

## EFFECT OF SALT AND WATER STRESS ON GROWTH AND BIOMASS PRODUCTION IN *HELIANTHUS ANNUUS* L.

SHAZIA HUSAIN AND SHOAB ISMAIL

*Biosaline Research Laboratories,  
Department of Botany, University of Karachi, Karachi, Pakistan.*

### Abstract

The effect of 0.25-1.0% NaCl salinity and water stress on growth and biomass production in *Helianthus annuus* L., was examined. All growth parameters showed reduction under both salt and salt water stress. One percent NaCl salinity adversely affected the relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR). Salt stress together with moisture stress also adversely affected the growth rate. Plants grown both under salt or salt and water stress conditions showed a better growth response after 1 month interval followed by a decline at subsequent harvests.

### Introduction

Sunflower (*Helianthus annuus* L.), an oil seed crop is cultivated over an area of 29.5 thousand hectares in Pakistan (Anon., 1990). It is as an ornamental plant, besides its use as a fodder crop in most of the Asian countries where it is fed green or converted into silage. The plant needs a higher quantity of water as it produces large amount of dry matter owing to its high transpiration co-efficient. However, it also endures drought well on account of its vigorous and well developed root system. This biological character is favourable for its production even on dry areas. Growth of the plant has been reported until the soil moisture drops to 14%, below which the leaves dry up and vital functions of the plant cells are stopped (Beg *et al.*, 1983). The yield of sunflower primarily depends on the quantity of water available to the plant at the stage of intensive growth and that of oil synthesis.

Although sunflower is cultivated on good agricultural areas, but the farmers have lately used the marginal and degraded soils which are mostly saline and waterlogged for its cultivation. In Pakistan, such salt-affected areas constitute about 5.82 m.ha. Experiments were therefore carried out to study the vegetative productivities of sunflower as affected by interactive effects of a combination to both salt and water stress conditions.

### Materials and Methods

A local commercial cultivar HO1 of sunflower (*Helianthus annuus* L.) was used. Earthen pots filled with 20 kg., garden soil were randomly arranged in three blocks, each block having 0, 0.25, 0.5, 0.75 and 1% NaCl levels with three replicates. The experiment was laid down using a Completely Randomized Design. Treatments and replicates included two separate sets for salt and salt  $\times$  water stress. Three seeds were sown in each pot and irrigated with tap water for a week until two leaf stage. Extra plants were removed leaving one uniform and healthy seedling in each pot. Salt treatments were initiated 3 days after thinning the plant using different salt levels, by

**Table 1. Analyses of different concentrations of sodium chloride solutions used during the growth of sunflower plants.**

Treatments NaCl (%)	Electrical Conductivity (dS/m)	TDS (g/L)	pH	Na <sup>+</sup> ---(Meq.L <sup>-1</sup> )---	K <sup>+</sup>
Control	0.430	0.30	8.05	3.46	0.06
0.25	3.690	2.41	7.95	18.18	0.06
0.5	6.740	4.62	7.85	33.76	0.05
0.75	9.910	6.31	7.85	43.29	0.05
1.0	12.920	8.64	7.95	47.61	0.07

gradually increasing the salt concentration for both salt and salt × water stress treatments. The chemical characteristics of irrigation water used in the experiment is given in Table 1. The pots were irrigated twice a week with the respective saline solutions to keep the moisture levels between field capacity and maximum water holding capacity. Three days prior to harvest, the plants were subjected to water stress (which was three irrigations less than the set for salt stress only). Plants in each block were harvested after 4 weeks growth interval with a total of 3 harvests in both salt and salt × water stressed plants for growth analyses and biomass production.

Plants were carefully removed from the pots and the fresh weight of root, stem and leaves was determined. Each fraction of the plants parts were kept in an oven at 60°C for 24 h for dry weight determination.

Functional growth analyses was performed as described by Hunt (1978) and outlined in Coombs *et al.*, (1985), by measuring the leaf area, fresh and dry weight of the leaf samples to calculate the relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR).

