

RESEARCH ARTICLE

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Biofertilizers as agent against the harmful effect of salinity on wheat

ABSTRACT:

The objective of this study was to investigate the ability of Biogen and Cyanobacteria as biofertilizers for substitution of normally used chemical fertilizer (Urea), and to ameliorate the harmful effect induced by salinity on two wheat cultivars Sakha 93 (salt tolerant) and Gemiza 10 (salt sensitive). Results showed that application of two salinity levels (100 and 200 mM NaCl) caused reductions in the germination percentage and the growth parameters (plant height, No. of leaves/plant, leaf area, No. of internodes, fresh and dry weights of shoot and root/plant) and in the rate of different metabolic processes (photosynthetic activity, total carbohydrates and protein content) and most yield parameters (plant height, No. of spike/m², spike length, No. of grain/spike, grain weight/spike, 1000-grain weight, carbohydrates and protein content). On the other hand, application of each biofertilizers significantly ameliorated the harmful effect induced by salinity on both wheat cultivars. However, the ameliorative effect was more significant with biogen than cyanobacterial fertilizers for both cultivars. In addition, the application of both biofertilizers could reduce the recommended dose of urea by about 50%. The ameliorative effect could be attributed to changes in the antioxidant enzyme activities and indicated by reduction of the phenolic compounds in response to the different biofertilizer treatment. Accordingly, we recommend the use of the two biofertilizers for partial substitution (50%) of the normally used chemical fertilizer (urea). Furthermore, we recommend the use of both biofertilizer for the salty land (100-200 mM NaCl).

KEY WORDS:

Biofertilizers, salinity, wheat

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INTRODUCTION:

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of crop land in the world is salt-stressed. Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. Also, it has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011).

Generally, salinity induces metabolic changes related to protein turnover (alterations in protein synthesis, maintaining the level of some proteins or protein degradation) (Demirevska *et al.*, 2008). Salinity affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition, stomata closure with consecutive reduction of transpiration, decrease in chlorophyll content

and inhibition of photosynthesis and protein changes (Yordanov *et al.*, 2003) to cope with osmotic changes in their tissues.

However, several investigations reported that chemical fertilizer could be harmful to human health, animals, plant and soil as it contaminates ground water and surface water. In addition, it acts as short supply and expensive charges for the agricultural products. Also, saline sodic soils after reclamation become infertile due to leaching of most of the nutrients along with salts from the rooting medium. However, the overuse of chemical fertilizers in farm lands is a major contributor to environmental problems such as ground water eutrophication, nitrate pollution, phosphate pollution, and others (Dat *et al.*, 2010; Mahmoud and Abd EL-Kader, 2012).

Due to increasing human health concerns and pressure in meeting stringent consumer standards, agricultural practices are shifting toward the development of nitrogen (N) fertilizers that are environmentally friendly, possess high N utilization efficiency, and can sustain crop production. Several strategies have been proposed to reduce N losses such as the use of biofertilizer. Therefore, biofertilizers are cheaper, fast, consume less energy than industrial process and appropriately effective (Al-Kasas, 2002) and reduce the environmental pollution from the high concentrations of chemicals and toxic NO^{-3} (Hammad, 1996). The additional advantages of biofertilizers include longer shelf life causing no adverse effects to ecosystem (Sahoo *et al.*, 2014). Biofertilizers could be generally defined as media containing microbial strains, which are efficient in fixing atmospheric nitrogen, solubilizing phosphate or decomposing plant cellulosic cells. Inoculating the soil or seeds in such a prepared media would increase the population of corresponding microorganism in the soil. Also, Cyanobacteria are oxygenic, photosynthetic prokaryotic organisms that are distributed worldwide and can inhabit a wide range of habitats including freshwater, marine and terrestrial environments (Pankratova, 2006; Nagarajan *et al.*, 2012; Whitton, 2012). In agricultural soils, they potentially contribute towards biological nitrogen fixation that improves soil fertility and crop productivity. Although, most of cyanobacteria that fix nitrogen are heterocystous (Granhall and Henriksson, 1969), non-heterocystous cyanobacteria can fix nitrogen as well (Kallas *et al.*, 1983).

This study aimed to evaluate the use of two biofertilizers (cyanobacteria or biogen), for substitution of normally used chemical fertilizer; urea, for two wheat cultivars Sakha 93 (salt tolerant) and Gemiza 10 (salt sensitive).

MATERIAL AND METHODS:

Pot experiment was carried out in Botany Department, Faculty of Science, and Tanta University in 2011 and 2012. Wheat grains were obtained from wheat research section, Agriculture Research Centre Egypt. Two biofertilizers were applied:

The first commercial biofertilizer (cyanobacteria) at its recommended dose (500 g/fed.) was applied at the beginning of cultivation. The most dominant cyanobacterial species in this biofertilizer were *Nostoc entophyllum*, *Nostoc muscorum*, *Anabaena variabilis*, *Oscillatoria acutissima*, and *Phormidium* sp.

The second commercial biofertilizer (Biogen) at its recommended dose (250 g/fed.) was applied also at the beginning of cultivation. The most dominant species in biofertilizer was *Azotobacter* sp.

Also, Nitrogen fertilizer (urea) was applied for all treatments in the field at half amount of the recommended dose used by the farmers.

Both cultivars were cultivated in plastic pots (44 x 105 cm) having depth of 40 cm and contains 58Kg of clay soil. The pots were left in an open greenhouse under the normal environmental condition of light and temperature. The plants were irrigated by the selected concentrations of NaCl (100 and 200 mM) once at the planting, after 15 days they were then irrigated again using water only. The experiments were carried out in randomize complete block design (RCBD) with three replicates taken at 70 days from planting.

Chemical analysis:

Leaf chlorophyll fluorescence as expression for photosynthetic activity was determined for each treatment at 70-days after sowing using Fluorometer (OS-30, Opti-Science, Inc. USA) in three plants by the formula of Maxwell and Johnson (2000).

Total carbohydrates in grains were determined as described by Nelsons (1944) method and modified by Naguib (1963).

Protein content was determined according to the method of Bradford (1976).

Phenolic content was carried out according to Jindal and Singh (1975).

Two antioxidant enzymes catalase and peroxidase (involved in scavenging of the reactive oxygen species) were assayed according to Kato and Shimizu (1978).

Lipid peroxidation was measured by determination the amount of malondialdehyde (MDA), as a product of peroxidation of unsaturated fatty acids. MDA concentration was estimated by the method of Heath and Packer (1968).

