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EXOGENOUSLY APPLIED SORBITOL ALLEVIATES THE SALT STRESS BY IMPROVING SOME BIOCHEMICAL PARAMETERS IN SPINACH (*SPINACIA OLERACEA* L.)

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ABSTRACT

Exogenous application of growth regulators under saline condition is an economically feasible approach to alleviate the adverse effects of salt stress in plants. The main objective of this study to evaluate the ameliorative role of foliar applied sorbitol in various biochemical mechanisms of salt stressed spinach (*Spinacia oleracea* L.). Seeds were grown in pots and irrigated with different salt concentrations (50mM, 100mM and 150mM NaCl). Various sorbitol concentrations (15, 30 and 45 mM) were applied on foliar parts after five weeks of germination. Increasing salt concentrations considerably decreased total chlorophylls, carotenoids, reducing sugars, non-reducing sugars, carbohydrates, and total proteins while flavonoids, phenols and total antioxidants showed significant increase with increasing salt concentrations. Foliar application of different concentrations of sorbitol showed significant improvement in all above mentioned parameters in saline and non-saline conditions. Sorbitol (30mM) showed better performance in both type of conditions. Therefore, foliar application of sorbitol play positive role to overcome salt stress in *Spinacia oleracea* L.

Keywords: Salinity, Chlorophyll, Proteins, Carbohydrates, Antioxidants

INTRODUCTION

Salinity is one of the most damaging problems in arid and semi-arid areas around the globe. It severely inhibits crop production due to higher salt accumulation in soil and water reservoirs (Zhao *et al.*, 2007). The two main causes of salinity are natural and anthropogenic processes that results in accumulation of dissolved salts in soil and water that negatively affects plant growth (Tilman *et al.*, 2002). It is estimated that about 50% of the world cultivated land which might be responsible for at least double the production of rain fed land and produce one-third of world's food is badly affected by salinity (Ghassemi *et al.*, 1995; Hillel, 2000).

Sorbitol is sugar alcohol which synthesized by reduction reaction from glucose in which the aldehyde group is replace by hydroxyl group. Mainly sorbitol is prepared from corn syrup beside this it is also found in apples, pears, peaches, and prune. It is a direct product of photosynthesis in leaves along with sucrose. Both of this have same functions and are responsible for translocation of carbon skeletons and energy from leaves to all other organs. Due to salt and drought stress the transport of Sorbitol is increased many folds both in xylem and phloem (Noiraud*et al*., 2001).

Spinacia oleracea L. (Spinach) of family Chenopodiaceae is erect, herbaceous and succulent plant used as carminative, laxative and alexipharmic. Stem used as therapeutic against asthma, leprosy, blood and brain diseases and urinary calculi. Leaves are emollient, wholesome, antipyretic, diuretic, laxative, digestible and anthelmintic. Seeds used in liver inflammation and jaundice (Kirtikar and Basu, 2005).

Present study was conducted to evaluate the effect of various doses of sorbitol on chlorophyll, carotenoids, reducing and non-reducing sugars, proteins, total carbohydrates, total phenols, flavonoids and total antioxidants of *Spinacia oleracea* grown under salt stress.

MATERIALS AND METHODS

Seeds of *Spinacia oleracea* L, were collected from Agriculture Research Institute Tarnab Peshawar, Khyber Pakhtunkhwa. The experiment was comprises of 48 pots which were divided into four set each set having 12 pots. Three replicates were maintained for each treatment i.e., (1) non saline (2) 50mM NaCl (3) 100mM NaCl (4) 150mM NaCl. Details of these four sets are given below:

Set 1: Without sorbitol and comprised of control and three salinity treatments (50mM, 100mM and 150mM).
Set 2: Sprayed with 15mM sorbitol solution and comprised of control and three salinity treatments (50mM, 100mM and 150mM).
Set 3: Sprayed with 30mM sorbitol solution and comprised of control and three salinity treatments (50mM, 100mM and 150mM).
Set 4: Sprayed with 45mM sorbitol and comprised of control and three salinity treatments (50mM, 100mM and 150mM).

This experiment was carried out in plastic pots (17.5 cm diameter, 15 cm depth) with basal outlet for proper drainage. Pots were filled with thoroughly washed sandy loam soil. Seeds of approximately equal size were selected and surface sterilized with 0.1% mercuric chloride for one minute followed by washing with distilled water and imbibed in distilled water for 30 minutes. Five seeds were sown in each pot and all 48 pots were placed in the wire-house of Botanical Garden, Department of Botany, Abdul Wali Khan University, Mardan, under natural climatic conditions in completely randomized design. They were irrigated with equal amount of tap water and 200mL hoagland's solution. Salt treatment was started at three leaves stage (after two weeks of germination) with two or three days of irrigation interval. After five weeks of germination various foliar spray treatments of sorbitol (100 mL/plant) were applied on both adaxial and abaxial surfaces of leaves. Control plants were sprayed with distilled water

Biochemical Analysis

Fresh leaves were collected at grand period of growth for biochemical analysis.

