *A. ilicifolius*. This increased activity of SOD indicates that it catalyzes the disproportion reaction of two superoxide radicals generated from the NaCl treatment, to generate O_2 and H_2O_2. As the rate of O_2 and H_2O_2 radicals increases by SOD pathway, the plant switch the GPX and CAT activity inorder to remove these deleterious free radicals from the cells. This accounts for the increased activity of GPX and CAT in the leaves and root tissues of *A. ilicifolius* upon treatment with NaCl. Coinciding with our results, a steep increase in total SOD activity levels has been recorded in two mangroves, *Bruguiera gymnorrhiza* and *Bruguiera parviflora* during NaCl stress (Takemura et al., 2000; Parida et al., 2004). Similarly, catalase activity was found to be increased under NaCl stress in *Bruguiera gymnorrhiza* (Takemura et al., 2000). Cherian et al. (1999), have reported the increased GPX activity in root and shoot tissues in *Avicennia marina* and Parida et al. (2004) have reported increase in APX content in *Bruguiera parviflora* under NaCl stress. However, in contrast to the enhanced activities of the above enzymes, activity of PPO was tend to show a decreased profile in both the leaf and root tissues as compared to the control plants. It was earlier reported that the decreased activity of polyphenol

oxidase may be due to the induced accumulation of total phenols in tissues (Ozturk and Demir, 2003). The increase of total phenol content in the leaf and root tissues (80 and 21 % respectively) and the associated decrease in activity of PPO under NaCl stress as compared to the control is in confirmation with the above statement.

Nonenzymatic antioxidants:-

Ascorbate is a ubiquitous soluble antioxidant in plant cells which can directly scavenge ROS and act as reducing substrate for APX and GPXs to detoxify H_2O_2 (Mittler, 2002). The decreased level of ascorbate in *A. ilicifolius* is likely to be due to its participation in reducing H_2O_2 to H_2O catalyzed by the increasing activity of APX. Prolonged treatment with high NaCl level caused a lowering of ascorbate and oxidized glutathione concentration without any changes in the reduced level of glutathione in *Bruguiera parviflora* (Parida et al., 2004). One of the main roles of many plant phenolics may be to protect leaves from photodamage, they can achieve this by acting as antioxidants; and their levels may vary with environmental conditions in order to counteract this potential photodamage (Close and McArthur, 2002). Thus, the increase in total phenol content of *A. ilicifolius* plants treated with NaCl may be an indication of the efficient antioxidant mechanism operational in the plant. According to Ksouri et al. (2007), polyphenols synthesis and accumulation in plants are generally stimulated in response to salinity stresses.

Figures:-

Figure 1: Effect of NaCl on proline content (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

Figure 2: Effect of NaCl on soluble sugar (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

Figure 3: Effect of NaCl on aminoacid content (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

Figure 4: Effect of NaCl on the activity of different antioxidant enzymes in *A. ilicifolius* L. SOD (A-leaf, B-root), CAT (C-leaf, D-root), GPX (E-leaf, F-root), APX (G-leaf, H-root) and PPO (I-leaf, J-root). Enzyme activity was expressed as unit per mg protein. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

Figure 5: Effect of NaCl on ascorbate content (µmol/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

Figure 6: Effect of NaCl on total phenolic content (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

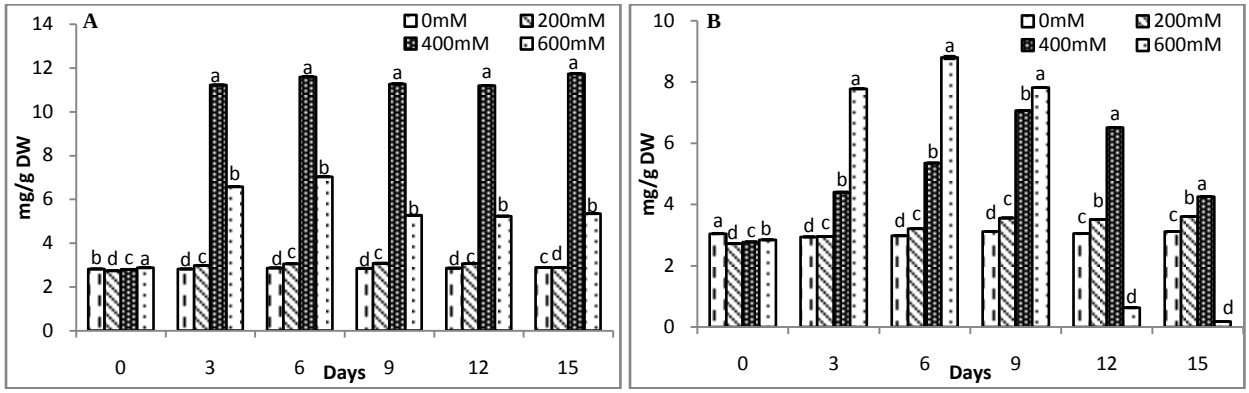

Figure 1 Effect of NaCl on proline content (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