Data were statistically analysed as described by Snedecor and Cochran (1980) and the means were compared using L.S.D. test at 5% significance level. Treatment means were compared according to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION:

Preliminary experiment:

The experiments were conducted in Petri dishes to show the effects of different concentration of NaCl (0, 50, 100, 150, 200, 250, 300, 400, and 500 mM) on germination percentage of eight cultivars of wheat grains (Seds 1, Sakha 61, Sakha 93, Sakha 94, Giza 168, Gemiza 7, Gemiza 9, and Gemiza 10) at 6 days.

Data in table 1 show the effect of different levels of salinity from (0–500 mM

Table 1. Effect of different salinity concentrations on germination percentage of eight cultivars at 6- day old of wheat grains

Cultivar NaCl conc.	Seds 1 %	Sakha 61 %	Sakha 93 %	Sakha 94 %	Giza 168 %	Gemiza 7 %	Gemiza 9 %	Gemiza 10 %
0 mM	100	90	100	100	90	80	80	80
50 mM	80	100	100	90	90	70	60	40
100 mM	50	80	100	80	70	50	60	40
150 mM	50	70	90	80	60	40	40	30
200 mM	40	50	100	80	60	40	30	20
250 mM	20	40	80	0	20	10	0	0
300 mM	0	20	30	0	10	10	0	0
400 mM	0	0	10	0	0	0	0	0
500 mM	0	0	0	0	0	0	0	0

Growth Parameter:

Results presented in tables 2 and 3 shows that salinity affected all growth parameters. The highest significant reduction was observed at 200 mM NaCl and 100 mM NaCl as well for both cultivars.

Data show that reductions by about 14.6%, 4.8%, 6 %, and 5.7% in plant height, no. of leaves/plant, leaf area/plant and no. of internodes/plant in Sakha 93, respectively.

NaCl) on germination percentage measured in 6- day old of grains of eight wheat cultivars. Results show that increasing NaCl concentrations caused reductions in germination percentage. The cultivar, Sakhs 93 recorded the highest germination percentage, 80% at 250 mM NaCl while Gemiza 9 and Gemiza 10 recorded the least, 30% and 10% at 200 and 150 mM NaCl concentrations respectively. This indicates that Sakha 93 is the tolerant cultivar and Gemiza 10 is the sensitive one. These results may suggest that salinity treatment caused reduction in the osmotic potential of the solution, resulting in delaying time of imbibition of the seeds and, consequently, of seedling emergency as reported by Ashraf and Ashraf (2012).

Also, there was a reduction by about 3.34%, 22.2%, and 25% in plant height, no. of leaves and leaf area and no reduction in no. of internodes observed in Gemiza 10, respectively. Also, data indicate that the highest significant reduction in fresh and dry weights was observed with 200 mM NaCl, as well for both cultivars by about 19.4%, 25.1%, 16.1%, and 16.9%, respectively, compared to control.

Table 2. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on some growth criteria of 70-day old wheat (Sakha 93) cultivated under salinity stress

Treatments	Stem length (Cm)	Root length (Cm)	Plant height (Cm)	No. of leaves/plant	Leaf area/plant (Cm ²)	No. of internodes
Control	57.6 ^f	6.3 ^d	63.9 ^f	6.3 ^d	18.2 ^d	5.3 ^{cd}
100 mM NaCl	53.1 ^g	6 ^{de}	59.1 ^g	6 ^d	17.5 ^e	5 ^d
200 mM NaCl	49 ^h	5.6 ^e	54.6 ^h	6 ^d	17.1 ^e	5 ^d
Urea (100%)	62.6 ^e	7 ^c	69.6 ^e	7.6 ^c	18.4 ^d	6.3 ^{abc}
Cyanobacteria (500 g/fed.)	65 ^c	7 ^c	72 ^d	9 ^b	18.9 ^d	6.6 ^{ab}
Cyanobacteria +100 mM NaCl	63 ^e	8.3 ^b	71.3 ^d	8.6 ^{bc}	18.9 ^d	6.6 ^{ab}
Cyanobacteria + 200 mM NaCl	65.3 ^c	8 ^b	73.3 ^c	8.6 ^{bc}	19.6 ^c	6.6 ^{ab}
Biogen (250 g/fed.)	64 ^d	7 ^c	71 ^d	8.6 ^{bc}	18.6 ^d	6.3 ^{abc}
Biogen + 100 mM NaCl	67 ^b	9 ^a	76 ^b	9.6 ^{ab}	21.6 ^b	6.6 ^{ab}
Biogen + 200 mM NaCl	68.3 ^a	9 ^a	77.3 ^a	10 ^a	23.3 ^a	7.3 ^a
F – value	1.132**	3.272*	1.837**	1.188**	0.426**	2.052*
L. S. D	0.641	0.346	0.721	0.760	0.587	0.788

Table 3. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on some growth criteria of 70-day old wheat (Gemiza 10) cultivated under salinity stress

Treatments	Stem length (Cm)	Root length (Cm)	Plant height (Cm)	No. of leaves/plant	Leaf area/plant (Cm ²)	No. of internodes
Control	51.5 ^g	8.25 ^d	59.75 ^g	9 ^g	17.1 ^h	5 ^c
100 mM NaCl	53 ^e	6.5 ^e	59.5 ^h	7 ^h	13.45 ⁱ	5 ^c
200 mM NaCl	51.25 ^h	6.5 ^e	57.75 ⁱ	7 ^h	12.83 ^j	5 ^c
Urea (100%)	51.75 ^f	8.25 ^d	60 ^f	13 ^f	17.13 ^g	6 ^b
Cyanobacteria (500 g/fed.)	59 ^c	9 ^c	68 ^c	16 ^d	25.63 ^d	7 ^a
Cyanobacteria +100 mM NaCl	57 ^d	10 ^a	67 ^d	15 ^e	23.68 ^e	7 ^a
Cyanobacteria +200 mM NaCl	59 ^c	9 ^c	68 ^c	17 ^c	26 ^c	7 ^a
Biogen (250 g/fed.)	57 ^d	9 ^c	66 ^e	15 ^e	17.43 ^f	7 ^a
Biogen + 100 mM NaCl	63.75 ^b	9.75 ^b	73.5 ^b	18 ^b	28.68 ^b	7 ^a
Biogen + 200 mM NaCl	68.50 ^a	9.75 ^b	78.25 ^a	20 ^a	32.66 ^a	7 ^a
F – value	4.448**	3.885**	4.448**	1.490**	0.583**	7.165**
L. S. D	9.737	0.006	0.004	3.989	0.002	3.614

Generally, increasing salinity levels causes a decrease in plant growth. The present results indicated that the most marked decreases were obtained at 200 mM NaCl which reduced growth parameters of wheat plants. The reduction was greater in, Gemiza 10 (salt sensitive) than in, Sakha 93 (salt tolerant). Similar data were obtained by Mansour *et al.* (2005). Also, salt in the soil solution may inhibit plant growth for two reasons. First, the presence of salt in the soil solution it reduces the ability of the plant to take up water, and this leads to reductions in the growth rate. This is referred to the osmotic or water-deficit effect of salinity. Second, if excessive amounts of salt enter the plant in the transpiration stream there will be injury to cells in the transpiring leaves and

this may cause further reductions in growth (Singh and Chatrath, 2001).