## Results

**A) Relative Growth Rate:** Relative growth rate in *H. annuus* showed variation at different concentrations of NaCl at both the harvest intervals. When plants were subjected to salt stress, the RGR at the first harvest interval exhibited an increase in 0.25% NaCl, followed by a decrease in 0.75% NaCl concentration (Fig.1). The RGR at the second harvest interval showed non-significant variation at all the NaCl concentrations studied, whereas plants grown under both salt × water stress treatment exhibited significant variation at 0.5 and 1% NaCl level with non-significant changes at 0 and 0.25% NaCl concentration at both the harvest period (Fig.1).

**B) Net Assimilation Rate:** The net assimilation rate (NAR) under salt stress at the first harvest interval showed a significant ( $P < 0.05$ ) increase at 0.25% NaCl only followed by non-significant reduction at high salt stress (Fig.2). At second harvest interval, the NAR exhibited non-significance in all the treatments. NAR under salt × water stress showed similar pattern to that of RGR. Almost same rate of NAR was evident at low salt level (0 and 0.25% NaCl) followed by a decline at 0.5% NaCl salinity (Fig.2).

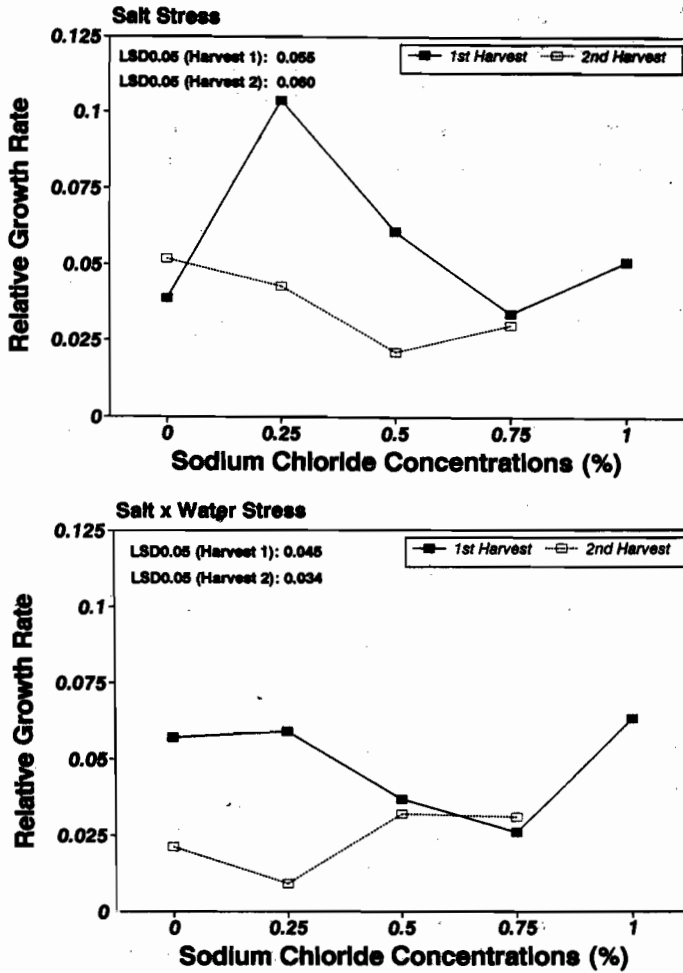


Fig.1. Effect of salt and salt x water stress on the relative growth rate ( $\text{g}^{-1} \cdot \text{g}^{-1} \cdot \text{month}^{-1}$ ) at different concentrations of sodium chloride.

**C) Leaf Area Ratio:** The leaf area ratio (LAR) under salt stressed conditions showed significant differences at first harvest interval with increase in salinity thus exhibiting a reduction in photosynthetic area of plants (Fig.3). LAR showed a reduction with increase in salt concentrations at both the salinity levels. LAR values under salt  $\times$  water stress in both first and second harvest intervals showed similar rate at low salt levels of 0 and 0.25% NaCl concentration with significant differences at high salt concentration of 0.75% at the second harvest period.