Determination of photosynthetic pigments

Estimation of photosynthetic pigments was performed by a method described by Maclachlam and Zalik (1963).

Determination of Reducing and non-reducing sugars

Determination of reducing sugars was performed by method described by Nelson-Somogy method (Nelson, 1944).

Extraction and estimation of Total Carbohydrates

The amount of total carbohydrates was analyzed using the method of Yemm and Willis (1956).

Determination of Proteins

The method is rapid and sensitive for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding (Bradford, 1976).

Total Flavonoids Estimation

Aluminum chloride method was applied to determine the total flavonoid content of the sample (Mervet*et al.,* 2009).

Total Phenol determination

The amount of total phenolics was analyzed using the Folin–Ciocalteu (FC) calorimetric method described previously by Malik and Singh (1980).

Total Antioxidants

The ferric ion reducing power capability of samples was determined by using modified method of Yen and Chen (1995).

Experimental design and statistical analysis

The experimental design was completely randomized Design (CRD) with two salt levels and three replicates. Collected data was analyzed statistically by using SPSS to analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Estimatiuon of Chlorophyll contents

Plants were grown under various salt concentrations showed non-significant reduction with increasing salinity whereas, highest salt concentration (150 mM NaCl) showed maximum reduction in chlorophyll a, chlorophyll b and

total chlorophylls. Carotenoid contents significantly (P<0.01) decreased with increasing concentration of salt as compared to its control plants (Fig. **1-4**). Plants treated with different doses of sorbitol showed non-significant increase in chlorophyll a, chlorophyll b and total chlorophylls while showed significant (P<0.05) increase under saline (50mM and 100mM NaCl) and non-saline condition. Photosynthetic rate is reduced due to salt stress (Ashraf and Harris, 2004; Parida and Das, 2005). It is a fact that salt stress is mainly responsible for decreased level of chlorophyll contents due to membrane deterioration (Ashraf and Bhatti, 2000). Khan (2003) also reported that salt stress is responsible for decrease production of chlorophylls in wheat. In salt stressed *Salvinia auriculata*, it was observed that chlorophyll-*a*, chlorophyll-*b*, total chlorophylls and carotenoid contents were decreased significantly (Maria *et al.,* 2011). Sharma and Hall (1991) investigated that salt stress is responsible for degradation of β-carotene which lead to decrease in amount of carotenoids which are the main constituents of thylakoid membranes and facilitate absorption and light transfer to chlorophylls and also protects chloroplast to photo-oxidation (Taiz and Zeiger, 2009; Lima *et al.,* 2004). Lima *et al.,* 2004 reported that degradation in carotenoid production is actually degradation of chlorophylls. Salt stress reduces the synthesis of chlorophyll-*a, b*, total chlorophylls and carotenoid contents (Maria *et al.,* 2011).