The highest significant increase in plant height, no. of leaves, leaf area and no. of internodes was observed with biogen treatments (250 g/fed) plus 200 mM NaCl by about 1.2, 1.6, 1.3, and 1.4-fold in Sakha 93 and by about 1.3, 2.2, 1.9, and 1.4-fold in Gemiza 10, respectively.

The effect of fertilization treatments (cyanobacteria, biogen, and urea) on total fresh and dry weights of the 70- day old wheat plant (Sakha 93 and Gemiza 10) is presented in tables 4 and 5. The results show that the maximum values of total fresh and dry weights were (1.7 and 1.5) and (1.8 and 2.3) fold for both cultivars, respectively, it was observed in case of biogen treatment (250 g/fed) plus 200 mM NaCl.

Table 4. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on fresh and dry weights (g) of 70-day old wheat (Sakha 93) cultivated under salinity stress

Treatments	Fresh weight (g) / plant				Dry weight (g) / plant			
	Shoot system		Root system	Total weight	Shoot system		Root System	Total Weight
	Stem	Leaves			Stem	Leaves		
Control	3.522 ^h	4.556 ^e	2.289 ^h	10.367 ^e	2.515 ^h	3.106 ^h	1.691 ^g	7.312 ^h
100 mM NaCl	2.757 ⁱ	4.243 ^{ef}	2.031 ⁱ	9.031 ^f	2.249 ⁱ	2.897 ⁱ	1.498 ^h	6.232 ⁱ
200 mM NaCl	2.561 ^j	3.973 ^f	1.819 ^j	8.353 ^g	1.837 ^j	2.563 ^j	1.339 ⁱ	5.476 ^j
Urea (100%)	4.384 ^g	5.323 ^d	3.011 ^g	12.718 ^d	2.533 ^g	3.389 ^g	1.273 ^f	7.871 ^g
Cyanobacteria (500 g/fed.)	5.117 ^e	6.623 ^{ab}	3.187 ^d	14.917 ^c	2.670 ^e	4.354 ^d	2.257 ^d	9.281 ^d
Cyanobacteria +100 mM NaCl	5.291 ^d	6.455 ^b	3.042 ^f	14.786 ^c	2.770 ^d	3.673 ^f	2.232 ^f	8.675 ^f
Cyanobacteria +200 mM NaCl	4.777 ^f	6.875 ^a	3.275 ^c	14.927 ^c	2.593 ^f	4.452 ^c	2.331 ^c	9.376 ^c
Biogen (250 g/fed.)	5.729 ^b	5.874 ^c	3.053 ^e	14.656 ^c	2.804 ^c	3.986 ^e	2.244 ^e	9.034 ^e
Biogen + 100 mM NaCl	5.467 ^c	6.888 ^a	3.434 ^a	15.789 ^b	3.127 ^a	4.456 ^b	2.450 ^a	10.033 ^b
Biogen + 200 mM NaCl	5.953 ^a	6.981 ^a	3.427 ^b	16.361 ^a	2.933 ^b	4.794 ^a	2.445 ^b	10.172 ^a
F – value	0.991**	1.000**	4.797**	1.825**	6.078**	0.113**	0.310**	3.771**
L. S. D	0.126	0.313	0.002	0.324	0.001	0.001	0.001	0.002

Biofertilizer could adverse the inhibitory effect of salinity on plant growth and yield because it has the ability to improve soil fertility as shown by Osman *et al.* (2010).The

use of cyanobacteria fertilizers is a promising alternative to avoid soil pollution caused by agrochemicals and recovering the nutrient content and structure lost after harvest as

they bring to soil combined N (some of them are N fixers), exopolysaccharide that improve soil structure and bioactive substances that enhance seedlings growth (Zaccaro *et al.*, 1999). In this connection, Gaballah *et al.* (2014) showed that cyanobacteria can improve several growth parameters in inoculated wheat plants by modifying their

endogenous phytohormones. Inoculation with cyanobacteria stimulated the accumulation of endogenous cytokinins and IAA (indole acetic acid) in wheat seedlings. Also, cyanobacteria undergo intimate associations with the roots of wheat and stimulate its growth under saline conditions (Table 5).

Table 5. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on fresh and dry weights (g) of 70-day old wheat (Gemiza 10) cultivated under salinity stress

Treatments	Fresh weight (g) / plant				Dry weight (g) / plant			
	Shoot system		Root system	Total weight	Shoot system		Root System	Total Weight
	Stem	Leaves			Stem	Leaves		
Control	3.570 ^g	4.060 ^g	2.115 ^g	9.745 ^h	2.470 ^h	2.316 ^g	1.711 ^g	6.497 ^h
100 mM NaCl	2.990 ^h	3.616 ^h	2.032 ^h	8.638 ⁱ	2.119 ⁱ	2.125 ^h	1.516 ^h	5.760 ⁱ
200 mM NaCl	2.860 ⁱ	3.506 ⁱ	1.815 ⁱ	8.181 ^j	1.969 ^j	2.078 ⁱ	1.351 ⁱ	5.398 ^j
Urea (100%)	3.905 ^f	4.060 ^g	2.975 ^f	10.940 ^g	2.713 ^g	2.316 ^g	2.233 ^f	7.262 ^g
Cyanobacteria (500 g/fed.)	6.162 ^e	5.737 ^d	3.132 ^d	15.031 ^d	4.180 ^e	3.037 ^d	2.352 ^d	9.569 ^d
Cyanobacteria + 100 mM NaCl	6.303 ^d	5.356 ^e	3.012 ^e	14.671 ^e	4.272 ^d	2.873 ^e	2.261 ^e	9.406 ^e
Cyanobacteria+200 mM NaCl	6.519 ^c	5.916 ^c	3.181 ^c	15.616 ^c	4.412 ^c	3.114 ^c	2.390 ^c	9.916 ^c
Biogen (250 g/fed.)	6.135 ^e	4.389 ^f	3.011 ^e	13.537 ^f	4.163 ^f	2.457 ^f	2.260 ^e	8.880 ^f
Biogen + 100 mM NaCl	6.571 ^b	6.111 ^b	3.201 ^b	15.883 ^b	4.466 ^b	3.198 ^b	2.405 ^b	10.069 ^b
Biogen + 200 mM NaCl	6.853 ^a	6.342 ^a	3.211 ^a	16.406 ^a	4.529 ^a	3.297 ^a	2.412 ^a	10.238 ^a
F – value	1.353 ^{**}	11.377 ^{**}	1.206 ^{**}	1.777 ^{**}	0.264 ^{**}	3.100 ^{**}	2.302 ^{**}	2.187 ^{**}
L. S. D	0.028	0.001	0.001	0.029	0.002	0.001	0.001	0.002