**D) Biomass Allocation:** Both shoot and root biomass (fresh and dry) decreased with an increase in salt concentration at the first harvest. Fresh weight of shoot decreased by 40% and root by 33% as compared to non-saline control (Table 2-A). Dry weight of plants were less affected in both shoot and roots of the plants indicating variation in moisture content in both the plant parts. Plants exposed to salt  $\times$  water stress also

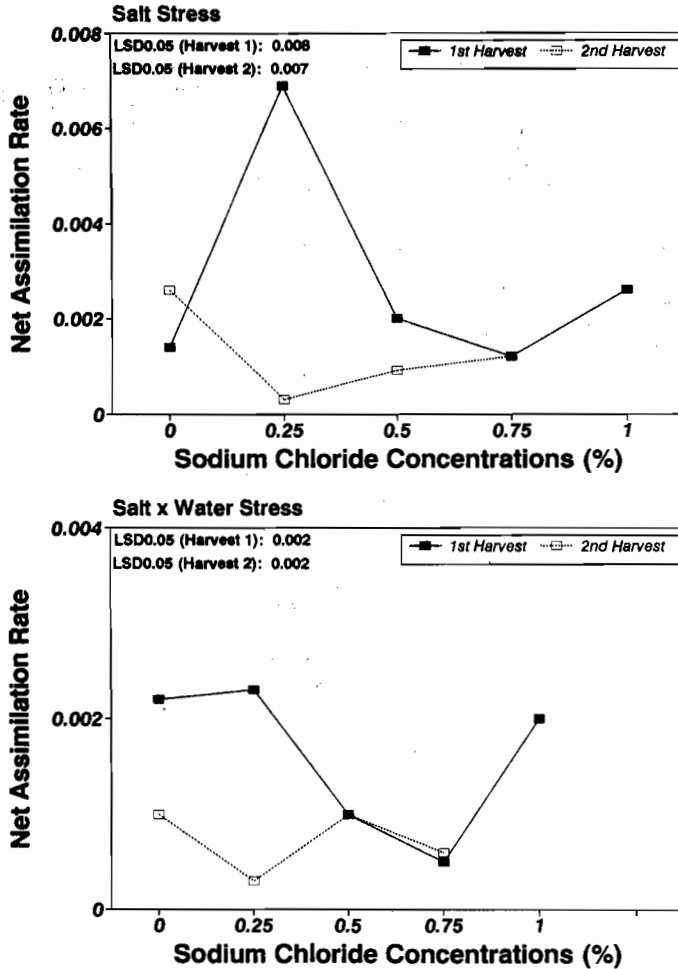


Fig.2. Effect of salt and salt x water stress on the net assimilation rate ( $\text{g}^{-1}.\text{cm}^{-2}.\text{month}^{-1}$ ) at different concentrations of sodium chloride.

showed similar response to the stresses, however, they were less affected even at 1% NaCl salinity as compared to salt stress alone (Table 2- A). Shoot/root ratio on fresh weight bases showed a gradual decrease with increase in NaCl concentration. In salt  $\times$  water stress treated plants, values of the ratio was comparatively less as compared to salt stress alone (Table 2-A). Fresh weight/dry weight ratio remained unaffected upto 0.25% NaCl level which showed an increase with an increase in salt level. Plants exposed to both salt and water stress also exhibited a similar response.