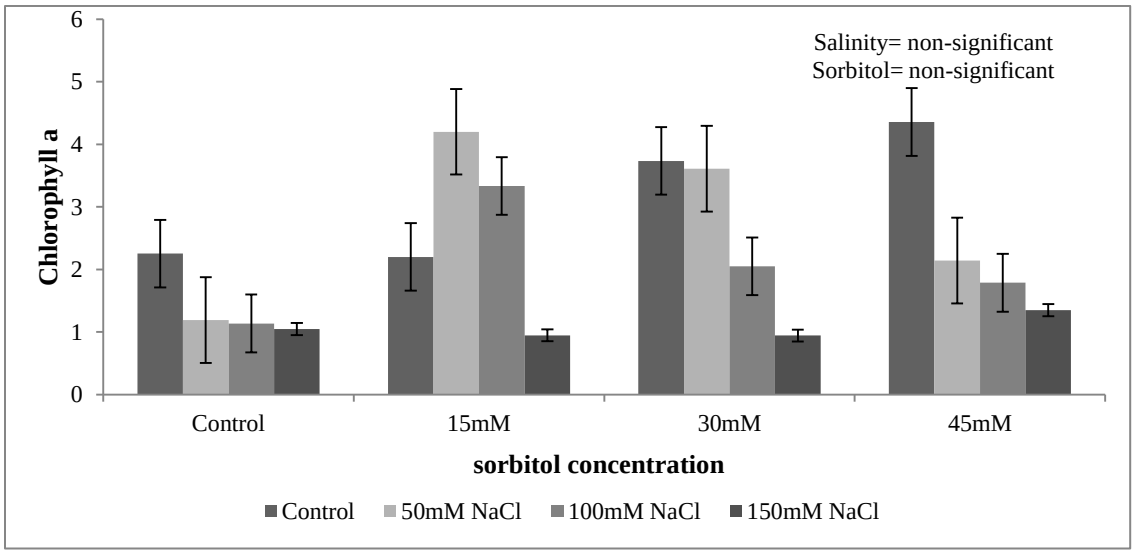

Fig. 1. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on chlorophyll a (mg/g F.W) of *Spinacia oleracea* L.

Reducing and None reducing sugars

Plants treated with different concentrations of salt showed significant (P<0.001) reduction in reducing and non-reducing sugars (Fig. **5 and 6**). Foliar applied Sorbitol (30mM) showed significant (P<0.01) promotion in 50 mM NaCl. . Data of reducing sugar presented by Nasser (2011) showed that the wheat plant organs grown under salinity stress exhibit significant decrease in reducing sugars.

Total carbohydrates

Total carbohydrates significantly (P<0.001) reduced under increasing salt concentrations as compared to the control plants (Fig. **7**). Plants treated with different concentration of sorbitol showed significant (P<0.001) decrease in carbohydrates in saline as well as non-saline conditions. Salinity is responsible for carbohydrates accumulation in many plants (Abd El- Samad and Azooz, 2002; Parida*et al.,* 2003; Azooz et al., 2004). Alternatively, Mostafa (2004) noted that even at low and moderate salinity levels total carbohydrates are reduced.

Increasing salt concentrations generally reduced the photosynthetic rate resulting decline in carbohydrate accumulation in plants (Ashraf and Harris, 2004; Parida and Das, 2005).

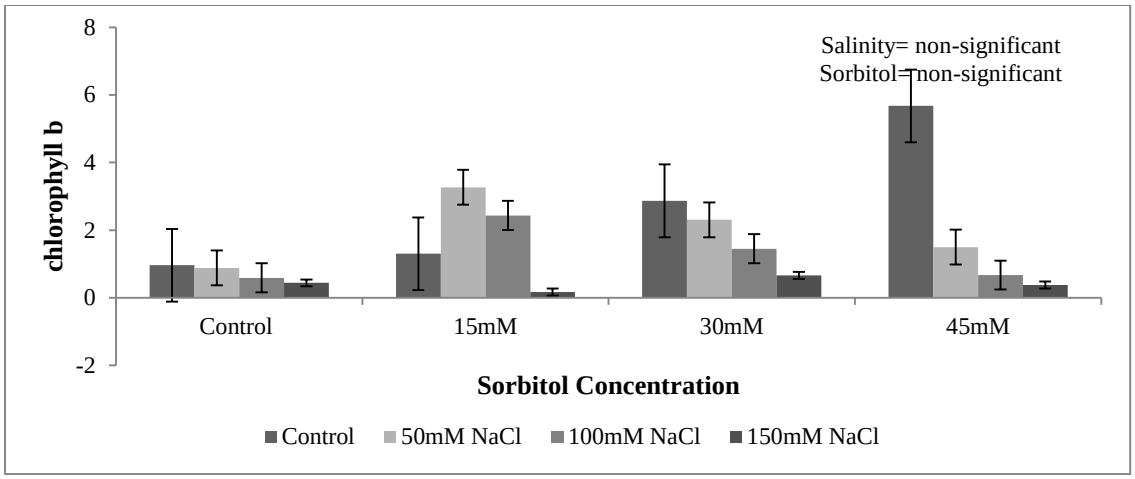

Fig. 2. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) andsorbitol (15mM, 30mM and 45mM) on chlorophyll b (mg/g F.W) of *Spinacia oleracea.*

