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Research Article

EFFECT OF SALT STRESS (NACL) ON DIFFERENT GROWTH PARAMETERS, PHOTOSYNTHETIC PIGMENTS AND LIPID PEROXIDATION IN THE LEAVES OF LOCAL CULTIVAR OF TOMATO (SOLANUM LYCOPERSICUM)

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ABSTRACT

The over salinity in the soil is one of the main factors that limits the spread of plants in their natural habitats. The NaCl stress was found to significantly decline the growth parameters and the photosynthetic pigment with compared to control. This present study was conducted to investigate the effects of salt stress on different growth parameters, photosynthetic pigments and lipid per oxidation of the local cultivar of Tomato. The results showed that as the salt concentration increased all the parameters get decrease significantly.

INTRODUCTION

The Abiotic stresses are major constraints for global crop production. Among various abiotic stresses the salinity has become a severe threat to ensure food security by affecting about one-third of the irrigated land on earth (Mortezai nejad F and P Rezai, 2009). About 20% of the world's cultivated land area and 50% of all irrigated land is affected by salinity (Moud and Maghsoudi, 2008). Saline soils are one of the major abiotic stresses that can adversely affect the overall metabolic activities and cause plant death (Roychoudury *et al*, 2008). Salinity problems can also occur in irrigated agriculture particularly when poor quality water is used for irrigation (Mitchell *et al*., 1991; Shibli, 1993). The scarcity of water resources in most countries of the arid and semiarid regions has led many farmers to use poor quality water for irrigation. Increasing the level of the soluble salts in the soil solution tends to increase its osmotic pressure and cause an individual ion toxicity (Greenway, 1973) which leads to decrease in the water and nutrient uptake by plants (Smith *et al*., 1992). It inhibits the uptake and transport of different ions. On improving salt tolerance the salt stress can also cause oxidative damage to membrane lipids, proteins and nucleic acids. Thus it is a serious threat to agricultural productivity especially in arid and semi-arid regions (Parvaiz *et al.*, 2008). High concentration of NaCl in saline soil subsequently sets up a severe competition between Na^+ and K^+ions which in turn disturbs the common transport system of plant roots. Ionic imbalance occurs in cells due to excessive accumulation $Na^+$and Cl^-ions that reduce uptake of K^+,Ca^{2+}, and Mn^{2+}(Bayuelo-Jimenez *et al*., 2003). Salinity delays the germination, emergence and the young salt-stressed seedlings may be more susceptible to hypocotyls and cotyledon injury (Miyamoto *et al*., 1985; Esechie *et al*., 2002). Shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point. Early flowering reduced dry matter, increased root shoot ratio and leaf size caused by salinity which may be considered as possible ways of decreasing yield in plant under salt stress condition (Mengel K, *et.al*; 2001). Salinity stress reduces elongation rate of the main stem in tomato (Tal and Shannon, (1983). Shoot length is one of the most reliable response indicators for a wide range of tomato genotypes under salinity stress (Cruz *et al*., 1990). Significant reductions in fresh and dry weight of tomato shoots were reported in response to salinity stress (Bolarin *et al*., 1991, 1993). Root fresh and dry weights were reported to decrease in response to increased induced salinity stress in other tomato genotypes (Shibli *et al.*, 1997). Root systems have been considered as the basic system to counteract salinity stress (Smith *et al*., 1992). The effect of salinity on plants was expressed as reduced shoot dry weight because vegetative growth in the most widely used index in studies on salt tolerance in tomato (Cruz *et al.*, 1990).