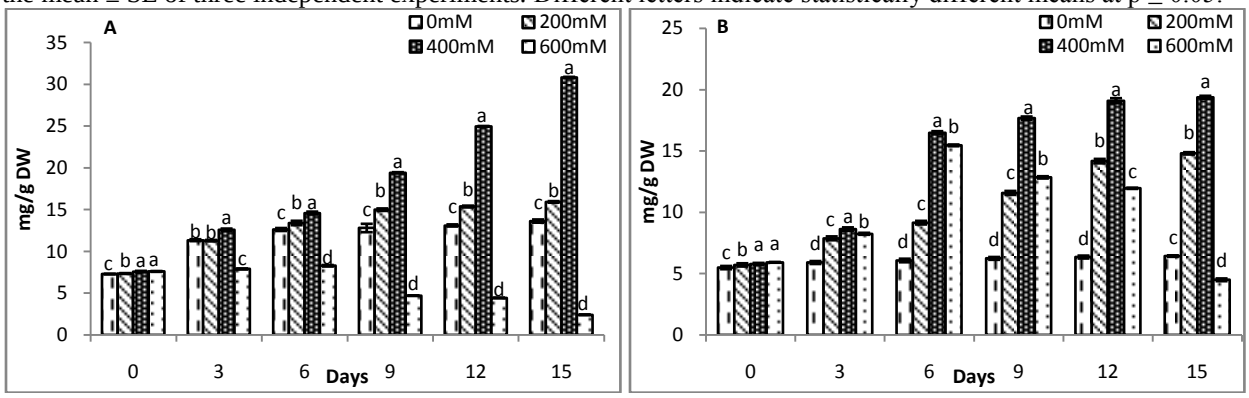

Figure 2 Effect of NaCl on soluble sugar (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

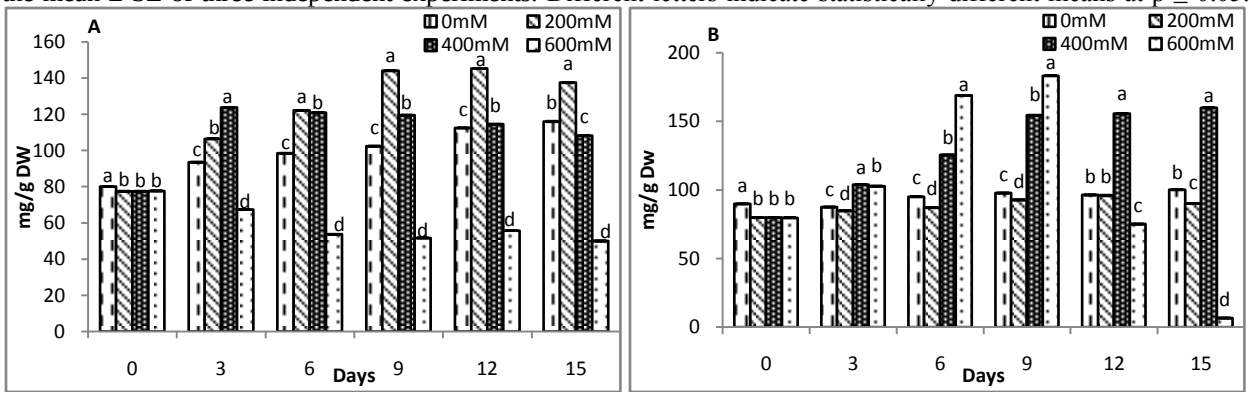

Figure 3 Effect of NaCl on aminoacid content (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