At second harvest, plants showed better performance in salt stress as compared to salt  $\times$  water stressed plants at all the salinity levels (Table 2-B). Salt stressed plants showed an increase in shoot biomass at 0.25% salt level. Root weight showed an increase at all the salinity levels as compared to control. Shoot/root (fresh) ratio increased in salt stressed plants as compared to non-saline control. Salt  $\times$  water

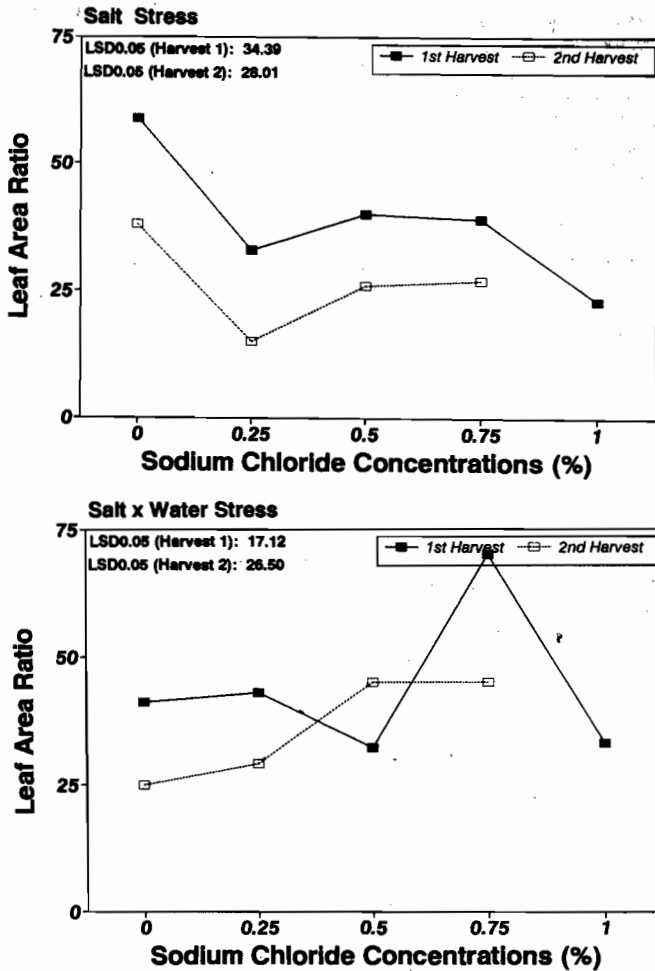


Fig.3. Effect of salt and salt x water stress on the leaf area ratio ( $\text{cm}^{-2} \cdot \text{g}^{-1} \cdot \text{month}^{-1}$ ) at different concentrations of sodium chloride.

stressed plants, exhibited a higher ratio of shoot/root ratio as compared to the first harvest. On dry weight bases, the ratio was low in salt stressed plants as compared to both salt and water stressed plants (Table 2-B). Fresh/dry weight showed a decrease in both salt and salt  $\times$  water stress plants. At third harvest, both salt and salt  $\times$  water stress plants showed a significant effect of salinity on shoot fresh and dry weight as compared to first and second harvest (Table 2-C). Root growth showed an increase at low salt levels (0.25% NaCl), both on fresh and dry weight bases. Salt  $\times$  water stressed plants showed a significant reduction of both shoot and root weight, on fresh and dry weight bases. Shoot/root ratio decreased in salt stress and remained unaffected in salt  $\times$  water stress plants. Fresh/dry weight ratio decreased both under salt and salt  $\times$  water stress conditions. (Table 2-C).

**Table 2. Changes in fresh and dry weight of shoot and root of sunflower in salt and salt x water stressed plants at different levels of sodium chloride salinity.**

