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B-Sitosterol Ameliorates The Chemical Constituents Of Sunflower (Helianthus Annuus L.) Plants, Grown Under Saline Condition

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Abstract: *The present experiment was carried out in fancied area at El-Mansoura University, Dakahlia Governorate during the growth period, from May to September (2013) to study the effect of different salinity levels (10, 20 and 30%sea water) and beta-sitosterol (10^{-5}, 10^{-7} and 10^{-9} molar) on some chemical aspect of sunflower plants (Helianthus annus L.). The results indicated that salinity caused a reduction in chlorophyll a, b, a+b, a/b and Carotenoids as well as total pigments, carbohydrates contents with significant increasing sea water concentration during two stages of growth, 45 and 85 days from planting. Also a marked reduction in unsaturated fatty acids content accompanied with a marked increase in saturated fatty acids as compared to untreated control plants especially myristic acid. Increasing β-sitosterol concentration significantly increased chlorophyll a, b, a+b and Carotenoids as well as total pigments content of sunflower leave which reflected on carbohydrates fractions during both stages except chlorophyll a/b which had no significant effect during 45 and 85 days from the planting. Marked increases were observed in unsaturated fatty acid contents, as compared with the control plants. It can be noticed that the plants treated with β-sitosterol concentration with(10^{-5} molar) attained the highest chlorophyll a, b, a+b, carotenoids and total pigments also, carbohydrates fractions as compared with the other treatments during both stages.*

Keywords*: salinity levels, β-sitosterol application, sunflower plant, pigments, carbohydrates, fatty acids.*

I. Introduction

Oil seed crops are very valuable for human food and they have the third position among different crops such as cereals and legumes (**Howard and Kinney, 2008**). Sunflower (*Helianthus annuus* L.) has a great importance all over the world as oil seeds but its production is decreasing in different areas where saline is rapidly increasing (**Caterina *et al.*, 2007**). Sunflower is high yielding, nonconventional oil seed crop. Sunflower is a fast- growing, herbaceous plant, whose water and nutrient uptake mechanisms are highly efficient (**Benlloch *et al.*, 1989**) It is one of the four most important oil crops in the world, beacause of its moderate cultivation requirements and high oil quality, its acreage has increased in both developed and un developed countries (Skoric,1992). Sunflower oil is highly demanded not only for human consumption, but also for chemical and cosmetic industries It is a short duration crop (90-120 days) and can be grown twice a year .It fits well in existing cropping systems and can be grown without replacing any major crop **(Ahmad *et al*., 2009**). Sunflower seed contains 25-48 % oil and 20-27 % protein. Its oil is quite palatable and contains soluble vitamins (A, D, E and K) .It is used in manufacturing of margarine. A fodder sunflower cake is used as cattle feed (**Hussain *et al*., 2006**).

Plant growth and productivity is adversely affected by nature worth in the form of various abiotic and biotic stress factors. Salinity stress is one of the serious threats which affect the plant growth and production all around the world (**Majeed *et al.*, 2010; Bahantana and Lazarovitch, 2010**). Total area of salt affected soil in the world is nearly 7% (**Hussain *et al.*, 2013**). Salinity is produced when low concentration of K^+ and Ca^{2+} contents are present and concentration of NaCl and SO_4 contents increases (**Dejampour *et al.*, 2012**).

Salinity causes many adverse effects on plant developmental stages which are due to many factors such as low water potential of soil solution, nutritional imbalance, ions toxicity (salt stress) and hormonal imbalance (**Miranda, 2011**). Salinity causes osmotic stress and ionic toxicity due to the accumulation of sodium ion which enhanced the formation of reactive oxygen species (ROS) such as superoxide(O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen which damage mitochondria, chloroplasts and cellular structure of the cell (**Sharma *et al.*, 2012**; **Malagoli *et al.*, 2008**). These after, ROS are scavenged by different antioxidant defense systems which consist of both enzymatic and non-enzymatic nature in plants (**Ashraf, 2009**).). Osmolytes are neutral small molecules which provide protection to proteins and membranes of plant cells against toxic effects when excessive salinity levels are present. Osmoregulation is a defense mechanism which is adopted by plants to eliminate the toxic

effects of salt, in which different osmotic regulators are used such as potassium, soluble sugar, proline and betaine (**Hong-Bo *et al*., 2006**).

Sitosterol is a phytosterol considered a structural component of the lipid core of cell membranes and the precursor of numerous secondary metabolites including plant steroid hormones or as carriers in acyl, sugar and protein transport (**Hassanein *et al.,* 2012**). Sterols play an important role in plant development including cell expansion, vascular differentiation, etiolation and reproductive development (**Abd El-Wahed *et al*., 2001**). Similar to the effect of brassinosteroids, sitosterol involved in the regulatory function of plant development, affected gene expression involved in cell expansion and cell division, vascular differentiation and other diverse developmental programs (**Sasse, 2003**). Sitosterol is known to influence permeability and fluidity characteristics of the plasma membrane and other organellar membranes in plants (**Senthil-Kumar *et al.,* 2013**). Interestingly, sitosterol also exhibit bacteriostatic or bactericidal activity against a broad range of gram-positive and gram-negative organisms, as well as *Candida albicans* (**Hoffman, 2003**). A number of studies have provided evidence that fluctuation in the sitosterol ratio plays a role in response to biotic and abiotic stresses. (**Arnqvist *et al.,* 2008**).