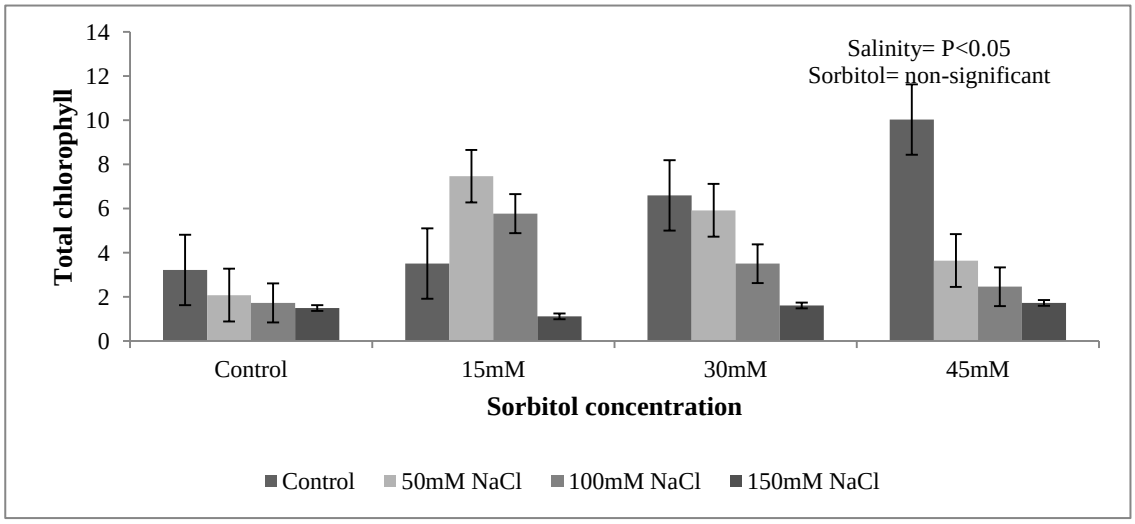

Fig. 3. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on total chlorophyll (mg/g F.W) of *Spinacia oleracea.*

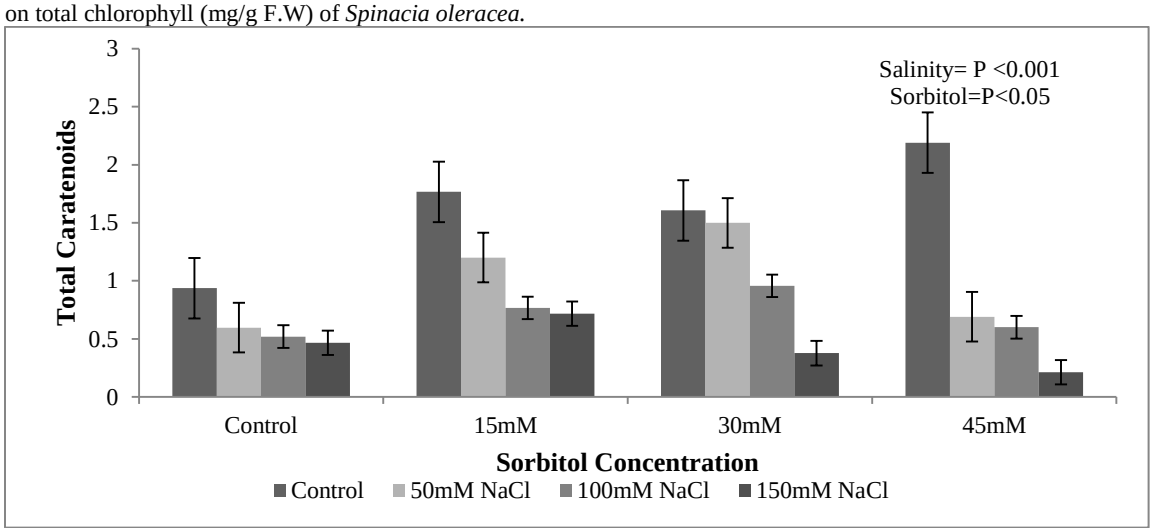

Fig. 4. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) andsorbitol (15mM, 30mM and 45mM) on total carotenoids (mg/g F.W) of *Spinacia oleracea.*