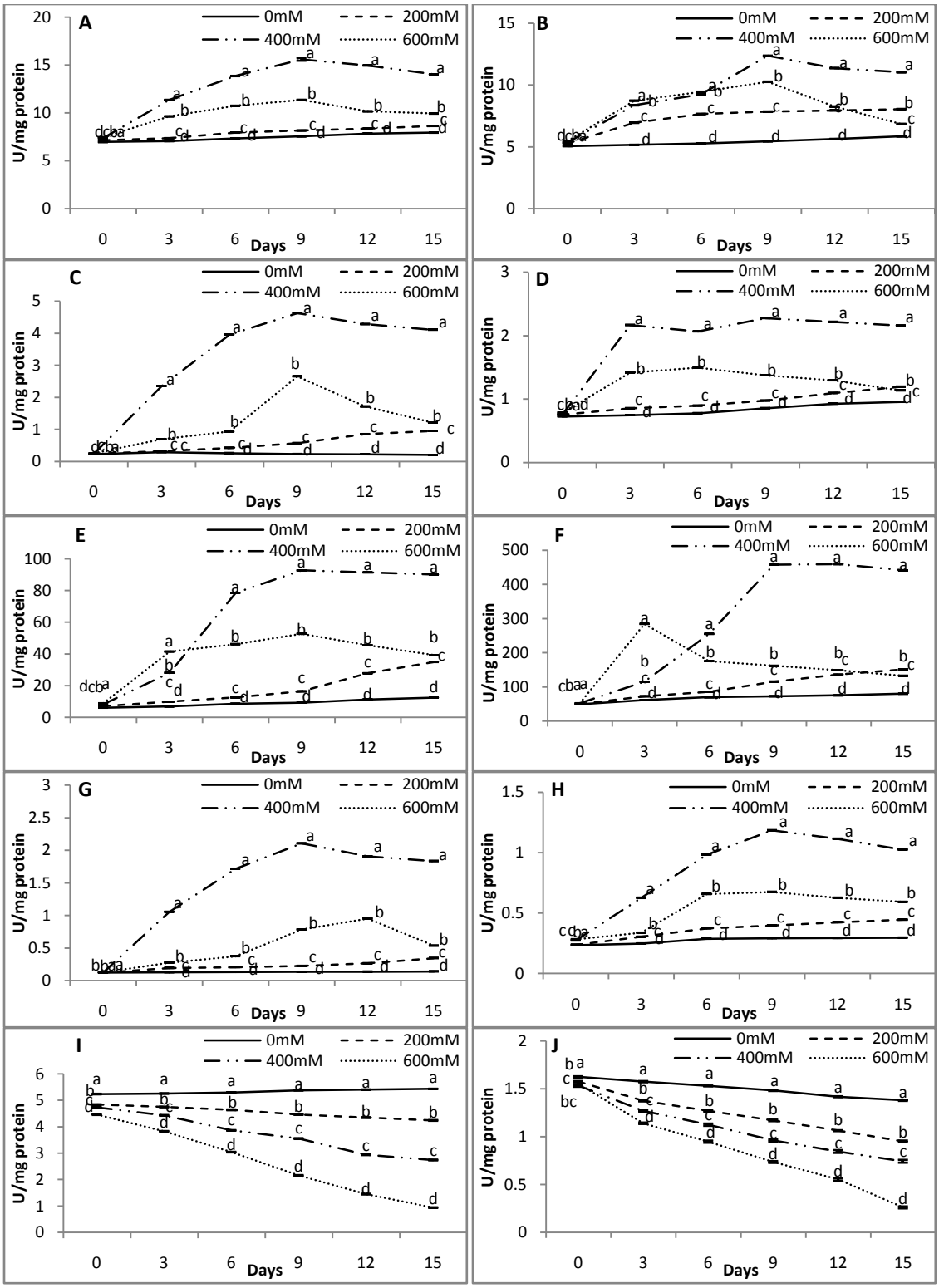

Figure 4 Effect of NaCl on the activity of different antioxidant enzymes in *A. ilicifolius* L. SOD (A-leaf, B-root), CAT (C-leaf, D-root), GPX (E-leaf, F-root), APX (G-leaf, H-root) and PPO (I-leaf, J-root). Enzyme activity was expressed as unit per mg protein. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