The objective of this study is to investigate the effect of beta-sitosterol on chemical constituents of sunflower plants, grown under saline conditions in order to highlight the possible mechanisms by which sitosterol adapt plant to stress.

II. Materials and methods

1.1. Materials

Pure strain of sunflower seeds (*Helianthus annus* L.) was soaked for 3 minutes in 0.01% $HgCl_2$ solution, and then washed thoroughly with distilled water. The seeds were then divided into four groups, the first group was soaked for 12 hours in tap water to serve as a control, the other three groups were soaked for the same time in different beta-sitosterol concentrations (10^{-5}, 10^{-7} and 10^{-9} molar) respectively. Each group was subdivided into four sub groups, the first subgroup was irrigated with tap water to the end of the experiment to serve as a control, the other subgroups 2^{nd}, 3^{rd} and 4^{th} were irrigated with diluted solution of sea water; 10%(10 ml sea water +90ml tap water), 20% (20 ml sea water +80 ml tap water), 30% (30 ml sea water +70 ml sea water) respectively.

Ten seeds of sunflower were sown at 20^{th} of May 2013 at equal distance and depth from the soil surface. The pots were irrigated with tap water up to7 days of germination then the plants were thinned to identical 5 plants/pot. After wards, the plants were irrigated when required either with tap water (control) or with one of the different concentrations of saline water to keep the soil at the level of 70% of field capacity. Saline soil was washed with tap water a day before irrigation with salt solution to decrease salt accumulation in the soil.

At the first and second stage; representing vegetative and flowering stage at 5^{th} of July (45 days old from the planting) samples of vegetative stage and at 20^{th} of August 2013 (85 days old from the planting) samples of flowering stage and 25^{th} of September 2013 (120 days old from the planting) samples of fruiting stage, from planting respectively. Three plants were taken from each pot and carried immediately to the laboratory. The collected samples were used for assessment of different metabolites as pigments, carbohydrates and fatty acids.

2.2. Analytical methods

2.2.1. Estimation of photosynthetic pigments

Photosynthetic pigments were determined using the spectrophotometric method as recommended and adopted by **Arnon (1949)** for chlorophylls and **Horvath *et al*. (1972)** for carotenoids as adopted by **Kissimon (1999)**.

2.2.2. Estimation of carbohydrates

The method used for estimation of carbohydrate fractions were essentially those of **Yamm and Willis (1954)** and **Handel (1968).**

2.2.3. Estimation of fatty acids

Whereas, methylation of fatty acids for gas-liquid chromatography analysis was essentially adopted by **Sink *et al*. (1964)**. The response of each fatty acid separated on the chromatogram was determined as peak area per unit weight of sample as recommended by **Radwan (1978).**

All data were statistically analyzed according to the technique of analysis variance (ANOVA) and the least significant difference (L.S.D) method was used to compare the deference between the means of treatment values to the methods described by **Gomez and Gomez, (1984)**. All statistical analyses were performed using analysis of variance technique by means of **CoSTATE** Computer Software.

III. Results and discussion

3.1. Pigments contents

3.1.1. Effect of salinity

Results presented in Table (1) declare the effect of various concentrations of salinity on photosynthetic pigments during two stages of growth, 45 and 85 days from the planting (vegetative and flowering).

Data showed generally that, there was a significant reduction in chlorophyll a, b, a+b, a/b and Carotenoids as well as total pigments with increasing Sea water concentration during two stages of growth.

The reduction of change in pigments was significantly decreased by increasing salinity levels. The significant reduction during both stages was found in plants grown under salinity levels i.e. 10, 20 and 30% of sea water. Chl a, Chl b and carotene were decreased at Sea water level (30%) by about (22.09, 21.89), (20.70, 20.05) and (30.54, 30.88), respectively during 45 and 85 days comparing with control.

The reduction in chlorophyll content under salinity conditions was reported by **Nazarbeygi *et al.* (2011)** and might have been due to salt-induced increase in the activity of the chlorophyll degrading enzyme, chlorophylase (**Yasar *et al.*, 2008**). Earlier studies reported that the reduction in leaf chlorophyll content of the plants grown in Sea water stress has been attributed to the destruction of chlorophyll pigment and instability of the pigment protein complex. Furthermore, increased salt content also interferes with protein synthesis and influences the structural component of chlorophyll. In this concern, a decrease in chlorophyll content (chl. a, b and total chl.) of fennel (**Rahimi *et al.*, 2012**) and faba bean (**Azooz *et al*., 2013**) under salt stress was observed.

In addition, **Sevengor *et al.* (2011)** ascribed the suppressed pigments content in salt-stressed rice plants to increased activity of chlorophyllase or disruption of the fine structure of the chloroplast, as well as instability of the chloroplast membrane and pigment protein complex.