* indicates a significant difference at $P \leq 0.05$

** indicates a significant difference at $P \leq 0.01$

- Means within the same column for each factor designated by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Photosynthetic activity:

Results presented in figure 1 show that salt stresses caused reduction in chlorophyll fluorescence in response to 200 mM NaCl treatments in both wheat cultivars (Sakha 93 and Gemiza 10) by about 7.8% and 31.1%, respectively, compared with control. Results show also that biogen treatment (250 g/fed) plus 200 mM NaCl induced the maximum increase in photosynthetic activity (24.2% and 65.6%) measured as fluorescence (Fv/Fm) of 70-day old leaves of two wheat cultivars

(Sakha 93 and Gemiza 10) respectively, compared with control. This increase in photosynthetic activity could be attributed to enhanced chlorophyll formation (Haroun and Hussein, 2003) induced by biofertilizers which cause increase in the photosynthetic electron transport (Bograh et al., 1997), leading to an increase in photosynthetic activity (Zheleva et al., 1994), besides an effect on the stomata and the photosynthetic apparatus (Singh, 2014).

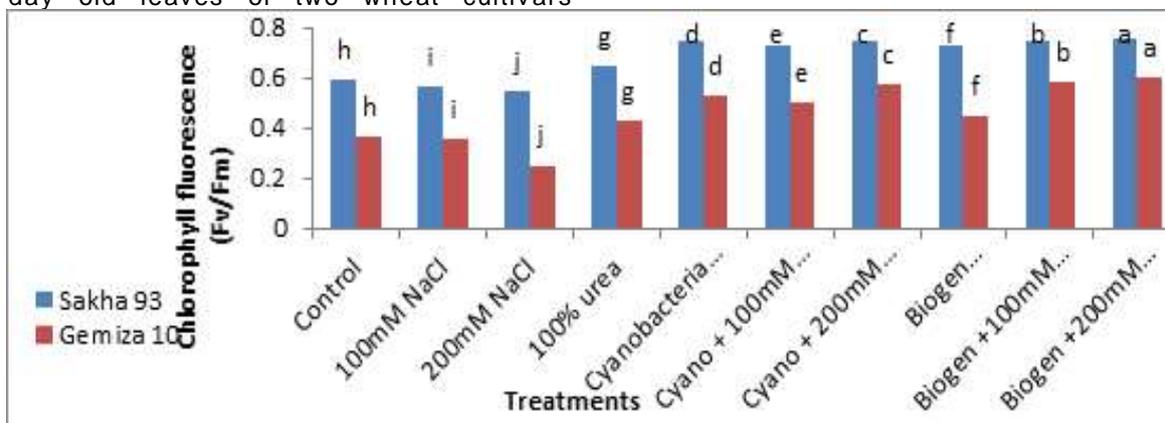


Fig. 1. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on photosynthetic activity measured as fluorescence (Fv/Fm) of leaves at 70-day old of two wheat cultivars Sakha 93 and Gemiza 10 cultivated under salinity stress.

Fv: Variable fluorescence, Fm: Maximum fluorescence, each column represents the mean value of three replicates, Different letters represent significant difference at $P \leq 0.05$ (Duncan's).

Carbohydrate content:

Results present in table 6 show that addition of biogen (250 g/fed) plus 200 mM NaCl induced the maximum level of total carbohydrate which increased by about 1.8-fold compared to control. Direct reducing sugar, sucrose and polysaccharides (starch)

increased in shoot system by about 1.4, 2.2, and 2.1-fold in Sakha 93.

Data show that the lowest reduction in the total carbohydrates was detected upon the addition of 200 mM NaCl which decreased by about 21.6% compared to control.

Table 6. Effect of fertilization treatments (Cyanobacteria, Biogen, and Urea) on carbohydrates and protein content (mg/g.d.wt) estimated in shoot of the 70-day old wheat (Sakha 93) cultivated under salinity stress

Treatments	Shoot system (mg/g.d.wt)				
	Direct reducing sugar	Sucrose	Polysaccharide (starch)	Total carbohydrates	Total soluble Protein
Control	3.261 ⁱ	2.406 ^g	1.841 ^h	7.508 ^h	48.66 ⁱ
100 mM NaCl	3.380 ^g	2.275 ⁱ	1.562 ⁱ	7.217 ⁱ	50.87 ⁱ
200 mM NaCl	2.166 ^j	2.395 ^h	1.324 ^j	5.885 ^j	55.04 ^h
Urea (100%)	3.357 ^h	1.767 ^j	2.515 ^g	7.639 ^g	69.32 ^g
Cyanobacteria (500g/fed.)	3.463 ^f	5.145 ^b	2.663 ^e	11.270 ^d	70.46 ^f
Cyanobacteria +100 mM NaCl	3.795 ^e	3.895 ^d	2.656 ^f	10.350 ^e	73.46 ^d
Cyanobacteria + 200 mM NaCl	4.501 ^c	3.187 ^e	3.674 ^b	11.360 ^c	74.38 ^c
Biogen (250 g/fed.)	3.928 ^d	3.145 ^f	2.819 ^d	9.892 ^f	71.85 ^e
Biogen +100 mM NaCl	4.589 ^b	4.718 ^c	3.542 ^c	12.850 ^b	75.76 ^b
Biogen + 200 mM NaCl	4.676 ^a	5.212 ^a	3.783 ^a	13.670 ^a	76.22 ^a
F – value	0.473*	1.328*	0.018***	0.008***	0.310**
L. S. D	0.005	0.005	0.005	0.005	0.005

Table 7 shows that maximum level of total carbohydrate was obtained by the addition of Biogen (250 g/fed) to 200 mM NaCl salinized plant which increased by about 1.8fold. Direct reducing sugars, sucrose and polysaccharides (starch) were increased in shoot system by about 2.6, 1.5, and 1.2-fold in Gemiza 10. Also, the lowest reduction in the total soluble carbohydrates was detected upon the addition of 200 mM NaCl which

caused reduction by about 16.3% compared to control. These increases may be presumably since biofertilizer is involved directly or indirectly in saccharides and nitrogen metabolism (Saswati and Radha, 2004). Also, carbohydrate can play a molecular role for sugar responsible genes that give different physiological responses like defensive response and cellular expansion (Simaei *et al.*, 2012).