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Excess sodium ions in cells cause enzyme inhibition and degradation of photosynthetic pigments (Chaves *et al*., 2009). Photosynthesis is one of the most severely affected processes due to more salinity induced decrease in stomatal conductance (Parida *et al*., 2004), internal CO_2 partial pressure and stomatal opening which affect the gaseous exchange (Iyenger and Reddy, 1996).Excess sodium ions in cells cause enzyme inhibition and degradation of photosynthetic pigments (Chaves *et al*., 2009).Its excessive exposure reduces tomato fruit size, total yield, and photosynthesis and increases blossom end rot. The damages to plants caused by saline irrigation may be enhanced by high temperatures and low relative humidity as well as long-term salinization of the soil that undergo to permanent modification of its physicochemical properties. Increasing salinity due to human activities i.e. natural processes and agricultural practices is a common environmental problem (Epstein *et al*, 1980). It exerts their effect by causing oxidative damage. This damage is caused by increased production of reactive oxygen species (ROS) (Smimoff, 1995). Excess production of ROS during stress results from impaired electron transport processes in chloroplast and mitochondria as well as from pathways such as photorespiration (Sanchez Rodriguez *et al.*, 2012). In the absence of a protective mechanism in plants ROS can cause serious damage to different aspects of cell structure and function such as initiating lipid per oxidation and damaging DNA, proteins and other small molecules (Arora *et al.*, 2002; Gill and Tuteja, 2010; Ahmad *et al.*, 2011).

Salinity induces biochemical changes in the exposed plants such as the activity of peroxidases as a group of enzymes affected by salt stress (Sancho *et al*., 1996). Tomato shoot and fruit physiological responses to salt stress conditions have been extensively investigated (Cruz *et al*., 1990; Mitchell *et al*., 1991; Niedziela *et al*., 1993). Nutritional imbalance caused by such ions leads to reduction in photosynthetic efficiency and other physiological disorders. Salt tolerance is higher in tomato plants that were treated at the germination stage than in plants treated after emergence. (Bolarin *et al*., 1993). In tomato, controlled Na^+ accumulation in the shoot may be an important factor in enhancing salt tolerance (Perez-Alfocea et al,. 1996; Cuartero and Fernandez-Munoz, 1999). Ionic toxicity is caused by excessive amount of salt entering the transpiration stream which injures the cell in transpiring leaves and may further reduce growth (Munns *et.al*, 2006). Plants under stress conditions with high salinity levels in their cytoplasm stored excessive Na^+ in their vacuoles to maintain metabolic functions (Parida and Das, 2005).Plants under salinity stress uptake lower levels of K^+ withincreasing uptake levels of Na^+(Yeo *et al*., 1991; Parida and Das, 2005; Mugdal *et al*., 2010) and they tend to accumulate simple sugars under stress conditions (Ashraf and Harris, 2004). Low salinity levels increase chlorophyll content (Franco *et al*., 1993; Ashraf, 2004). Excess sodium chloride inhibits synthesis of new chlorophyll and increases destruction of chlorophyll and the structure of chloroplasts (Reddy and Vora, 1986; Sakaki *et al*., 1983). The chlorophyll content of tomato plants decreased with increasing NaCl concentration. 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). 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). Depending on increasing salinity levels there is a decrease in vegetative growth parameters have been observed in plants (Sixto *et al.*, 2005; Pessarakli & Touchane, 2006). The survival of tomato plants in saline soils depends on their ability to synthesize organic compounds e.g. proline and sugars (Fariba and Ehsanpour, 2005; Flowers, 2004) and accumulation of Na^+ and K^+ (Borsani *et al.,* 2003) to decrease salt stress damage. Salinity can change the uptake mechanisms of nutrient elements, resulting in variations in relationships between ions (Lauchli and Epstein, 1990). However, high concentrations of Na in the soil inhibit plant growth and reduce commercial yield (Graifenberg *et al*., 1993, 1996). Lipids are among the most prominent constituents of cell membrane which play a fundamental role in cell permeability (Baybordi *et al*, 2010). It is stated that when a plant is subject to salinity stress for a long period, ion toxicity and water deficiency in mature leaves and carbohydrate deficiency and related signs are observed in young leaves (Greenway & Munns, 1980; Munns & Termaat, 1986; Cramer *et al*., 1988; Shannon & Grieve, 1999; Chookhampaeng, 2011).