Ebrahimi *et al.* (2014) investigated the response of sunflower to water stress. Water stress reduced leaf relative water content, chlorophyll a and b, chlorophyll a/b, total chlorophyll. Also, **Abu-Muriefah (2015**) showed that the contents of photosynthetic pigments such as chl a, chl b and carotenoids, total chlorophyll and chl a:chl b ratio were significantly reduced in pepper plants with increasing salinity level from from 50 to 100 and 200 mM compared with those of non-salt-stressed plants.

Table 1. Effect of salinity on photosynthetic pigments (mg/g fresh weight) of sunflower plant.

Stage	Treatments	Ch. a	Chl. b	Chl a+b	Chl. a/b	Carotenoids	Total pigments
45 days	Tap water	0.688	0.459	1.147	1.499	0.239	3.427
	10%	0.639*	0.434*	1.073*	1.475*	0.215*	3.354*
	20%	0.585*	0.394*	0.979*	1.486*	0.194*	3.279*
	30%	0.536*	0.364*	0.900*	1.472*	0.166*	3.202*
	LSD at 5%	0.006	0.004	0.006	0.017	0.007	0.009
85 days	Tap water	0.548	0.364	0.912	1.505	0.204	1.116
	10%	0.509*	0.346*	0.855*	1.473 n.s	0.184*	1.039*
	20%	0.469*	0.321*	0.790*	1.465*	0.166*	0.957*
	30%	0.428*	0.291*	0.719*	1.467*	0.141*	0.860*
	LSD at 5%	0.002	0.009	0.009	0.035	0.005	0.007

3.1.2. Effect of different concentration of β-sitosterol

Data in Table (2) show the effect of β-sitosterol with various concentration on pigment fractions. It was noticed that, β-sitosterol treatments markedly increased chlorophyll a, b, a+b, a/b and carotenoids as well as total pigments in sunflower plants with increasing β-sitosterol concentration during two stages,(45 and 85 days) from the planting.

The data in Table (2) show a significant increase with increasing β-sitosterol concentration for chlorophyll a, b, a+b and carotenoids as well as total pigments during both stages except chlorophyll a/b which had no significant effect.

In the present study application of β-sitosterol alleviated the damage effects of salt stress on photosynthetic pigment contents by increasing the membrane stability index compared with those of the control plants. Moreover, the chlorophyll content may be protected probably because of the high antioxidant enzyme activities that increased with β-sitosterol and prevented degradation of leaf chlorophyll (**Sevengor *et al*., 2011**). The results showed that β-sitosterol could stabilize the integrality of chloroplast membrane and protect the chloroplasts from salt stress. In agreement with these results, **Hassanein *et al.* (2012)** found that sitosterol caused a significant increase in photosynthetic pigment contents of salt stressed faba bean plants. In this regard, **Senthil-Kumar *et al.* (2013)** reported that sitosterol may have a role in abiotic stress tolerance by enhancing membrane and chlorophyll stability. **Rashad *et al.* (2008)** stated that Pigments (Chlorophyll (Chl) a and b, and carotenoids) were significantly enhanced by β-sitosterol (20 or 40 mg L-1) as a foliar application.

Gamel (2011) found that stigmasterol or sitosterol increased significantly photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls and carotenoids and consequently total pigments).

Table2: Effect of β-sitosterol on photosynthetic pigments (mg/g fresh weight) of sunflower plant.

Stage	Treatments	Ch. a	Chl. b	Chl a+b	Chl. a/b	Carotenoids	Total pigments
45 days	Control	0.597	0.403	0.999	1.481	0.194	1.790
	B^{-9}	0.608*	0.410*	1.018*	$1.483^{n.s}$	0.200*	2.808*
	B^{-7}	0.613*	0.414*	1.027*	$1.479^{n.s}$	0.205*	3.818*
	B^{-5}	0.630*	0.423*	1.054*	$1.489^{n.s}$	0.215*	4.846*
	LSD $_{at\ 5\%}$	**0.005**	**0.003**	**0.008**	**0.017**	**0.006**	**0.009**
85 days	Control	0.474	0.321	0.795	1.479	0.169	0.964
	B^{-9}	0.484*	0.331*	0.815*	$1.463^{n.s}$	$0.171^{n.s}$	0.986*
	B^{-7}	0.490*	0.332*	0.822*	$1.477^{n.s}$	0.175*	0.998*
	B^{-5}	0.506*	0.339*	0.844*	$1.492^{n.s}$	0.180*	1.024*
	LSD $_{at\ 5\%}$	**0.004**	**0.007**	**0.008**	**0.032**	**0.006**	**0.011**

3.1.3. Interactive effect

Table 3 (a & b) shows the interaction between Sea water concentration in irrigation water and application different concentrations of β-sitosterol on pigments fractions of sunflower plants of the two taken samples. It can be found that chlorophyll a, b, a+b and carotenoids as well as total pigments significantly increased in salinized plants treated with β-sitosterol under various sea water concentrations compared to the corresponding control, except with chlorophyll a/b which had no significant effect. It can be noticed that the plants grown under zero Sea water and treated with 10^{-5} molar attained the highest chlorophyll a, b, a+b, carotenoids and total pigments as compared with other treatments during 45 and 85 days-old-plants. This result was in agreement with those, **Hassanein *et al.* (2012)** whose found that sitosterol caused a significant increase in photosynthetic pigment contents of salt stressed faba bean plants. Also, **Bassuany *et al.*, (2014)** revealed that application of stigmasterol to salt - stressed flax plants causing significant icrease in photosynthetic pigment.