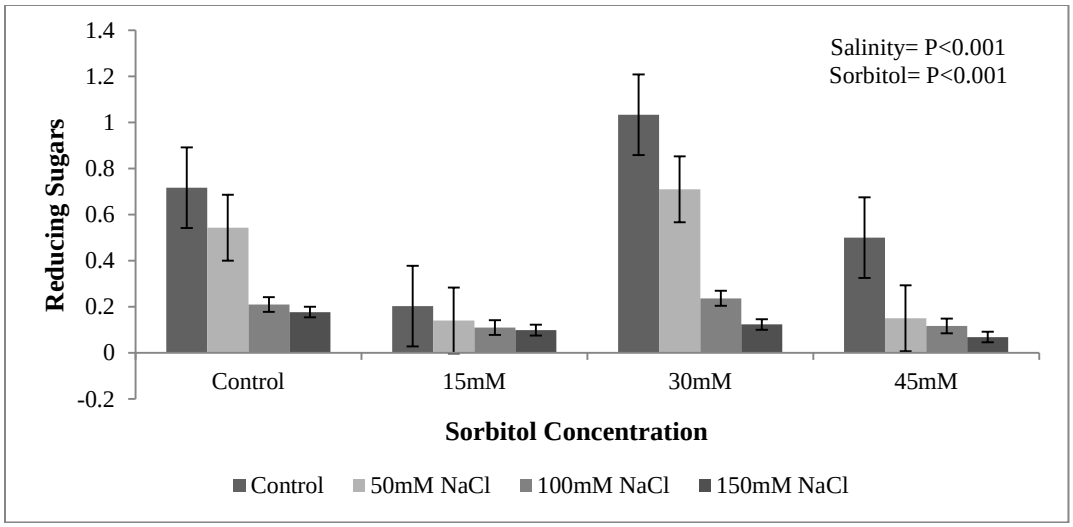

Fig. 5. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on reducing sugars of *Spinacia oleracea.*

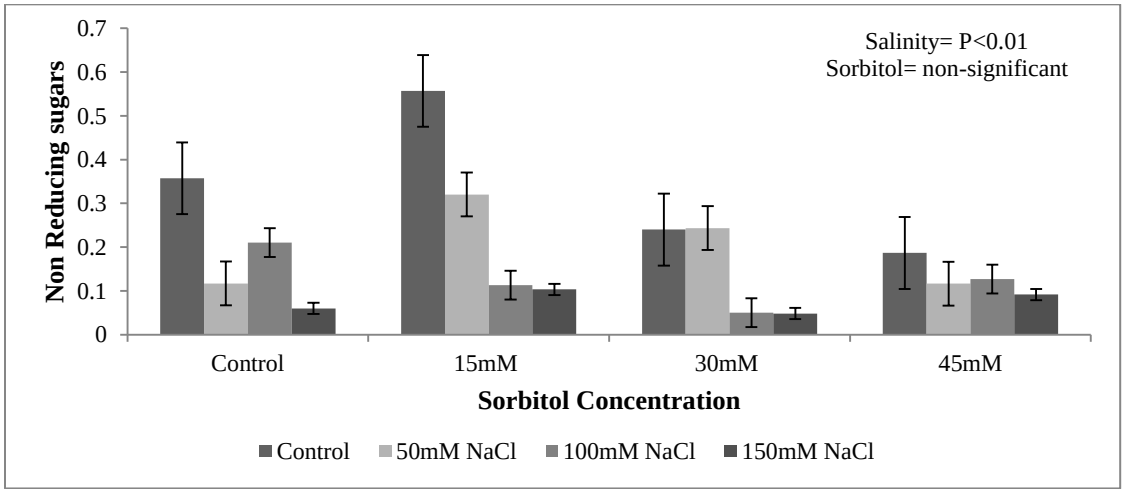

Fig. 6. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on non-reducing sugars of *Spinacia oleracea*.

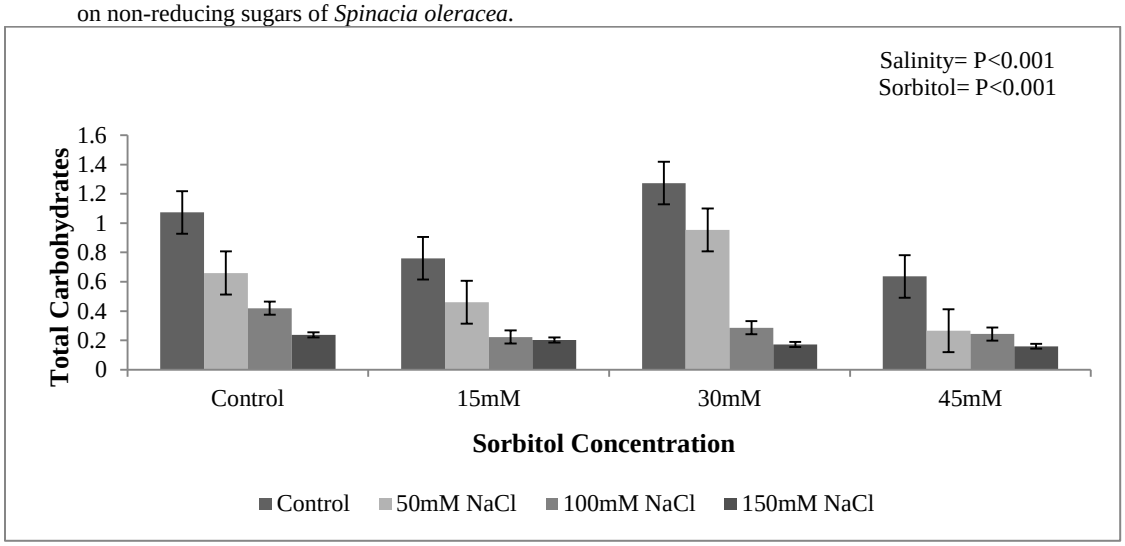

Fig. 7. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on total carbohydrates of *Spinacia oleracea.*