**A. HARVEST - I**

Treatments (% NaCl)	Fresh weight (g)			Dry weight (g)			F.wt./ D.wt.
	Shoot	Root	Shoot/ Root	Shoot	Root	Shoot/ Root	
<b>S A L T</b>							
0	3.086	0.706	4.316	0.669	0.174	3.688	4.718
	+0.642	+0.064	+0.729	+0.188	+0.025	+0.711	+0.434
0.25	2.009	0.564	3.555	0.389	0.167	1.654	4.623
	+0.187	+0.027	+0.220	+0.016	+0.013	+0.589	+0.305
0.50	2.439	0.829	2.964	0.402	0.199	2.237	5.401
	+0.426	+0.163	+0.100	+0.034	+0.056	+0.414	+0.474
0.75	1.589	0.589	2.805	0.312	0.153	1.963	5.451
	+0.133	+0.078	+0.496	+0.032	+0.015	+0.082	+0.362
1.00	1.844	0.471	3.902	0.251	0.103	2.470	6.641
	+0.188	+0.020	+0.266	+0.032	+0.015	+0.082	+0.036
LSD <sub>0.05</sub>	1.164	0.276	1.363	0.318	0.109	0.977	
<b>S A L T x W A T E R</b>							
0	2.040	0.556	4.298	0.502	0.130	3.883	4.770
	+0.454	+0.069	+0.533	+0.124	+0.022	+0.612	+0.349
0.25	2.306	0.623	3.776	0.553	0.190	3.045	3.990
	+0.415	+0.033	+0.812	+0.035	+0.029	+0.466	+0.462
0.50	2.408	0.419	1.975	0.336	0.158	2.209	5.477
	+0.124	+0.179	+0.183	+0.044	+0.015	+0.490	+0.495
0.75	1.528	0.446	3.484	0.295	0.084	3.496	5.690
	+0.132	+0.060	+0.331	+0.084	+0.021	+0.434	+0.881
1.00	1.851	0.498	3.692	0.287	0.123	2.375	5.860
	+0.303	+0.072	+0.166	+0.063	+0.027	+0.221	+0.438
LSD <sub>0.05</sub>	1.000	0.182	1.122	2.438	0.076	1.948	

Analysis of variance calculated for fresh and dry biomass of shoot and root to salt and/or water stress and harvest period showed significant variation with reference to salt stress and harvest periods (Table 3). Interactions between salt stress and harvest periods were also significant ( $P < 0.01$ ) in both fresh and dry weight of shoot. Roots also exhibited variation to salt stress and harvest period. Dry weight of root, also exhibited variation at  $P < 0.01$  for water stress.

**E) Changes in Soil Characteristics:** Plants grown under salt and salt  $\times$  water stress conditions exhibited variation in salt accumulation pattern. Electrical conductivity increased as the concentration of NaCl increased in the external medium (Table 4). At second harvest, no change in  $EC_e$  at 0 and 0.25% NaCl was observed, whereas, at concentration greater than 0.25% NaCl, it showed accumulation of salt which was evident by increase in the  $EC_e$  of soil. Plants subjected to both salt and water stress, exhibited

Table 2 (Cont'd)

Treatments (% NaCl)	Fresh weight (g)			Dry weight (g)			F.wt./ D.wt.
	Shoot	Root	Shoot/ Root	Shoot	Root	Shoot/ Root	
<b>SALT</b>							
0	18.733	3.066	5.900	2.480	0.996	2.281	7.160
	+8.383	+0.949	+1.217	+1.149	+0.372	+0.327	+1.452
0.25	57.400	7.800	7.440	7.160	3.380	2.100	6.240
	+20.916	+3.156	+0.950	+2.642	+1.294	+0.430	+0.299
0.50	21.166	3.830	5.284	2.560	1.627	1.470	6.150
	+7.194	+0.808	+0.752	+1.077	+0.472	+0.190	+0.299
0.75	7.900	1.350	5.890	1.090	0.791	1.400	4.910
	+0.300	+0.150	+4.336	+0.050	+0.135	+0.251	+0.015
1.00	15.200	2.500	6.380	2.070	0.961	2.400	5.640
	+5.577	+0.950	+0.554	+0.691	+0.363	+0.344	+0.267
LSD <sub>0.05</sub>	36.098	5.236	2.706	4.683	2.191	1.028	
<b>SALT x WATER</b>							
0	21.766	2.260	10.030	2.890	1.168	3.120	7.560
	+4.841	+0.676	+0.805	+0.739	+0.591	+0.716	+1.352
0.25	24.330	2.930	7.550	3.430	1.134	2.97	5.710
	+11.199	+0.731	+1.646	+1.647	+0.150	+1.202	+0.510
0.50	9.340	2.230	4.360	1.670	1.120	1.590	4.230
	+4.551	+0.120	+2.282	+0.143	+0.202	+0.289	+1.584
0.75	4.766	0.933	5.240	0.660	0.328	1.980	5.830
	+0.927	+0.120	+1.007	+0.142	+0.022	+0.354	+0.572
1.00	15.433	2.200	6.880	1.500	0.767	2.030	7.830
	+2.994	+0.305	+0.474	+0.219	+0.181	+0.182	+1.152
LSD <sub>0.05</sub>	18.007	1.487	4.413	2.578	0.941	2.090	