Table 3a. Interaction effect of salinity and β-sitosterol on photosynthetic pigments (mg/g fresh weight) of sunflower plant after 45 days.

Stages	Treatments	Ch. a				Chl. b				Chl a+b			
		Control	B^{-9}	B^{-7}	B^{-5}	Control	B^{-9}	B^{-7}	B^{-5}	Control	B^{-9}	B^{-7}	B^{-5}
45 days	Tap water	0.669	0.683*	0.691*	0.708*	0.452	$0.454^{n.s}$	0.463*	0.467*	1.121	1.137*	1.154*	1.175*
	10%	0.627*	0.634*	0.642*	0.654*	0.420*	0.430*	0.437*	0.448*	1.047*	1.064*	1.079*	1.102*
	20%	0.570*	0.575*	0.592*	0.604*	0.384*	0.388*	0.398*	0.405*	0.954*	0.963*	0.990*	1.009*
	30%	0.520*	0.539*	0.527*	0.556*	0.355*	0.368*	0.359*	0.374*	0.875*	0.907*	0.886*	0.930*
	LSD $_{at\ 5\%}$	**0.012**				**0.007**				**0.015**			
		Chl. a/b				**Carotenoids**				**Total pigments**			
	Tap water	1.481	$1.506^{n.s}$	$1.491^{n.s}$	1.518^{*}	0.231	$0.239^{n.s}$	0.243*	0.245*	1.900	2.922*	3.934*	4.953*
	10%	$1.494^{n.s}$	$1.475^{n.s}$	$1.468^{n.s}$	$1.461^{n.s}$	0.205*	0.211*	0.218*	$0.226^{n.s}$	1.832*	2.845*	3.860*	4.880*
	20%	$1.482^{n.s}$	$1.483^{n.s}$	$1.488^{n.s}$	$1.491^{n.s}$	0.179*	0.188*	0.193*	0.216*	1.749*	2.763*	3.785*	4.820*
	30%	$1.467^{n.s}$	$1.467^{n.s}$	1.468*	$1.485^{n.s}$	0.159*	0.162*	0.168*	0.175*	1.679*	2.701*	3.695*	4.731*
	LSD $_{at\ 5\%}$	**0.033**				**0.012**				**0.019**			

Table 3b. Interaction effect of salinity and β-sitosterol on photosynthetic pigments (mg/g fresh weight) of sunflower plant after 85 days.

Stages	Treatments	Ch. a				Chl. b				Chl a+b			
		Control	B^{-9}	B^{-7}	B^{-5}	Control	B^{-9}	B^{-7}	B^{-5}	Control	B^{-9}	B^{-7}	B^{-5}
85 days	Tap water	0.530	0.542*	0.549*	0.572*	0.357	$0.354^{n.s}$	$0.371^{n.s}$	0.375*	0.887	$0.896^{n.s}$	0.920*	0.946*
	10%	0.500*	0.503*	0.515*	0.518*	0.337*	0.342*	$0.347^{n.s}$	$0.356^{n.s}$	0.837*	0.846*	0.862*	$0.874^{n.s}$
	20%	0.454*	0.461*	0.472*	0.490*	0.306*	0.332*	0.322*	0.324*	0.760*	0.793*	0.794*	0.814*
	30%	0.413*	0.430*	0.425*	0.442*	0.282*	0.296*	0.288*	0.300*	0.695*	0.726*	0.713*	0.742*
	LSD $_{at\ 5\%}$	**0.007**				**0.015**				**0.017**			
		Chl. a/b				**Carotenoids**				**Total pigments**			
	Tap water	1.482	$1.530^{n.s}$	$1.482^{n.s}$	$1.526^{n.s}$	0.198	$0.204^{n.s}$	$0.207^{n.s}$	$0.207^{n.s}$	1.085	$1.099^{n.s}$	1.127*	1.153*
	10%	$1.485^{n.s}$	$1.470^{n.s}$	$1.485^{n.s}$	$1.454^{n.s}$	0.176*	0.181*	$0.187^{n.s}$	$0.192^{n.s}$	1.013*	1.026*	1.049*	$1.066^{n.s}$
	20%	$1.485^{n.s}$	$1.398^{n.s}$	$1.467^{n.s}$	$1.511^{n.s}$	0.169*	0.161*	0.165*	0.170*	0.930*	0.955*	0.959*	0.984*
	30%	$1.464^{n.s}$	$1.454^{n.s}$	$1.474^{n.s}$	$1.476^{n.s}$	0.135*	0.137*	0.141*	0.150*	0.830*	0.862*	0.855*	0.892*
	LSD $_{at\ 5\%}$	**0.064**				**0.012**				**0.022**			

3.2. Change in carbohydrate fractions content

3.2.1. Effect of salinity

Data recorded in Table 4 show the effect of different salinity concentrations on carbohydrate contents in sunflower plants during 45, 85 and 120 days-old-plants. Results declare that there was a significant decrease in the content of carbohydrates fraction i.e. glucose, sucrose, total soluble, polysaccharides (starch) and total carbohydrates by increasing Sea water concentration in growth medium. This result was true during three taken samples.