MATERIALS AND METHODS

Plant material and Experimental pots

The plant material which was included in our study was a tomato variety **C-21** purchased from market in the form of seeds and their viability test was confirmed in Agriculture mall, Rohtak. The seeds were surface sterilized with dilute solution of sodium hypo chlorite (NaOCl) to prevent any fungal contamination and then rinsed three times with distilled water. Then seeds were grown in Petri dishes containing double layered wet filter paper with tap water in order to check the viability of seeds. The seeds were sown in five sets in an earthen pot containing equal quantities (4kg) of loamy sand soil. Salt treatment of NaCl was prepared using sodium chloride salt in concentrations of 60, 90, 120 and 150mM in soil, leaving one set as a control. The samples were taken from two week old seedlings for physiological analysis.

Effect on seed germination and growth rate

Germination percentage

Seed germination was recorded daily up to 12 days after the initial day of the experiment. Seeds were considered as germinated when the radical reached a length of 1 mm (Kabir *et al.*, 2008) and the germination percentage was calculated as per the following formula:

Germination percentage = Number of Germinated seeds× 100/ Total number of seeds.

Root and shoot length

Length of root and shoot was measured with the help of a scale and reading was taken from both treated and controlled seedlings of 10 days.

Biomass production

Biomass production was obtained by taking out the seedlings from the pots, washed under running tap water, dried on the

blotting sheets and weighed. They were dried in hot air oven at 60ºC for 48 hours or till their weight became constant.

To estimate the chlorophyll content in Tomato

Amount of leaf sample used for extraction depended upon the availability and other requirements which ranged from 100-500 mg. The chlorophylls and carotenoids were extracted by the method of Arnon (1949) and Holden (1965). For extraction, 50 mg of plant sample was homogenized with about 10 ml of ice cold 80% acetone (AR Grade). A pinch of $CaCO_3$ was added to avoid the destruction of chlorophylls and other pigments. Extraction has to be carried out under dim light to avoid photoxidation of the pigments. It was centrifuged in a Remi centrifuge at low speed (5000 rpm) for about 20 min. Ice cold pestle and mortar were used for grinding the samples. After centrifugation cell wall debris were settled down and was discarded. Only supernatant was taken out and raised to a specific volume of 10 ml with ice cold 80% acetone. Absorbance was recorded soon after extraction was over with the help of UV-Vis spectrophotometer (Specord-205 Analytik Jena, Germany) at wavelengths of 663, 645, 510 and 480 nm. The amount of total chlorophylls and carotenoids were estimated by the formula of Arnon D.I. (1949). Pigments were calculated in terms of mg/gm plant tissue on fresh weight and dry weight basis.

Mg chlorophyll a/g tissue = 12.7(A663) - 2.69(A645)×V/1000×10
Mg chlorophyll b/g tissue =22.9(A645) - 4.68(A663) ×V/1000×10
Mg total chlorophyll /g tissue =20.2(A645) +8.02(A663) ×V/1000×10
Mg caretonoids/g tissue = 7.6(A480) – 1.49(A510) ×V/1000×10
Where
A = Absorbance of specific wavelength.
V = Final volume of chlorophyll and caretonoids in 80% acetone.
W = Fresh weight of tissue extract.

Lipid Peroxidation

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA). MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modification of the method of Heath and Packer (1968).

Reagents

0.1% Trichloroacetic acid (TCA)
20% TCA containing 0.5% Thiobarbituric acid (TBA)

Extraction

100 mg of fresh leaves were homogenized separately with 5ml of 0.1% TCA. Homogenate was centrifuged at 8000 xg for 15 min. The supernatant was read at 532 nm & 600 nm, the value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using its extinction coefficient of 155 nM^{-1} cm^{-1}. The lipid content can be calculated by:

Lipid content= specific-nonspecific × volume taken × total volume/155 nM^{-1} cm^{-1}

Where 155 nM^{-1} cm^{-1} is the extinction coefficient.

RESULTS

Effect on seed germination and growth rate

Germination percentage

The deleterious effect of different concentrations (60mM, 90mM, 120mM and150mM) of NaCl salt on seed germination and seedling growth in **C-21** variety of tomato is presented in Figure-1 & Table-1.1 it is clear from figure-2 that NaCl salt reduced the seed germination percentage. Plants were more sensitive to 150mM of NaCl concentration as compared to all others. The maximum seed germination percentage was shown in control set which is free from NaCl treatment. Table- 1.1 clearly shows that plants exhibited a marked decrease in percentage of germination rate and shoot length due to imposition of NaCl salt.