differences in the  $EC_e$  values at the first harvest from 0.5% NaCl. At the second harvest, again a significant accumulation of salts was evident as a result of saline water irrigation (Table 4). Though soil salinity increased at third harvest also, but total accumulation of salts was comparatively less as compared to first and second harvest period.

Total soluble salts showed a higher value at 1% NaCl salinity only in salt x water stressed plants. Little differences were observed in plants at different harvest periods (Table 4). At first harvest, the salt x water stressed plants, showed non-significant differences in TSS at various salinity levels except at 1% NaCl level. At the second harvest, non-significant variation was observed at 0.25% and 0.75% NaCl in the external medium. The pH of both salt and salt x water stressed plants did not exhibit any difference at all the salinity levels in all the harvest periods (Table 4).

Table 2 (Cont'd)

## C. HARVEST - III

Treatments (% NaCl)	Fresh weight (g)			Dry weight (g)			F.wt./ D.wt.
	Shoot	Root	Shoot/ Root	Shoot	Root	Shoot/ Root	
<b>SALT</b>							
0	35.330	8.130	4.050	10.200	2.700	3.690	5.020
	+12.498	+1.775	+0.784	+2.013	+0.305	+0.371	+1.855
0.25	35.600	17.560	3.000	7.430	4.600	2.170	4.040
	+14.837	+11.794	+0.727	+2.194	+2.542	+0.506	+0.499
0.50	23.660	7.160	3.360	5.300	2.400	2.270	4.020
	+2.316	+1.120	+0.198	+0.585	+0.450	+0.218	+0.142
0.75	12.660	2.960	4.290	3.660	1.360	2.710	3.030
	+2.868	+0.717	+0.058	+0.272	+0.166	+0.130	+0.419
1.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	0.000
LSD <sub>0.05</sub>	32.199	4.349	15.945	4.968	2.248	8.678	
<b>SALT x WATER</b>							
0	44.530	16.400	2.690	10.060	4.760	2.080	3.580
	+20.711	+6.993	+0.276	+3.909	+1.683	+0.218	+0.702
0.25	49.300	12.400	3.975	9.800	3.500	2.800	4.630
	+0.000	+0.000	+1.647	+0.000	+0.000	+0.000	+0.000
0.50	29.550	8.550	3.530	6.850	2.850	2.530	3.950
	+1.850	+1.050	+0.531	+0.050	+0.530	+0.559	+0.367
0.75	10.230	2.430	4.230	3.130	1.230	2.580	2.890
	+0.983	+0.290	+0.140	+0.185	+0.145	+0.200	+0.175
1.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
LSD <sub>0.05</sub>	22.447	2.801	19.950	6.320	3.570	1.203	

## Discussion

Increasing salinity of soil and water threatens crop productivity both in arid and semi-arid regions of the world either due to low internal water potential, ion toxicity and/or ion imbalance (Greenway & Munns, 1980). The degree to which each of these factors inhibit plant growth, depends on the plant genotype and environmental conditions. The most distinct effect of salinity on the plant is the reduction in growth primarily due to limited supply of metabolic energy for maintenance of normal growth process. Results related to functional growth analyses as well as those related to fresh and dry biomass showed a reduction in growth of sunflower with increase in salt concentration in the external media. Generally all type of stress including salinity increases the amount of work necessary to combat with osmotic and ionic stresses for