The lowest value of carbohydrates fractions was recorded at the highest level of salinity stress compared with control plants. In this regard, the 30% of Sea water resulted in a decrease of about (43.07, 32.93, 30.04), (45.00, 50.42, 58.62), (32.28, 23.73, 19.88), (22.91, 15.90, 10.64) and (24.85, 16.39, 10.60) respectively for glucose, sucrose, total soluble, polysaccharides (starch) and total carbohydrates during 45, 85 and 120 days-old-plants as compared with control plants. Such inhibition in carbohydrates accumulation was recorded by other authors (**Hassanein *et al.*, 2009b**). The decrease in carbohydrate and photosynthetic pigments content were directly proportional to the applied concentration of sea water. These results led to the conclusion that Sea water may inhibit photosynthetic activity or increase partial utilization of carbohydrates in other metabolic pathways.

In this respect, **Sadak *et al.* (2012)** stated that increasing salinity level from 3000 to 6000 mg/l caused significant reduction in polysaccharides, total carbohydrates, protein contents, also caused significant increases in total polyamine levels, total soluble sugars, and total amino acids. Also**, Amini *et al.*, (2014)** reported significant effects on soluble carbohydrate, and protein content. Cluster analysis divided genotypes into 3 groups of sensitive, tolerant, and semi tolerant genotypes under water-deficient conditions. The superior seed yield and quality was closely related to the physiological properties of the plants, resulting in higher carbohydrate. The results suggest that leaf carbohydrate content would be useful traits to select water stress-tolerant plants in safflower.

Table 4. Effect of salinity on carbohydrates content (mg/100g dry weight) of sunflower plant.

Stage	Treatments	Glucose	Sucrose	Total soluble sugars	Polysaccharides (starch)	Total carbohydrates
45 days	Tap water	0.202	1.600	1.865	17.299	20.715
	10%	0.176*	1.353*	1.659*	15.981*	18.997*
	20%	0.145*	1.118*	1.459*	14.670*	17.259*
	30%	0.115*	0.880*	1.263*	13.335*	15.567*
	LSD $_{at\,5\%}$	0.004	0.010	0.010	0.008	0.029
85v days	Tap water	0.249	1.313	2.916	23.915	29.114
	10%	0.223*	1.086*	2.740*	22.663*	27.520*
	20%	0.197*	0.873*	2.479*	21.393*	25.930*
	30%	0.167*	0.651*	2.224*	20.112*	24.342*
	LSD $_{at\,5\%}$	0.003	0.009	0.144	0.048	0.014
120 days	Tap water	0.273	0.928	3.330	32.624	36.110
	10%	0.243*	0.746*	3.105*	31.469*	34.852*
	20%	0.216*	0.602*	2.818*	30.309*	33.562*
	30%	0.191*	0.384*	2.668*	29.154*	32.280*
	LSD $_{at\,5\%}$	0.003	0.065	0.116	0.028	0.026

3.2.2. Effect of different concentration of β-sitosterol

Table (5)declare the effect of β-sitosterol treatments on concentration of carbohydrate fractions i.e. glucose, sucrose, total soluble, polysaccharides (starch) and total carbohydrates by increasing Sea water concentration in growth medium during three stages of growth.

Carbohydrate fractions significantly affected with application of β-sitosterol under all treatments. It can be also noticed that plants treated with 10^{-5} molar β-sitosterol had the highest carbohydrate fractions values comparing with the other treatments. This was true at different growth stages.

Application of β-sitosterol stimulated the accumulation of sugars in salt-treated sunflower plants and the inhibitory effects of salt stress were partially alleviated. In this connection, **Abd El-Wahed (2001)** found that treated maize plant with sitosterol resulted in significant increases in total soluble and non-soluble sugar contents and accumulation of sucrose at the tasselling stage compared with the controls. **Abd El-Wahed and Gamal El-Din (2004)** stated that sitosterol concentration (100 mg/l)strongly affect growth and consequently the biochemical constituents of leaves (total sugars, phenols and indoles), of which the contents were increased. In addition, the enhancement of carbohydrate biosynthesis by sitosterol, especially soluble sugars that are

considered to be the principle organic osmotica in a number of glycophytes subjected to saline conditions (**Hassanein *et al.*, 2012**), highlight a possible mechanism by which sitosterol plays a positive role in alleviation of the harmful effects of salt stress. This effect might be due to stimulation of the metabolic processes.

The present study shows that with β-sitosterol application, the leaves fill up more soluble sugar and proline. The increasing of carbohydrate is a signal for water deficiency tolerance. The high carbohydrate concentration with its role to reduce water potential helps to prevent oxidative losses and protein structure maintenance during water shortage. Also, carbohydrates play a molecule role for sugar responsible genes that give different physiological response like defensive response and cellular expansion (**Simaei *et al.*, 2012).**

Table 5: Effect of β-sitosterol on carbohydrates contents (mg/100g dry weight) of sunflower plant.