Fig 1 Germinated seeds of first seven days.

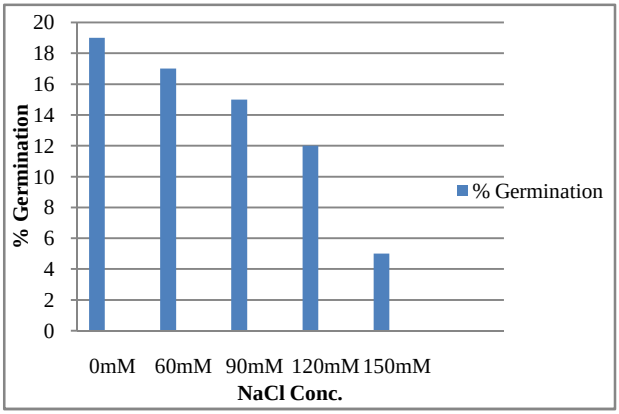

Figure: - 2 Effect of different concentrations of NaCl on percentage germination rate.

Table 1 Analysis of variance table for seed germination

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	16614.93	2	8307.467	7.37022	0.008167	3.885294
With in groups	13526	12	1127.167			
Total	30140.93	14				

Since $p \leq 0.05$. We can reject the null hypothesis and say that there is an effect of NaCl on seed germination.

Root and Shoot length

Table no. - 1.2(a) and1.2(b) & fig. - 3 show the decrease in root and shoots development with increasing concentrations of NaCl. Root length was found to be more affected than shoot length with increase in the concentration of NaCl.

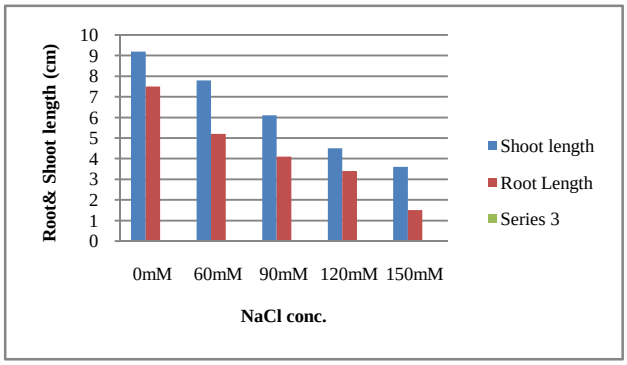

Figure3 Effect of different concentrations of NaCl on shoot and root length.

Table 2 (a) Analysis of variance table for Shoot length.

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	20129.51	2	10064.75	9.037313	0.004035	0.004035
With in groups	13364.26	12	1113.689			
Total	33493.77	14				

Since p ≤ 0.05. We can reject the null hypothesis and say that there is an effect of NaCl on Shoot length.

Table 2 (b) Analysis of variance table for Root length.

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	21131.16	2	10565.58	9.489376	0.003378	3.885294
With in groups	13360.94	12	1113.412			
Total	34492.1	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on root length.

Biomass production

An increase in different concentrations of NaCl decreased the fresh and dry mass of the C-21 variety of tomato. As we increase the concentration of NaCl, mass was decreased. Maximum effects were shown at 150mM NaCl concentration as shown in in fig.4. Table- 1.3(a) and1.3 (b) clearly shows that plants exhibited a marked decrease in fresh weight and dry weight due to imposition of NaCl salt.