**Table 3. FANOVA for shoot and root biomass and its interaction with salt-water-stress and harvest periods.**

Source of variation	Sum of squares	df	Mean Squares	F-value
<b>Fresh Weight of Shoot</b>				
Salt Stress (S)	4730.60	4	1182.65	6.01***
Water Stress (W)	347.66	1	347.66	1.77 N.S.
Harvest (H)	6080.81	2	3040.41	15.46***
S x W	1197.41	4	299.35	1.52 N.S.
S x H	4944.07	8	618.01	3.14
W x H	265.16	2	132.58	0.67 N.S.
S x W x H	765.26	8	95.66	0.49 N.S.
Error	11797.78	60	196.63	
Total	30128.78	89		
<b>Dry Weight of Shoot</b>				
Salt Stress (S)	161.02	4	40.26	8.37***
Water Stress (W)	13.61	1	13.60	2.83 N.S.
Harvest (H)	270.11	2	135.06	28.09***
S x W	18.65	4	4.66	0.97 N.S.
S x H	195.56	8	24.45	5.09***
W x H	7.19	2	3.59	0.75 N.S.
S x W x H	13.40	8	1.67	0.35 N.S.
Error	288.45	60	4.81	
Total	967.99	89		
<b>Fresh Weight of Root</b>				
Salt Stress (S)	320.39	4	80.10	3.44*
Water Stress (W)	21.95	1	21.95	0.94 N.S.
Harvest (H)	536.34	2	268.17	11.51***
S x W	177.27	4	44.31	1.90 N.S.
S x H	384.23	8	48.09	2.06 N.S.
W x H	8.52	2	4.26	0.19 N.S.
S x W x H	209.46	8	26.18	1.12 N.S.
Error	1397.63	60	23.29	
Total	3055.80	89		
<b>Dry Weight of Root</b>				
Salt Stress (S)	78.20	4	19.55	4.93**
Water Stress (W)	20.24	1	20.24	5.11***
Harvest (H)	115.05	2	57.52	14.53***
S x W	11.70	4	2.92	0.74 N.S.
S x H	114.69	8	14.34	3.62
W x H	18.37	2	9.18	2.32 N.S.
S x W x H	7.53	8	0.94	0.23 N.S.
Error	237.52	60	3.96	
Total	603.30	89		

Table 4. Effect of salt and salt x water stress on some properties of the soil at the termination of the harvest periods.