Stage	Treatments	Glucose	Sucrose	Total soluble sugars	Polysaccharides (starch)	Total carbohydrates
45 days	Control	0.148	1.154	1.482	14.838	17.475
	B^{-9}	0.157*	1.201*	1.528*	15.152*	17.917*
	B^{-7}	0.162*	1.269*	1.592*	15.482*	18.357*
	B^{-5}	0.170*	1.327*	1.642*	15.815*	18.788*
	LSD $_{at\,5\%}$	**0.004**	**0.009**	**0.004**	**0.013**	**0.025**
85v days	Control	0.198	0.900	2.506	21.534	26.127
	B^{-9}	0.206*	0.954*	$2.582^{N.S}$	21.860*	26.525*
	B^{-7}	0.214*	1.007*	$2.555^{N.S}$	22.182*	26.923*
	B^{-5}	0.219*s	1.061*	2.715*	22.507*	27.331*
	LSD $_{at\,5\%}$	**0.003**	**0.008**	**0.115**	**0.023**	**0.022**
120 days	Control	0.220	0.634	2.916	30.450	33.723
	B^{-9}	0.227*	$0.625^{N.S}$	2.967*	30.749*	34.039*
	B^{-7}	0.234*	$0.678^{N.S}$	2.959*	31.033*	34.364*
	B^{-5}	0.242*	0.724*	3.078*	31.326*	34.678*
	LSD $_{at\,5\%}$	**0.002**	**0.059**	**0.010**	**0.019**	**0.016**

3.2.3. Interactive effect.

As regard, the interaction effect between salinity levels and application of various concentrations of β-sitosterol on carbohydrate fractions content is shown in tables 6 (a, b &c). It could be observed that using the different concentration of β-sitosterol significantly increased all carbohydrate fractions i.e. glucose, sucrose, total soluble, polysaccharides (starch) and total carbohydrates in most cases of sunflower plants.

At all stages of growth and development, carbohydrate fractions content were significantly increased in plants treated with different β-sitosterol under any Sea water concentration in most cases, the increase was significant with all treatments specially under the highest level of sitosterol which was (10^{-5}) molar and control plant and the lowest value of carbohydrates was recorded at the highest level of salinity stress compared with control plants.

3.3. Effect of salinity and different concentrations of □-sitosterol on fatty acids

Gas liquid chromatography analysis cleared mainly eighteen fatty acids in sunflower seeds oil. Namely n-carpoic acid (C6:0), carprylic acid (C8:0), capric acid (C10:0) , lauric acid (C12:0), tridecanoic acid (C13:0), and myristic acid (C14:0) as well as tetradecenoic acid (14:1), pentadecanoic acid (15:0), 14-pentadecen ooic acid (C15:1), palmitic acid (C16:0), 9 hexldecenoic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), 5-ecosenic acid (C20:1) and heneicosanoic acid (C21:0) as well as erucic acid (C22:1) at Table 7.

All the applied concentrations of Sea water were found to cause a marked reduction in unsaturated fatty acids content accompanied with a marked increase in saturated fatty acids content as compared with untreated control plants.

The most affected unsaturated fatty acid was linolic acid which decreased by 8.64%, 19.28 % and 21.31% in response to 10, 20 and 30% sea water, respectively, as compared with control plants. On the other hand, Myristic acid (C14:0) was the most increased saturated fatty acid in response to different concentrations of NaCl, the highest value obtained in response to 30% Sea water and was estimated by 90.91%, as compared the control.

This indicates that mystiric acid may play an important role in salt tolerance mechanism of plants. In this respect, **Ishitani *et al.* (2000)** reported evidence for an essential role of protein N-myristoylation (refers to the covalent attachment of myristic acid by an amide bond to N-terminal glycine residue of a nascent polypeptide) in salt tolerance plant.

The effect of β-sitosterol treatment was found to be contrary to that of salinity; as marked increases were observed in unsaturated fatty acid contents, as compared with the reference control plants. In this respect, oleic acid was increased by 28.39%, 22.07%, 27.72% and 12.58% and linolic acid by 14.42%, 17.42%, 29.24% and 10.22%, as compared with plant treated with,(10, 20 and 30 %)Sea water under the suitable addition of β-sitosterol, respectively.

Similar results were obtained by **Abd El-Wahed and Gamal El-Din (2004**). **Putanam *et al*. (1990)** ,they stated that the good quality of sunflower oil is, principally, due to its high level of unsaturated fatty acids. The increase in oleic acid and linolic acid in response to β-sitosterol treatment, increases the nutritional value and the economic importance of the sunflower plants seed oil, as the sunflower seed oil health benefits are primarily due to it being the highest food source of crucially needed omega 3 fatty acids. There is a terribly widespread deficiency of the omega 3 source essential fatty acid (alpha linolic acid) and the omega 3 fatty acids that are derived from it. Oleic acid (omega 9) has also received a great attention lately due to its ability to lower blood pressure and the level of cholesterol in the body being rich in antioxidants that help in fighting the effects of free radicals in the body. It also boosts the immune system reduces the inflammation of joints and other complications related to arthritis reducing the resistance of insulin, thereby, improving glucose (blood sugar) maintenance (**Thompson and Cunnane, 2003; Berab *et al*., 2006**). **El –Lethy *et al.* (2010)** found that foliar application of putrescine, stigmasterol or α-tocopherol, significantly, affected growth criteria and seed yield of flax plant and linolenic acid was found to be the main fatty acid in the seeds of all treatments under study.