Table 7. Effect of fertilization treatments (Cyanobacteria, Biogen, and Urea) on carbohydrates and protein content (mg/g.d.wt) estimated in shoot system of the 70-day old wheat (Gemiza 10) cultivated under salinity stress

Treatments	Shoot system (mg/g.d.wt)				
	Direct reducing sugar	Sucrose	Polysaccharide (starch)	Total carbohydrates	Total soluble Protein
Control	2.011 ⁱ	2.146 ^h	2.324 ^g	6.481 ^h	42.420 ⁱ
100 mM NaCl	2.186 ^g	1.676 ^j	2.067 ⁱ	5.929 ⁱ	43.454 ⁱ
200 mM NaCl	1.485 ^j	1.813 ⁱ	2.128 ^h	5.426 ^j	48.283 ^h
Urea (100%)	2.157 ^h	2.541 ^g	2.877 ^d	7.575 ^g	57.939 ^g
Cyanobacteria (500 g/fed.)	3.128 ^e	3.156 ^e	2.930 ^a	9.214 ^d	59.319 ^f
Cyanobacteria +100 mM NaCl	3.238 ^d	2.794 ^f	2.568 ^f	8.600 ^e	63.917 ^d
Cyanobacteria + 200 mM NaCl	3.613 ^c	3.493 ^a	2.919 ^b	10.025 ^c	64.377 ^c
Biogen (250 g/fed.)	2.223 ^f	3.167 ^d	2.865 ^e	8.225 ^f	62.078 ^e
Biogen + 100mM NaCl	4.053 ^b	3.343 ^b	2.880 ^c	10.276 ^b	65.527 ^b
Biogen + 200mM NaCl	5.298 ^a	3.267 ^c	2.879 ^{cd}	11.444 ^a	73.114 ^a
F – value	2.007*	1.551*	0.536**	3.768**	2.632**
L. S. D	0.002	0.002	0.002	0.003	0.002

* indicates a significant difference at $P \leq 0.05$

** indicates a significant difference at $P \leq 0.01$

- Means within the same column for each factor designated by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Protein contents:

Table 6 shows that addition of biogen (250 g/fed) increased protein content in 70-day old wheat plants. The maximum value of total soluble proteins was detected by the addition of biogen (250 g/fed) to 200 mM NaCl salinized plant as it increased by 1.6 times in Sakha 93 compared to control. On the other hand, the lowest increase of total soluble proteins was observed in 200 mM NaCl treated plant as it increased by 1.3-fold compared to control. Also, addition of biogen (250 g/fed) or cyanobacteria (500 g/fed.) increased proteins content in the 70-day old wheat plants (Table 7). Results show that the maximum value of total soluble protein content was detected by the addition of biogen (250 g/fed) to 200 mM NaCl salinized plant as it increased by 1.7-fold in Gemiza 10 compared to control. The lowest increase of total soluble proteins was observed in 200 mM NaCl treated plants as it increased by 1.1-fold compared to the control. Such increase in protein content may be explained on the basis that nitrogen can play a prominent role in building protein structure and could activate some enzymatic reaction related to protein metabolism (El-Kalla *et al.*, 2002; Zeidan and Nofal, 2002).

**120- day old plant: (Yield components):
Growth parameters:**

Results present in tables 8 and 9 shows that both concentrations of NaCl reduced all yield parameters. The highest significant reduction was observed in plants

salinized with 200 mM NaCl for both cultivars. However, treatment with different biofertilizers ameliorated the harmful effect of salinity on different yield parameters. The highest ameliorating effect was observed with biogen treatment of soil salinized with 200 mM NaCl for both wheat cultivars. Results show increases in the different yield parameters by about 1.3, 1.4, 1.2, 1.7, 1.4, and 1.7-fold in plant height, No. of spikes/m², spike length, No. of grains/spike, grains weight/spike and 1000-grain weight in Sakha 93, respectively in response to biogen treatment. Also, there was an increase by about 1.3, 1.4, 1.1, 1.8, 1.3, and 1.7-fold in plant height, no. of spikes/m², spike length, No. of grains/spike, grains weight/spike and 1000-grain weight observed in Gemiza 10, respectively, compared to control. These results may be attributed to more uptakes of nutrients which in turn reflected on more growth activity, nitrogenous compounds assimilation, forming more growth substances, more cell division and enlargement, more growth of tissues and organs and plant elongation. These results are in harmony with those obtained by Abd El-Hady *et al.* (2006). Also, biofertilizers have increased the fertilizer use efficiency of the crop plants and reduced the loss of nitrogen fertilizer as shown by Carreres *et al.* (1996). The increase in 1000- grain weight might be attributed to the favourable effect of biofertilizer on the development and maturation of grains, leading to larger grains, (El-Hag, 2008; Koriem, 2008).

Table 8. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on some yield characters of wheat (Sakha 93) cultivated under salinity stress

Treatments	Plant Height (Cm)	No. of spikes/m ²	Spike length (Cm)	No. of grains/spike	Grains weight/spike (g)	1000-grain weight (g)
Control	88.71 ^h	208 ^h	9.89 ^h	41.33 ^f	2.349 ^g	33.51 ^h
100 mM NaCl	79.23 ⁱ	192 ⁱ	9.11 ⁱ	36.33 ^g	2.308 ^g	29.85 ⁱ
200 mM NaCl	70.25 ^j	186 ^j	8.93 ^j	31.66 ^h	2.209 ^h	28.77 ^j
Urea (100%)	103.66 ^g	255 ^g	10.59 ^g	52.33 ^e	2.422 ^f	38.23 ^g
Cyanobacteria (500 g/fed.)	108.64 ^d	275 ^d	10.88 ^d	60.66 ^c	2.874 ^d	48.56 ^d
Cyanobacteria + 100 mM NaCl	107.13 ^e	267 ^e	10.76 ^e	57.66 ^d	2.793 ^e	47.26 ^e
Cyanobacteria + 200 mM NaCl	111.43 ^c	278 ^c	11.52 ^c	64.33 ^b	2.991 ^c	51.39 ^c
Biogen (250 g/fed.)	105.26 ^f	263 ^f	10.68 ^f	56.66 ^d	2.802 ^e	45.83 ^f
Biogen + 100 mM NaCl	115.22 ^b	281 ^b	11.87 ^b	67.66 ^a	3.100 ^b	54.63 ^b
Biogen + 200 mM NaCl	115.61 ^a	285 ^a	12.3 ^a	68.66 ^a	3.275 ^a	56.88 ^a
F – value	0.438**	0.661**	4.243**	0.032**	0.998**	1.140**
L. S. D	0.029	2.405	0.010	1.743	0.056	0.026

Table 9. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on some yield characters of wheat (Gemiza 10) cultivated under salinity stress