Treatment (% NaCl)	Electrical Conductivity (dS.m <sup>-1</sup> )			Total Soluble Salts (g/L)			pH		
	Harvest	Salt Stress	Salt x Water Stress	Salt Stress	Salt x Water Stress	Salt x Water Stress	Salt Stress	Salt x Water Stress	Salt x Water Stress
0	I	1.800 ± 0.271	1.693 ± 0.629	0.810 ± 0.201	1.430 ± 0.429	8.450 ± 0.050	8.450 ± 0.050	8.320 ± 0.070	
	II	1.386 ± 0.421	1.553 ± 0.354	0.883 ± 0.263	0.993 ± 0.228	8.450 ± 0.050	8.450 ± 0.050	8.480 ± 0.200	
	III	1.030 ± 0.010	0.980 ± 0.118	0.680 ± 0.006	0.653 ± 0.083	7.710 ± 0.050	7.710 ± 0.050	7.800 ± 0.100	
0.25	I	3.420 ± 2.422	2.296 ± 0.532	2.200 ± 1.521	1.510 ± 0.320	8.460 ± 0.010	8.460 ± 0.010	8.430 ± 0.020	
	II	3.243 ± 0.600	3.053 ± 0.109	2.087 ± 0.388	1.973 ± 0.071	8.380 ± 8.160	8.380 ± 8.160	8.160 ± 0.200	
	III	1.610 ± 0.110	2.230 ± 0.264	1.070 ± 0.072	1.500 ± 0.168	7.380 ± 0.150	7.380 ± 0.150	7.710 ± 0.100	
0.50	I	4.873 ± 1.047	5.060 ± 0.142	3.573 ± 0.661	3.300 ± 0.092	8.510 ± 0.010	8.510 ± 0.010	8.520 ± 0.110	
	II	6.530 ± 1.089	4.380 ± 1.630	4.080 ± 0.638	2.786 ± 1.006	7.250 ± 0.150	7.250 ± 0.150	7.570 ± 0.810	
	III	1.210 ± 0.782	1.620 ± 0.325	0.803 ± 0.520	1.080 ± 0.216	7.810 ± 0.030	7.810 ± 0.030	7.630 ± 0.040	
0.75	I	6.167 ± 0.981	5.217 ± 1.126	3.960 ± 0.666	3.446 ± 0.780	8.580 ± 0.030	8.580 ± 0.030	8.650 ± 0.020	
	II	8.697 ± 1.253	9.043 ± 0.883	5.577 ± 0.833	5.683 ± 0.661	7.930 ± 0.060	7.930 ± 0.060	8.320 ± 0.040	
	III	1.810 ± 0.440	2.510 ± 0.433	1.200 ± 0.208	1.444 ± 0.082	7.930 ± 0.060	7.930 ± 0.060	7.850 ± 0.090	
1.00	I	6.197 ± 1.791	10.167 ± 0.354	3.776 ± 1.151	6.287 ± 0.913	8.500 ± 0.800	8.500 ± 0.800	8.530 ± 0.040	
	II	10.850 ± 1.448	10.973 ± 1.537	7.167 ± 0.898	7.610 ± 1.088	8.400 ± 0.050	8.400 ± 0.050	8.350 ± 0.030	
	III	4.080 ± 0.543	6.640 ± 0.753	4.230 ± 0.565	4.630 ± 1.560	7.810 ± 0.080	7.810 ± 0.080	7.083 ± 0.030	

normal cellular maintenance and as a consequence, there is less energy left for growth requirements (Nieman & Maas, 1978).

The RGR in *H. annuus* decreased with increase in salt levels at all the harvest periods irrespective of salt and salt  $\times$  water stress. Due to increase in the photosynthetic efficiency of the plant as evident by reduced NAR, decrease in RGR can be attributed to reduced leaf area, decrease in synthesis of primary and secondary metabolites and their utilization for growth. Differences in RGR at different salinity treatments and harvest periods may be due to difference in net assimilation rate, which is largely the balance in the rate of photosynthesis and that of respiration in the entire plant. Values of NAR are dependent on different environmental factors including light intensity (Waring *et al.*, 1985; Hiroi & Monsi, 1963, Evans & Hughes, 1961), nitrogen supply (McDonald, 1989; Sage & Percy, 1987), as well as species (Konings *et al.*, 1989). Differences in RGR may not necessarily be due to variation in NAR. Such difference may also arise from the relative amount of biomass a plant invest in the leaf area. Value of LAR vary widely in species (Koning, 1989). Biomass production which is a function of carbon fixation in photosynthesis is determined by the rate of photosynthesis per leaf area, and the area of leaf surface (Terry & Waldron, 1984) and is affected in the glycophytes by any of these two factors. This may also be due to decrease in leaf area and increase in leaf thickness thus reducing area for light interception and carbon-dioxide fixation.

Salt accumulation in the soil as a consequence of use of saline water is frequently observed. The extent of salt accumulation depends upon the quality and quantity of water used; physiological age of the plant; and a number of environmental factors specially temperature and humidity. Plants grown in soil having both salt and salt  $\times$  water stress exhibited accumulation during the first two months at first and second harvest. Significant reduction was observed in pots harvested after three months at all the salinity levels. This period coincided with moderate climatic conditions thus reducing salt accumulation. It would suggest that both salt and water stress effects the normal growth and metabolism of sunflower plants. Where salt stress is associated with water stress, there is additional stress to the plant and hence more diversions of energy. However, plant species/cultivars which are drought tolerant are able to cope with the water stress to some extent.

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