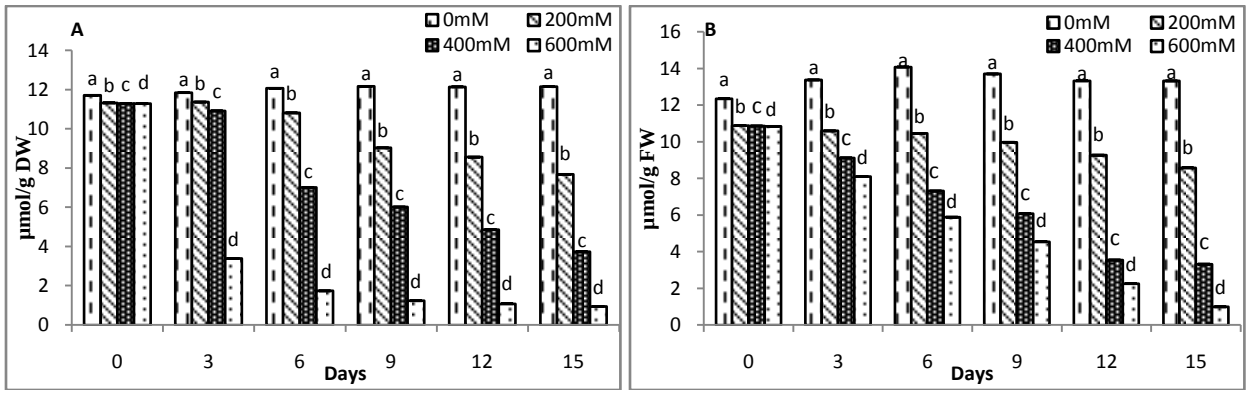

Figure 5 Effect of NaCl on ascorbate content (µmol/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

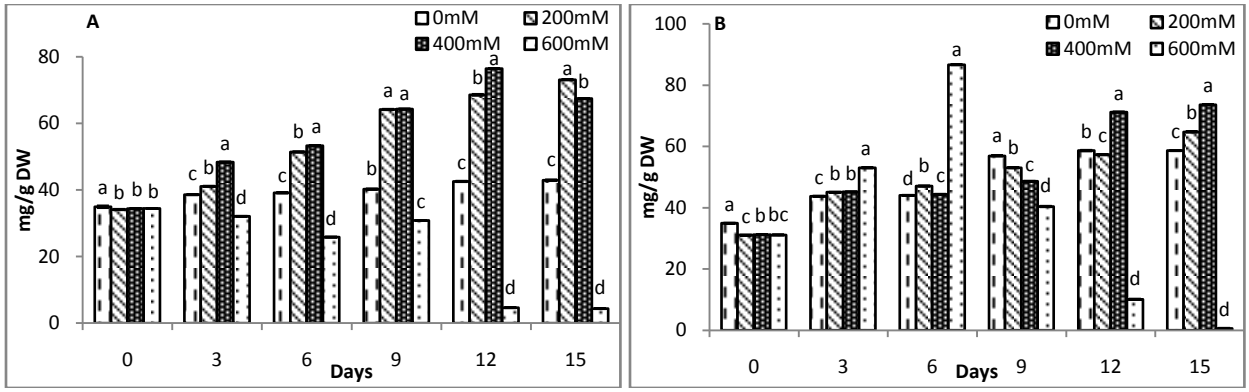

Figure 6 Effect of NaCl on total phenolic content (mg/g DW), in the leaves (A) and roots (B) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means at $p \geq 0.05$.

Table:-

MDA:-

Table 1 Effect of NaCl on MDA content (µmol/g DW), in the leaves (a) and roots (b) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments. Different letters indicate statistically different means as at $p \geq 0.05$.

Duration of treatment (Days)	NaCl (mM/L)							
	0		**200**		**400**		**600**	
	Leaf	**Root**	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
0	42.75± 0.132a	16.37± 0.092a	41.93± 0.064c	15.27± 0.062c	42.38± 0.049b	15.63± 0.141b	42.55± 0.049ab	16.08± 0.110a
3	43.68± 0.066d	16.21± 0.091d	44.67± 0.243c	23.12± 0.131c	93.04± 0.522a	36.035± 0.176b	53.04± 0.129b	61.17± 0.173a
6	44.33± 0.186d	17.19± 0.216d	49.74± 0.111b	26.22± 0.172c	110.25± 1.177a	46.81± 0.095b	46.50± 0.054c	69.17± 0.247a
9	45.76± 0.042d	19.15± 0.064d	55.71± 0.213b	26.20± 0.229c	114.02± 1.450a	60.79± 0.261b	48.65± 0.043c	79.03± 0.300a
12	45.58± 0.216d	19.17± 0.062d	64.44± 0.082b	28.16± 0.311c	96.02± 0.043a	58.88± 0.121b	61.69± 0.095c	96.82± 0.143a
15	50.17± 0.185d	20.88± 0.109d	68.41± 0.163c	32.67± 0.146c	84.15± 0.038b	55.51± 0.111b	87.12± 0.051a	59.25± 0.318a

Conclusion:-

The high media salinity usually affects plant growth due to low water potential, ion toxicities, nutrient deficiencies or a combination of these. In the case of *A. ilicifolius*, the tolerance limit falls at 400 mM NaCl almost near to sea water salinity of 500 mM as assessed by the accumulation and activity of antioxidant compounds and the increased efficiency of osmoregultion mechanism. This indicate that *A. ilicifolius* is moderately saline tolerant and it may be intolerant to a concentration of above 400 mM NaCl.

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