Treatments	Plant Height (Cm)	No. of spikes/m ²	Spike length (Cm)	No. of grains/spike	Grains weight/spike (g)	1000-grain weight (g)
Control	84.43 ^g	204 ^h	9.56 ^g	38.66 ^{bc}	2.251 ^{cd}	30.06 ^h
100 mM NaCl	75.56 ^h	188.33 ⁱ	8.87 ^h	34.33 ^c	2.168 ^d	28.56 ⁱ
200 mM NaCl	70.36 ⁱ	182.33 ^j	8.73 ⁱ	29.66 ^c	2.110 ^d	26.49 ^j
Urea (100%)	96.52 ^f	248.66 ^j	10.21 ^f	48.66 ^b	2.396 ^{bc}	32.12 ^g
Cyanobacteria (500 g/fed.)	101.88 ^d	266.33 ^d	10.42 ^d	61.33 ^a	2.791 ^a	44.60 ^d
Cyanobacteria+ 100 mM NaCl	100.73 ^e	255 ^e	10.41 ^d	46.66 ^b	2.429 ^{bc}	39.43 ^e
Cyanobacteria + 200 mM NaCl	102.55 ^c	268.33 ^c	10.66 ^c	63.33 ^a	2.888 ^a	45.62 ^c
Biogen (250 g/fed.)	100.58 ^e	251.33 ^f	10.37 ^e	58.66 ^a	2.563 ^b	36.71 ^f
Biogen + 100 mM NaCl	108.60 ^b	274.66 ^b	10.84 ^b	66.33 ^a	2.960 ^a	49.19 ^b
Biogen + 200 mM NaCl	110.06 ^a	277.66 ^a	10.88 ^a	67.66 ^a	2.973 ^a	52.04 ^a
F – value	0.858**	2.007**	0.375**	1.473**	0.979**	1.116**
L. S. D	0.646	1.230	0.030	8.552	0.170	0.023

* indicates a significant difference at $P \leq 0.05$

** indicates a significant difference at $P \leq 0.01$

- Means within the same column for each factor designated by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Carbohydrate contents of grains:

Results indicated that the maximum level 1.4-fold of carbohydrates in the grain was attained in case of Biogen treatment of the (200 mM NaCl) salinized soil as presented in table 10. Direct reducing sugar, sucrose and polysaccharides (starch) in grains of wheat plants increased by about 1.3, 1.2, and 2.7-fold in (Sakha 93). Table 11 shows that the maximum level of total soluble carbohydrates was recorded at Biogen treatment (250 g/fed) of plants salinized with 200 mM NaCl which increased by about 2.1-

fold. Direct reducing sugar, sucrose and polysaccharides (starch) in grains of wheat plant were increased by about 3.6, 2 and 1.1-fold in (Gemiza 10). This increase may be presumably due to increased CO₂ fixation which in turn led to a consequence increase in carbohydrate reserved for plant growth (Chaves *et al.*, 1995; Martínez Lozano *et al.*, 1999). These results confirm the above-mentioned results of the increased photosynthetic activity of both wheat cultivars cultivated under salinity conditions in response to treatment with biofertilizers.

Table 10. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on carbohydrate, protein (mg/g.d.wt) and Phenol content estimated in wheat grains (Sakha 93) cultivated under salinity stress

Treatments	Direct reducing sugar	Sucrose	Polysaccharide (starch)	Total carbohydrates	Total soluble Protein	Phenol
Control	4.632 ⁱ	5.762 ^h	1.563 ^h	11.96 ^h	17.24 ⁱ	54.6 ^e
100 mM NaCl	5.331 ^g	5.144 ⁱ	1.257 ⁱ	11.73 ⁱ	21.27 ^h	83.6 ^b
200 mM NaCl	5.094 ⁱ	5.123 ^j	1.233 ^j	11.45 ^j	21.38 ^g	102.3 ^a
Urea (100%)	5.137 ^h	6.168 ^f	3.582 ^e	14.89 ^g	23.45 ^f	67.7 ^{cd}
Cyanobacteria (500 g/fed.)	5.909 ^a	5.826 ^g	4.208 ^c	15.94 ^d	42.77 ^e	63.3 ^{cde}
Cyanobacteria + 100 mM NaCl	5.845 ^d	6.721 ^b	3.276 ^f	15.84 ^e	43.45 ^d	74.6 ^{bc}
Cyanobacteria + 200 mM NaCl	5.640 ^e	6.209 ^e	4.105 ^d	15.95 ^c	46.21 ^c	75.1 ^{bc}
Biogen (250 g/fed.)	5.535 ^f	6.485 ^c	2.894 ^g	14.91 ^f	42.77 ^e	58.9 ^{de}
Biogen +100 mM NaCl	5.897 ^b	6.262 ^d	4.226 ^a	16.39 ^b	51.04 ^b	72.7 ^{bc}
Biogen +200 mM NaCl	5.860 ^c	6.887 ^a	4.214 ^b	16.96 ^a	58.63 ^a	73.4 ^{bc}
F – value	0.245**	0.375**	2.923*	0.034**	4.724**	1.215**
L. S. D	0.005	0.005	0.005	0.005	0.005	9.381

Table 11. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on carbohydrate, protein (mg/g.d.wt) and Phenol content estimated in wheat grains (Gemiza 10) cultivated under salinity stress

Treatments	Direct reducing sugar	Sucrose	Polysaccharide (starch)	Total carbohydrates	Total soluble Protein	Phenol
Control	1.167 ⁱ	1.680 ^g	1.831 ^c	4.678 ^h	45.213 ⁱ	144.146 ⁱ
100 mM NaCl	1.940 ^h	1.578 ^h	0.825 ^j	4.343 ⁱ	46.213 ⁱ	191.643 ^b
200 mM NaCl	1.397 ⁱ	1.556 ⁱ	1.312 ^h	4.265 ^j	51.042 ^h	220.326 ^a
Urea (100%)	3.121 ^g	1.472 ⁱ	1.995 ^b	6.588 ^g	53.801 ^g	154.376 ^g
Cyanobacteria (500 g/fed.)	3.518 ^e	2.722 ^b	1.631 ^e	7.871 ^d	55.870 ^f	149.206 ^h
Cyanobacteria +100 mM NaCl	3.444 ^f	2.639 ^c	1.351 ^g	7.434 ^e	57.249 ^d	172.946 ^d
Cyanobacteria + 200 mM NaCl	4.373 ^b	2.180 ^e	1.758 ^d	8.311 ^c	58.629 ^c	177.053 ^c
Biogen (250 g/fed.)	3.892 ^d	1.805 ^c	1.258 ⁱ	6.955 ^f	56.560 ^e	144.206 ⁱ
Biogen + 100 mM NaCl	4.855 ^a	2.215 ^d	1.509 ^f	8.579 ^b	65.297 ^b	158.546 ^f
Biogen + 200 mM NaCl	4.244 ^c	3.361 ^a	2.007 ^a	9.612 ^a	67.596 ^a	161.578 ^e
F – value	2.250**	6.483**	2.392**	6.714**	5.843**	1.341**
L. S. D	0.001	0.001	0.001	0.002	0.001	0.266

* indicates a significant difference at $P \leq 0.05$

** indicates a significant difference at $P \leq 0.01$

- Means within the same column for each factor designated by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Protein contents:

Results in tables 10 and 11 show increased protein content in grains of both cultivars in response to biogen treatments of salinized plant. The maximum value of total soluble protein content was detected after biogen treatment (250 g/fed) of 200 mM salinized soil as it increased by 3.4 and 1.5-fold in Sakha 93 and Gemiza 10, respectively, compared to control. Such increase in protein content may be explained on the basis that nitrogen plays a prominent role in building protein structure and could activate some enzymatic reaction related to protein metabolism (El-Kalla *et al.*, 2002; Zeidan and Nofal, 2002).