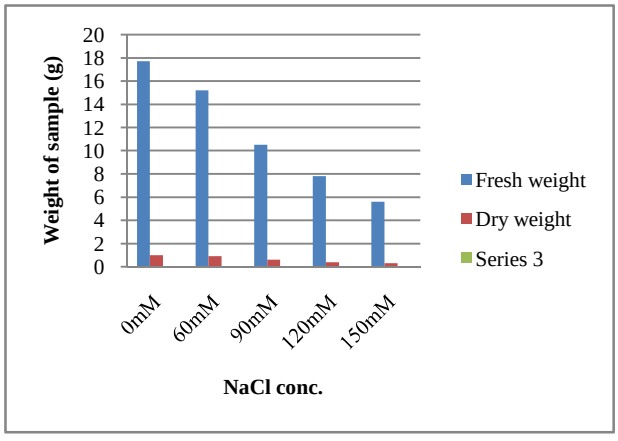

Figure 4 Effect of different concentrations of NaCl on biomass production.

Table 3 (a) Analysis of variance table for fresh weight.

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	18122	2	9060.998	8.046579	0.006074	3.885294
With in groups	13512.82	12	1126.068			
Total	31634.82	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on fresh weight

Table 3 (b) Analysis of variance table for dry weight.

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	23129.67	2	11564.83	10.41781	0.002382	3.885294
With in groups	13321.22	12	1110.102			
Total	36450.89	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on dry weight.

To estimate the chlorophyll content in tomato

Different concentrations of NaCl affected the chlorophyll amount of leaves (Fig.5). The reduction of chlorophyll *a* and *b* contents of leaves was detected with enhanced NaCl accumulation in leaves. Light-induced chlorophyll accumulation was inhibited by increasing the concentration of NaCl. Under salt stress, the chlorophyll *b* content of leaves was more affected than the chlorophyll *a* content. Total chlorophyll content of the leaves decreased significantly with increased the concentration of NaCl as represented in table 2.1. The 150mM NaCl concentration caused the maximum decrease of Chlorophyll amounts as compared to all others. The highest carotenoid content was measured in control plants and it decreased with increasing salt concentration.

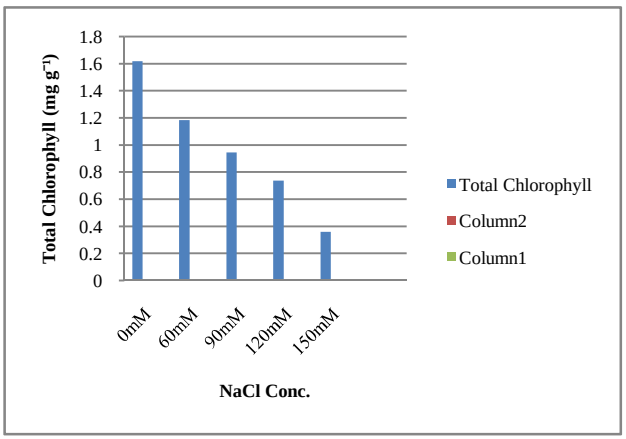

Figure 5 Effect of different concentrations of NaCl on chlorophyll content

Table 2 Analysis of variance table for Total chlorophyll.

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	23016.62	2	11508.31	10.36625	0.002428	3.885294
With in groups	13322.06	12	1110.172			
Total	36338.68	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on total chlorophyll.

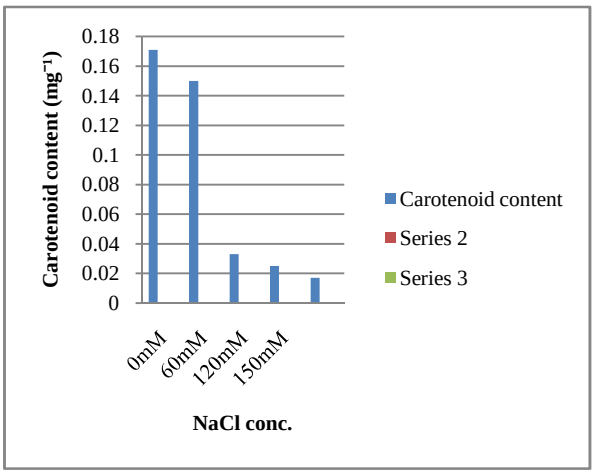

Figure 6 Effect of different concentrations of NaCl on carotenoid content.