Total protein

Plants treated with various salt concentrations showed significant (P<0.001) decline in total protein as compared to the control plants (Fig **8**). Severe reduction was found at highest salt concentration (150 mM). Whereas, Plants treated with different concentration of sorbitol showed increase the total protein contents in control and 50mM salt, while 100mM and 150mM NaCl showed decrease at 15mM and 30mM sorbitol treatments and increase at 45mM sorbitol in control plants. Merril (1990) reported that salt stress is also accountable for decreasing protein contents in many plant species. It was reported in *Catharanthus roseus* where NaCl treatment significantly reduced protein contents of plant (Osman *et al.*, 2007). Similar results were found in *Achillea fragratissima* where salt treatment of 4000 ppm considerably inhibited protein contents (Abd EL-Azim and Ahmed, 2009). Parida and Das (2005) reported that at low concentration soluble protein exhibited increase but as the salt concentrations increased the amount of soluble protein decreased in mulberry. The work of Al-Aghabary*et al*. (2004) showed that salt stress decreased soluble protein contents in leaves of tomato.

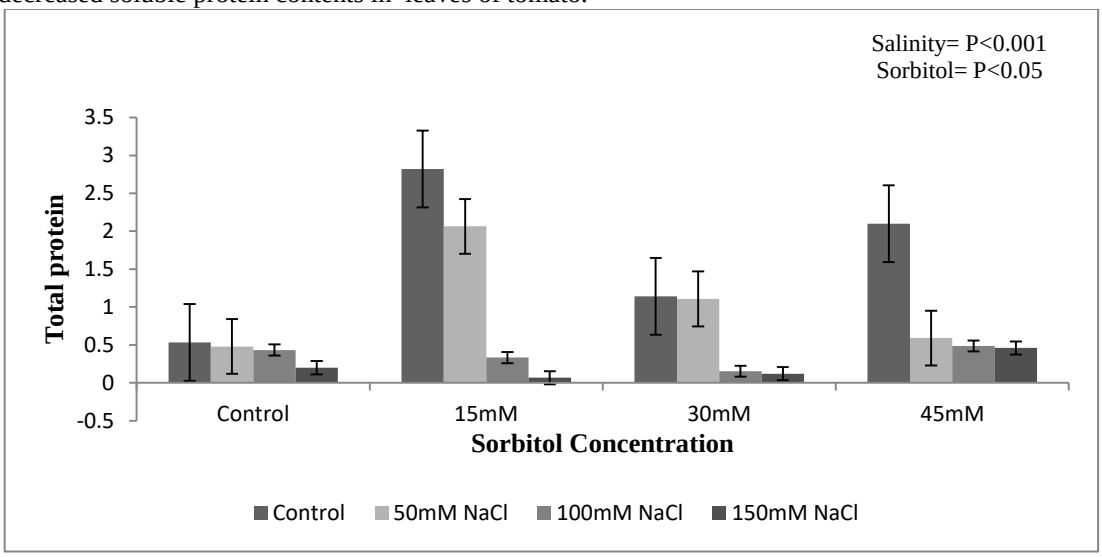

Fig. 8. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on total protein of *Spinacia oleracea.*