Phenolic compounds content:

The results (Tables 10 & 11) indicate that the maximum value of Phenolic compounds content was detected with 200 mM NaCl salinized plants as increased by 1.9 and 1.5-fold in Sakha 93 and Gemiza 10, respectively, compared to control. This increase in phenolic compounds under increasing salinity levels could suggest induction of secondary products as a one of the defence mechanisms adapted by the plants to face saline condition as reported by Radi *et al.* (2013). Also, the lowest gained value of Phenolic compounds contents was detected with Biogen (250 g/fed.) by 1.1 and 1.0-fold in Sakha 93 and Gemiza 10, respectively, compared to the control.

Antioxidant enzymes and lipid peroxidation:

Data presented in figures 2-4 show catalase and peroxidase activity as affected

by salinity and biofertilizers treatments. The effect of salinity varied significantly in response to salt concentrations. The highly significant increase was observed in case of 200 mM NaCl compared with control. Catalase and peroxidase activities were increased by about 1.8 and 1.5-fold in Sakha 93 and 1.5, and 1.4-fold in Gemiza 10, respectively. The same trend was observed in lipid peroxidation which was significantly increased by about 1.1 and 1.2-fold in both cultivars, respectively. Results show highly significant reduction in catalase, peroxidase activity and lipid peroxidation in response to Cyanobacterial treatment (500 g/fed) by about 92%, 96%, and 19% in Sakha 93, 80%, 97%, and 29% in Gemiza 10. This is followed by biogen treatment (250 g/fed) which shows significant reduction in catalase, peroxidase activity and lipid peroxidation by about 90%, 94%, and 18% in Sakha 93, 61%, 95%, and 20% in Gemiza 10. It is known that salinity can cause oxidative stress by inhibition of CO₂ assimilation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of reactive oxygen species (ROS) from triplet chlorophyll (Asada, 1994; Gosse *et al.*, 1994). It could be suggested that cyanobacteria react positively plant antioxidative defence system via enhancing the activities of superoxide dismutases, peroxidases, catalases, glutathione S-transferases and glutathione reductases, (Chen *et al.*, 2004; Pflugmacher *et al.*, 2007).

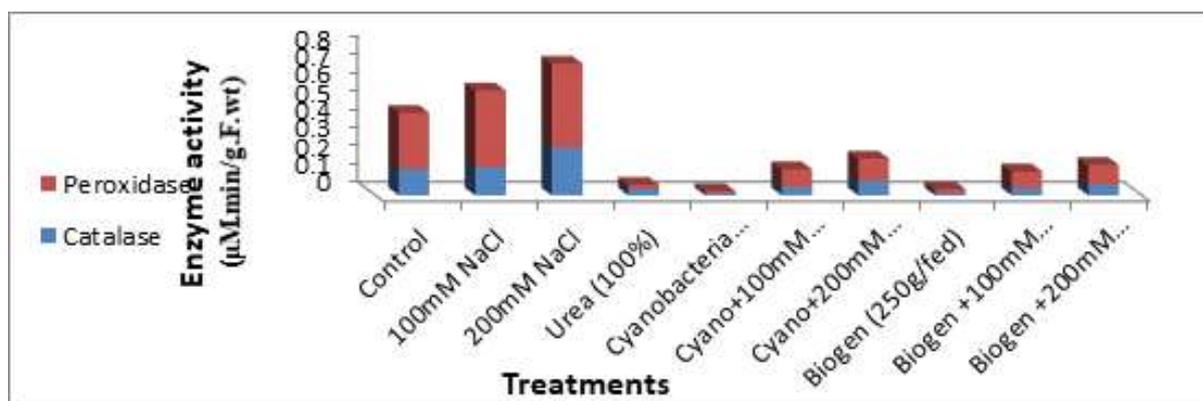


Fig. 2. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on enzymes activity (catalase and peroxidase) ($\mu\text{M}/\text{min}/\text{g}$ FW) in leaves of 30-day old wheat (Sakha 93) cultivated under salinity stress

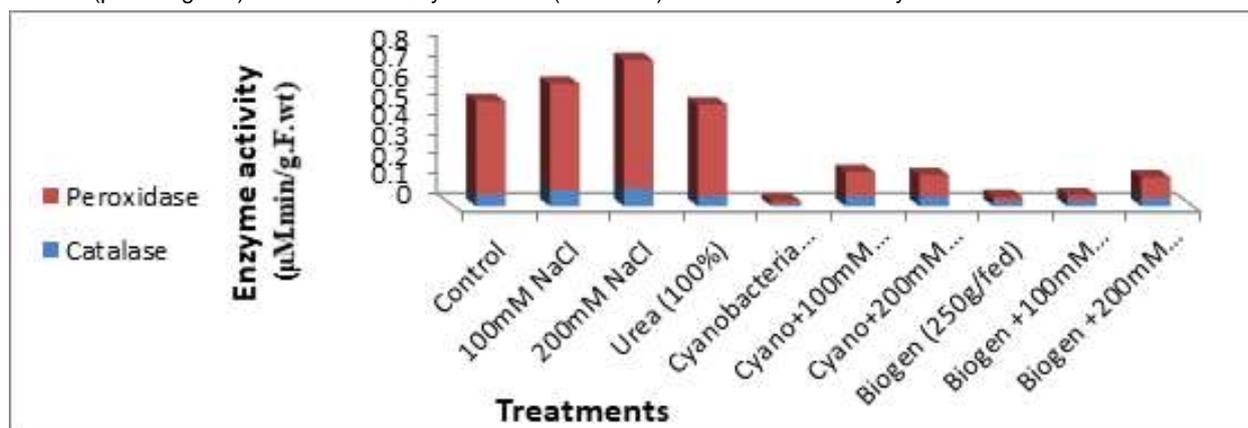


Fig. 3. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on enzymes activity (catalase and peroxidase) ($\mu\text{M}/\text{min}/\text{g}$ FW) in leaves of 30-day old wheat (Gemiza 10) cultivated under salinity stress