Table 2 Analysis of variance table for Carotenoid content

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	23468.29	2	11734.14	10.57127	0.002253	3.885294
With in groups	13320.04	12	1110.003			
Total	36788.33	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on carotenoid content.

Lipid peroxidation

From table-3 and figure-7 it was clearly shown that the lipid content decreases with increase in NaCl concentration. The maximum content was shown in control at 0mM concentration and minimum content was shown at 150mM concentration.

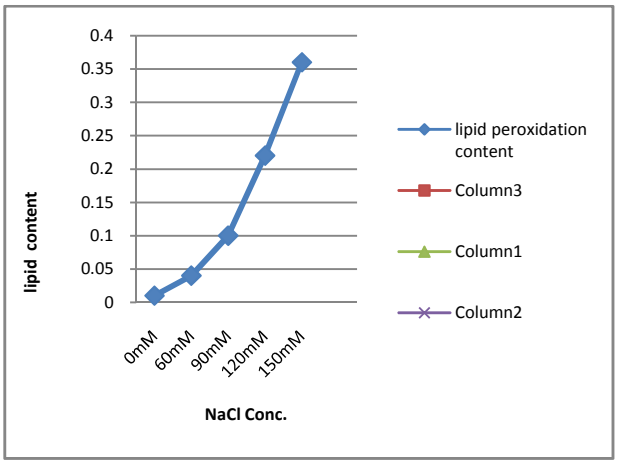

Figure 7 Effect of different concentrations of NaCl on lipid peroxidation

Table 3 Analysis of variance table for lipid peroxidation content

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	23369.2	2	11684.6	10.52637	0.00229	3.885294
With in groups	13320.38	12	1110.032			
Total	36689.58	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on lipid peroxidation content.

DISCUSSION

The extent of plant injury by elevated concentration is specific and strongly depends on the environmental conditions and on the availability of salt concentration. In the present study we took the one variety of tomato **(C-21).** This variety was grown under **NaCl salt stress**. Different studies on salt stress indicate that salinity have an adverse effect on the growth of tomato plant. In this study we have taken five concentrations **(0mM, 60mM, 90mM,120mM,150mM) of NaCl** were used in order to evaluate its effect on growth parameters, photosynthetic rate, lipid content . The result of current study indicated that an increase of salt concentration delayed **seed germination** at various concentration of NaCl especially at highest concentration **(150mM**).When we increase the concentration of salt, it inhibits the germination of the seed. **Table 1.1** and **fig. 2** clearly shows the adverse effect of NaCl on the seed germination of tomato. NaCl affect the Shoot length and root length of the Tomato varieties. **Table no. 1.2(a)** and **1.2(b)** shows the decrease in root and shoot development with increasing concentrations of NaCl stress. Root length was found to be more affected than shoot length. Effect of NaCl on root and shoot are also represented in **fig. 3**. At **150 mM** concentration of NaCl plant showed a **shoot length** of **3.6** and **root length** of **1.5 cm**. The effects of NaCl were also viewed on the biomass of the tomato variety. Maximum reduction was observed in fresh weight at **150mM** of concentration **(5.6).** As represented in **table 1.3(a)** and **fig 4**. The reduction of **chlorophyll *a* and *b*** contents of leaves was detected with enhanced NaCl accumulation in leaves. Under NaCl stress, it was observed that the **chlorophyll *b*** content of leaves was more affected than the **chlorophyll a** content. Total chlorophyll content of the leaves decreased significantly with increasing NaCl concentration as represented in **table 2.1**. The NaCl concentration of **150mM** caused the maximum decrease of Chlorophyll amounts. The highest **carotenoid content** was measured in control plants and it decreased with increasing NaCl concentration. Malondialdehyde (MDA) content, a product of **lipid peroxidation,** has been considered as an indicator of oxidative damage. MDA is the decomposition product of polysaturated fatty acids of bio membranes and its increase shows plants under high-level antioxidative stress. Thus, the increased in lipid content with increase in NaCl conc. highest at150mM **(Table 3)** indicates oxidative stress and this may be one of the possible mechanisms by which toxicity due to salinity could be manifested in the plant tissues.

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