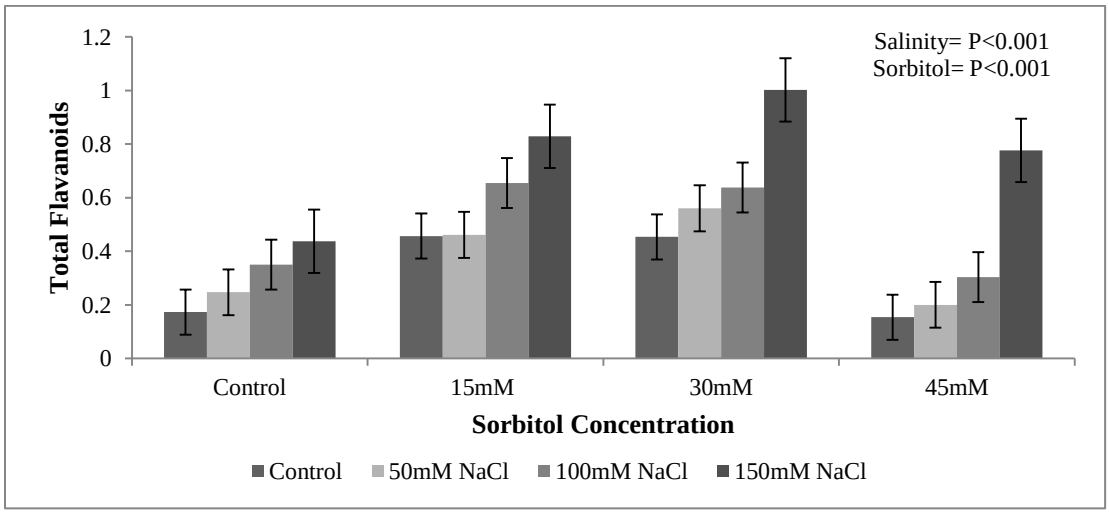

Fig. 9. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on total flavanoids of *Spinacia oleracea.*

Total flavonoids

Plants treated with different concentration of salt showed significant (P<0.001) increase in total flavonoids as compared to the control plants (Fig. **9**). Sorbitol treatment showed increase in flavonoid contents at 15 and 30 mM concentrations while decline was observed at higher concentration (45 mM sorbitol). Rajamane and Gaikwad (2014)

studied the effect of salinity on *Simarouba glauca* biochemistry and found that control plants showed relatively low flavonoids as compared to salt stressed plants. Ali and Abbas (2003) studied effect of salt stress (50 and 100 mM NaCl) on flavonoid contents of barley. They noticed significant increase in flavonoid contents in barley in response to salt stress. This may contribute to the antioxidant metabolism in salt stressed leaf tissue (Rajamane and Gaikwad, 2014). Abiotic stress was found to play significant role in induction of Flavonoids which helps in plant protection (Dixon and Paiva, 1995; Grace and Logan, 2000).

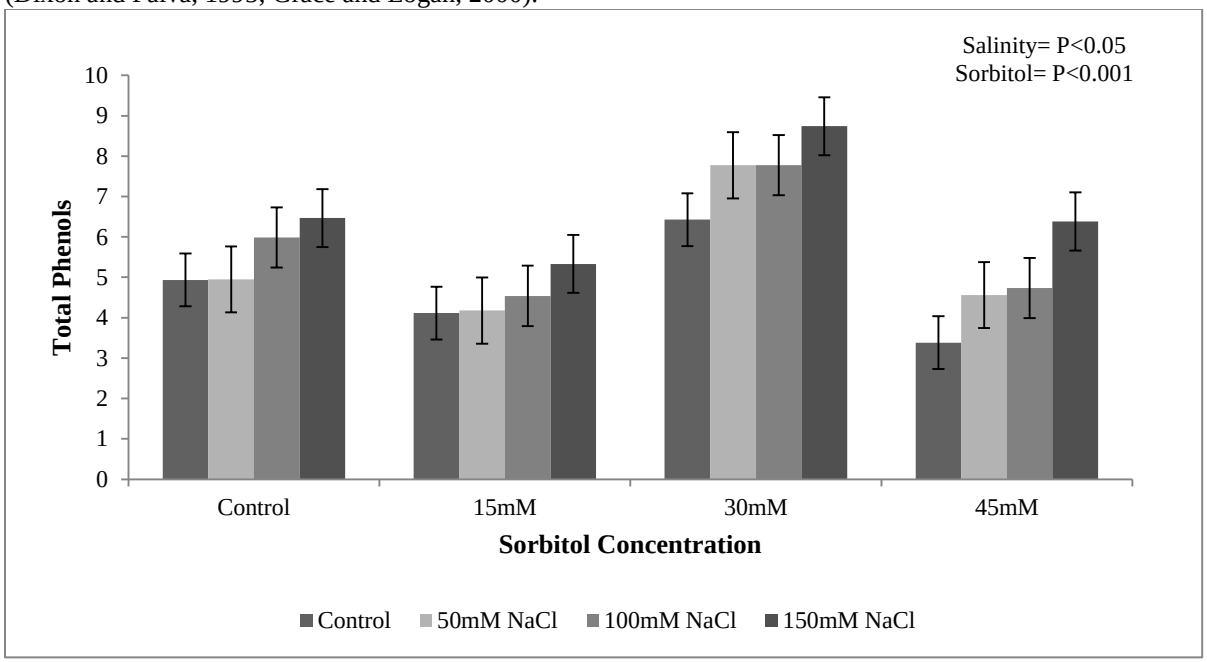

Fig. 10. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on total phenols of *Spinacia oleracea*.