Table 6a. Interaction effect of salinity and β-sitosterol on carbohydrates contents (mg/100g dry weight) of sunflower plant after 45 days.

	Glucose			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	0.191	0.199*	0.204*	0.214*
10%	0.16**	0.175*	0.179*	0.182*
20%	0.134*	0.141*	0.145*	0.159*
30%	0.102*	0.112*	0.119*	0.127*
$LSD_{at\ 5\%}$	**0.008**			
	Sucrose			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	1.519	1.552*	1.623*	1.697*
10%	1.269*	1.314*	1.385*	1.443*
20%	1.035*	1.090*	1.145*	1.203*
30%	0.793*	0.845*	0.914*	0.966*
$LSD_{at\ 5\%}$	**0.019**			
	Total soluble sugars			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	1.785	1.813*	1.887*	1.975*
10%	1.581*	1.638*	1.965*	1.721*
20%	1.379*	1.429*	1.494*	1.532*
30%	1.183*	1.233*	1.292*	1.341*
$LSD_{at\ 5\%}$	**0.020**			
	Polysaccharides (starch)			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	16.819	17.134*	14.462*	17.78*
10%	15.492*	15.792*	16.159*	16.483*
20%	14.177*	14.506*	14.833*	15.163*
30%	12.863*	13.175*	13.473*	13.831*
$LSD_{at\ 5\%}$	**0.026**			
	Total carbohydrates			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	19.992	20.508*	20.942*	21.419*
10%	18.334*	18.796*	19.208*	19.647*
20%	16.651*	17.023*	17.498*	17.862*
30%	14.921*	15.341*	15.781*	16.224*
$LSD_{at\ 5\%}$	**0.049**			

Table 6b. Interaction effect of salinity and β-sitosterol on carbohydrates contents (mg/100g dry weight) of sunflower plant after 85 days.

	Glucose			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	0.239	$0.245^{n.s}$	0.254*	0.258*
10%	0.211*	0.221*	0.226*	$0.234^{n.s}$
20%	0.188*	0.193*	0.200*	0.206*
30%	0.153*	0.166*	0.174*	0.177*
$LSD_{at\ 5\%}$	**0.007**			
	Sucrose			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**

Tap water	1.231	1.284*	1.341*	1.394*
10%	1.003*	1.063*	1.110*	1.170*
20%	0.796*	0.845*	0.898*	0.950*
30%	0.571*	0.624*	0.677*	0.732*
LSD $_{at\ 5\%}$	1.231			
	Total soluble sugars			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	2.890	$2.971^{n.s}$	$2.691^{n.s}$	$3.110^{n.s}$
10%	2.644*	$2.703^{n.s}$	$2.770^{n.s}$	$2.845^{n.s}$
20%	2.362*	2.466*	2.509*	2.579*
30%	2.127*	2.189*	2.250*	2.328*
LSD $_{at\ 5\%}$	**0.230**			
	Polysaccharides (starch)			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	23.381	23.760*	24.085*	24.436*
10%	22.185*	22.503*	22.820*	23.146*
20%	20.923*	21.234*	21.545*	21.869*
30%	19.649*	19.943*	20.279*	20.576*
LSD $_{at\ 5\%}$	**0.046**			
	Total carbohydrates			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	28.502	28.912*	29.37*	29.724*
10%	26.932*	27.319*	27.706*	28.122*
20%	25333*	25.726*	26.126*	26.535*
30%	23.740*	24.142*	24.543*	24.942*
LSD $_{at\ 5\%}$	**0.044**			

Table 6c: Interaction effect of salinity and β-sitosterol on carbohydrates contents (mg/100g dry weight) of sunflower plant after 120 days.

	Glucose			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	0.263	0.269*	0.277*	0.282*
10%	0.232*	0.239*	0.245*	0.256*
20%	0.204*	0.215*	0.20*	0.226*
30%	0.182*	0.186*	0.19*	0.202*
LSD $_{at\ 5\%}$	**0.005**			
	Sucrose			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	0.871	$0.906^{n.s}$	$0.949^{n.s}$	$0.985^{n.s}$
10%	0.684*	0.718*	$0.764^{n.s}$	$0.820^{n.s}$
20%	0.659*	0.537*	0.584*	0.630*
30%	0.323*	0.338*	0.414*	0.462*
LSD $_{at\ 5\%}$	**0.117**			
	Total soluble sugars			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	3.248	$3.297^{n.s}$	$3.366^{n.s}$	$3.408^{n.s}$
10%	3.017*	$3.074^{n.s}$	$3.140^{n.s}$	$3.187^{n.s}$
20%	2.812*	2.854*	2.632*	2.972*
30%	2.587*	2.641*	2.698*	2.744*
LSD $_{at\ 5\%}$	**0.199**			
	Polysaccharides (starch)			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	32.170	32.941*	32.765*	33.072*
10%	31.036*	31.343*	31.607*	31.891*
20%	29.875*	30.156*	30.457*	30.749*
30%	28.718*	29.005*	29.301*	29.591*
LSD $_{at\ 5\%}$	32.170			
	Total carbohydrates			
Treatments	**Control**	**B^{-9}**	**B^{-7}**	**B^{-5}**
Tap water	35.634	335.946*	336.270*	336.589*
10%	34.372*	34.692*	35.017*	35.328*
20%	33.083*	33.398*	33.723*	34.044*
30%	31.803*	32.119*	32.447*	32.075*
LSD $_{at\ 5\%}$	**0.032**			