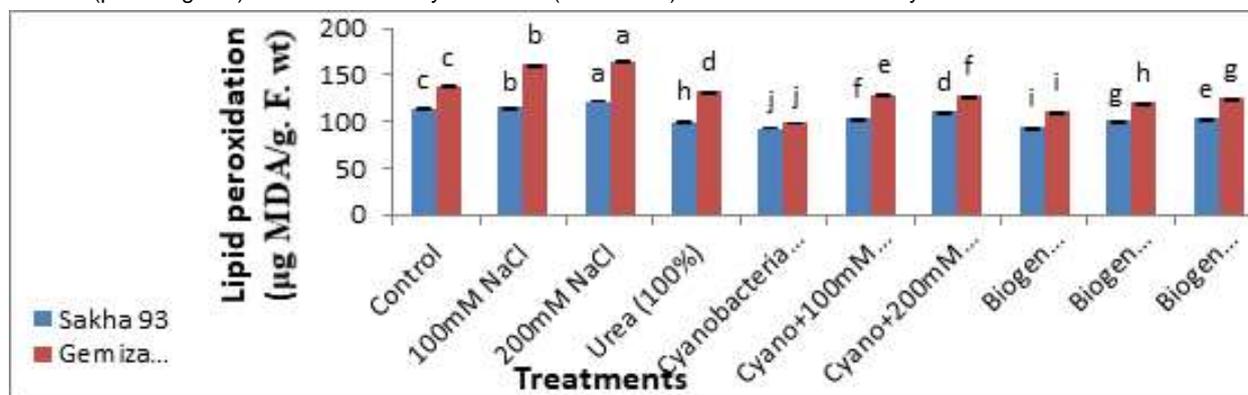


Fig. 4. Effect of fertilization treatments (Cyanobacteria, Biogen, Urea) on lipid peroxidation (μg MDA/g FW) in leaves of 30-day old wheat (Sakha 93 and Gemiza 10) cultivated under salinity stress

- Each column represents the mean value of three replicates.
- Different letters represent significant difference at $P \leq 0.05$ (Duncan's)

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المخصبات الحيوية كوسيلة ضد التأثير الضار للملوحة على نبات القمح

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أعلى قيمة لنشاط انزيم الكاتاليز وانزيم البيروكسيداز والدهون المتأكسدة ناتجة من المعاملة بالملوحة عند تركيز 200 مللي مول كلوريد صوديوم وأيضا أقل قيمة ناتجة كانت من المعالجة بالتسميد الحيوي (السيانوبكتريا 500 جم/فدان) وتليها المعاملة بالبيوجين 250 جم/ فدان وذلك عند عمر 30 يوما من الانبات مقارنة بالكنترول. وقد تم دراسة مقاييس المحصول عند عمر 120 يوما من الزراعة وأوضحت النتائج أن أفضل قياسات للنمو (طول النبات عند الحصاد، عدد وطول السنابل، عدد الجيوب في السنبل، وزن الجيوب بالسنبل (جم) ووزن 1000 حبه (جم) والوزن الطازج والجاف) كانت عند المعاملة بالتسميد الحيوي (البيوجين 250 جم/ فدان) في وجود 200 مللي مول كلوريد صوديوم وذلك مقارنة بالكنترول وبجميع المعاملات. كما دلت النتائج أن أعلى محتوى فينولي للحبوب لسنفي القمح مع المعاملة ب 200 مللي مول كلوريد صوديوم وأقل كميته من المركبات الفينولية مع المعاملة بالتسميد الحيوي (البيوجين 250 جم/ فدان). مقارنة بالكنترول وبجميع المعاملات، ومن الجدير بالذكر أن نقص الفينولات، ونشاط انزيمات الأكسدة عند المعاملة بالمخصب الحيوي هو دليل على تخفيض الاجهاد الناتج من تأثير الملوحة على نبات القمح، وبناء على نتائج هذه الدراسة يمكن التوصية باستخدام المخصب الحيوي البيوجين (250 جم/ فدان) كسماد حيوي في الأراضي الملحية التي لا يتعدى فيها تركيز الملوحة عن 200 مللي مول كلوريد صوديوم ويعتبر ذلك أوفر من الناحية الاقتصادية كما أوضحت النتائج علي اختزال كميته السماد الكيماوي (اليوريا) الي 50 % عند استعمال الطرق الموصي بها.

أجريت هذه الدراسة لمعرفة تأثير بعض المخصبات الحيوية (سيانوبكتريا، البيوجين) على صنفين من القمح أحدهما مقاوم للملوحة (سحا 93) والآخر حساس للملوحة (جميزه 10) النامية تحت ظروف الاجهاد الملحي من خلال دراسة دالات النمو ونشاط البناء الضوئي والمحتوي الكربوهيدراتي والبروتيني والمحصول ومكوناته. وقد تم عمل تجريب ميدئية لاختيار أصناف القمح وكذلك تركيزات الملوحة التي سيتم استخدامها، ومن خلال هذه التجربة تم اختيار صنف القمح صنف سحا 93 كصنف مقاوم للملوحة وصنف جميزه 10 كصنف حساس للملوحة وذلك لدراستهما تحت تأثير تركيزين من الملوحة وهما (100 و 200 مللي مول) من كلوريد الصوديوم. وقد تم دراسة تأثير المعاملة بنوعين من التسميد الحيوي (سيانوبكتريا 500 جم/فدان) و (البيوجين 250 جم/ فدان) علي صنف القمح (سحا 93 و جميزه 10) المعرضان لتركيزي الملوحة (100 و 200 مللي مول) من كلوريد الصوديوم علي دالات النمو المختلفة. وقد أوضحت النتائج أن افضل قيم لدالات النمو لكلا الصنفين (طول النبات، طول الساق، طول الجذر، عدد ومساحة الاوراق و عدد السلميات) والوزن الخضري والجاف الناتج من السيقان والأوراق والجذور وكفاءة عمليه البناء الضوئي (مقاسه علي اساس التفلور الضوئي) والمحتوي الكربوهيدراتي والبروتيني لأجزاء النبات مع المعاملة بالتسميد الحيوي (البيوجين 250 جم/ فدان) في وجود 200 مللي مول كلوريد صوديوم ويليها المعاملة بالتسميد الحيوي (سيانوبكتريا 500 جم/فدان) عند نفس التركيز وذلك خلال جميع الأعمار مقارنة بالكنترول وبجميع المعاملات. وقد أوضحت نتائج نشاطات انزيمات الأكسدة أن