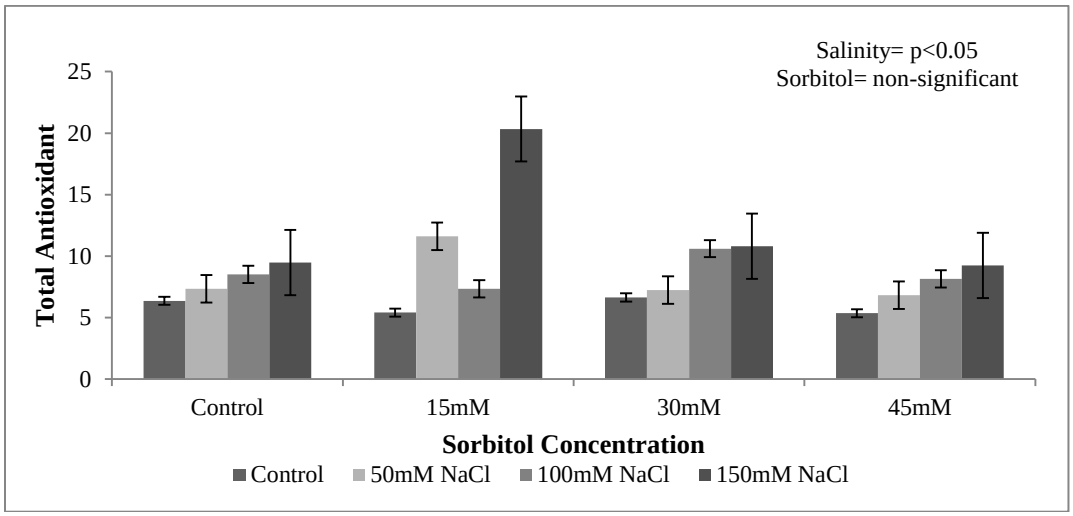

Fig. 11. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and sorbitol (15mM, 30mM and 45mM) on total antioxidants of *Spinacia oleracea*.

Total phenols

Total Phenolic contents was significantly (P<0.05) increased with increasing salt treatments and maximum phenols were observed at higher salt concentration (150 mM NaCl (Fig **10**). Plants treated with 45 mM sorbitol showed significant (P<0.05) decrease at control and salt treatments as compared to 30 mM soritol. While 30mM sorbitol showed maximum accumulation of phenols at 150 mM NaCl. Several workers have observed increased levels of polyphenols under salinity stress in plants like *Aegiceros corniculatum* (Parida, *et al.*, 2004). In *Bruguiera*

parviflora Parida and Das (2002) noticed an increase in polyphenols with the increasing levels of salinity. Salt stress found to cause increase in total phenolic content in the halophytic species such as *B. parviflora, Agiceroscorniculatum, Cakile maritime and Salvadora persica* (Parida and Das, 2002; Parida *et al*., 2004; Ksouri *et al.,* 2007; Sharma and Ramawat, 2012). Rajamane and Gaikwad (2014) stated increased phenolic content in *S. glauca*clearly and indicates a stimulation of secondary metabolism under salinity stress and evaluate the possible role of phenolic compounds as defense against oxidative stress.

Total Antioxidants

Plants irrigated with various salt concentrations showed significant increase ($P<0.05$) in total antioxidants with increasing salinity levels (Fig. **11**). Maximum increase was found at higher salt concentration (150 mM NaCl). Hernandez *et al.,* (2000) showed that due to various environmental stresses enhanced the enzymatic and non enzymatic antioxidants in plants. This was also supported by the work of Nasser (2011) who reported the increased activity of antioxidant enzymes such as catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) in different NaCl concentrations. This collaboration between enzymatic and non-enzymatic antioxidant systems supply to the cell a complex and effective system to control reactive oxygen species (ROS) levels (Miller *et al.,* 2010; Shao *et al.,* 2007).

Sorbitol (15 mM) at 50 and 150 mM NaCl showed significant increase in total antioxidants. It was also found that the exogenous application of mannitol protected wheat plants from the detrimental effects of salt-induced oxidative stress by promoting the activity of Antioxidant enzymes (Seckin*et al*., 2009). Mitoi *et al.,* (2009) and Srivastava *et al.,* (2010) reported that the foliar applications of mannitol and thiourea was useful to decrease the harmful effects of oxidative stress on membranes through scavenging of reactive oxygen species.

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