Table 7. Interaction effect of salinity and β-sitosterol on fatty acids of sunflower seeds.

Common name	symbol	Control	10%	20%	30%	10^{-5} molar				10^{-7} molar				10^{-9} molar			
						Control	10%	20%	30%	Control	10%	20%	30%	Control	10%	20%	30%
n- Carpoic Acid	C6:0	0.032	0.017	0.011	0.0055	0.006	0.008	0.029	-	0.009	0.024	0.028	0.024	-	-	-	-
Caprylic Acid	C8:0	0.101	0.074	0.059	0.051	0.040	0.038	0.053	0.048	0.021	0.017	0.036	0.023	0.028	0.056	0.025	0.030
Capric Acid	C10:0	0.023	0.022	0.047	0.033	0.018	0.019	-	-	-	-	0.038	0.035	-	0.031	-	0.039
Lauric Acid	C12:0	0.085	0.122	0.133	0.129	0.096	0.132	0.163	0.152	0.182	0.0844	0.144	0.103	0.089	0.117	0.073	0.208
Tridecanoicc Acid	C13:0	0.250	0.175	0.323	0.187	0.114	0.199	0.291	0.212	0.318	0.404	0.221	0.168	0.269	0.187	0.128	0.555
Myrisitic Acid	C14:0	0.338	0.609	0.517	0.630	0.440	0.617	1.117	0.646	0.969	0.520	0.688	0.554	0.455	0.521	0.456	0.874
Tetradecenoic	C14:1	0.109	0.181	0.161	0.221	0.126	0.217	0.221	0.147	0.240	0.094	0.135	0.094	0.075	0.186	0.123	0.306
Pentadecanoic	C15:0	0.202	0.385	0.277	0.343	0.240	0.352	0.688	0.439	0.597	0.253	0.342	0.3134	0.273	0.293	0.376	0.443
14,Pentadecenooic	C15:1	0.063	0.126	0.092	0.125	0.083	0.129	0.236	-	0.144	0.090	0.110	0.093	0.067	0.112	-	0.186
Palmitic Acid	C16:0	6.321	7.260	7.123	8.081	6.393	7.534	6.583	8.047	6.096	6.884	7.359	7.322	6.561	7.059	6.568	8.929
9 Hexldecenoic	C16:1	0.050	0.077	0.081	0.099	0.060	0.074	0.063	0.091	0.075	0.098	0.119	0.090	0.081	0.104	0.088	0.275
Stearic Acid	C18:0	4.754	4.222	4.038	3.461	4.054	4.532	3.069	4.243	3.396	3.904	3.080	3.304	1.935	3.256	3.015	2.514
Oleic Acid	C18:1	30.952	35.516	40.411	42.110	39.740	43.356	29.210	36.813	39.337	40.760	32.619	39.073	36.044	42.736	44.523	36.499
Linolic Acid	C18:2	54.461	49.758	43.957	42.856	46.606	41.088	56.810	47.236	47.209	45.436	53.309	47.616	52.797	43.339	42.176	47.527
Arachidic Acid	C20:0	0.425	0.357	0.567	0.282	0.343	0.332	0.415	0.331	0.304	0.353	0.353	0.324	0.344	0.331	0.456	0.475
5-Ecosenic Acid	C20:1	0.205	0.173	0.249	0.191	0.228	0.204	0.242	-	0.275	0.309	0.208	0.265	0.218	0.221	0.244	0.276
Heneicosanoic	C21:0	0.591	-	0.769	0.588	0.662	0.446	-	0.723	-	-	-	-	-	0.567	0.643	-
Erucic Acid	C22:1	1.029	0.918	1.177	0.600	0.732	0.711	0.796	0.657	0.819	0.761	0.718	0.589	0.756	0.875	1.098	0.857

IV. Conclusion

The adverse effects of salt stress on the growth of sunflower can be mitigated by soaking the seeds in β-sitosterol. However, effectiveness of β-Sitosterol in alleviating the adverse effects of salinity stress was salt-dose dependent. β-sitosterol treatment induced augment of enzymatic antioxidant system, reducing oxidative damage (membrane integrity and MDA) in Sea water stress conditions. The increase in the degree of salt tolerance induced by β-sitosterol was also reflected in the improvement in the photosynthetic pigments content and consequently the carbohydrate pool and fatty acid content under saline condition. Thus, our data provide evidence for the stimulatory effects of β-sitosterol to induce salt tolerance in sunflower plants.

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