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Beneficial Effects of Calcium Chloride on Two Cyanobacterial Species under Sodium Chloride Stress

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EXPOSURE of *Anabaena constricta* and *Nostoc linckia* to 0.2M NaCl induced a significant decrease in cell number, dry weight and pigments (chlorophyll "a" and carotenoids,). Also there was a decrease in glucose, protein and nitrogen contents. Addition of two different concentrations of $CaCl_2$ (40 and 60 mM) to the salinized cultures with both organisms induced a significant increase in growth and metabolites activities. Protein electrophoretic patterns of culture of *A. constricta* exposed to 0.2 M NaCl showed disappearance of one protein band at 77 KD_a and appearance of two protein bands at 171 and 70 KD_a. The addition of 40 mM $CaCl_2$ to the salinized culture of *A.constricta* showed disappearance of one protein at 77 KDa and appearance of three protein bands at 17, 61 and 162 KDa, while addition of 60 mM $CaCl_2$ to salinized culture caused appearance of three protein bands at 18, 58 and 138 KD_a as compared with control (salinized culture of 0.2 M NaCl alone). Addition of 0.2 M NaCl to culture of *N linckia* and addition of 40 mM or 60 mM $CaCl_2$ to salinized culture showed no change of protein patterns as compared with control (culture without NaCl) but differed in the percentage of intensity of proteins.

Keywords: *Anabaena* sp., *Nostoc* sp., *Cyanobacteria*, Salinity stress, Protein profile, Mitigate effect of $CaCl_2$.

Salinity is an important deterrent to agriculture in many areas of the world. Salts not affect only the growth of plants but also inhibit the proliferation and activity of native or introduced microorganisms. Among these organisms, cyanobacteria have a fundamental role in supplying the crop plants with both nitrogen and growth regulators. This can increase crop yield and indirectly maintains the fertility status of soil.

High concentration of NaCl inhibits growth by increasing ionic and osmotic stress on cells (Brownell & Nicolas, 1967). Since high intercellular concentrations of Na^+ are toxic to most biological systems, organisms that can live in Na rich environments. The ability to produce organic osmolytes to cope with ionic and osmotic stresses in the environment is common in N-fixing cyanobacteria (Reed *et al*., 1986).

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Exposure of *Chlorococcum sp.* to 0.2 M NaCl caused an increase in the biomass dry weight due to an increase in the cell size accompanied by massive appearance of secondary carotenoids. Maximum size was obtained after 2-3 days of cultivation (Masojidek *et al.,* 2000). However, addition of 40 mM NaCl did not increase the carotenoids biosynthesis in the flagellated alga *Haematococcus pluvialis* (Hagen *et al*., 2001). The influence of salinity (0.03-0.5M NaCl) on the physiological characteristics of fresh water cyanobacterium *Synechococcus* 6311 showed that intercellular granules disappeared, the density of the cytoplasm decreased and the appearance of DNA material was changed (Lefort –Tran *et al*., 1988).

Rai & Abraham (1993), observed that with the increase in NaCl concentration (beyond 200 mM), the filaments of *Anabaena doliolum* were shorter with less heterocysts. Anand *et al*. (1994) studied the effect of salinity on the growth of cyanobacteria *Chroococus minor, Gloeocapsa polydermatica, Oscillatoria salina, Lyngbya spiralis, Nostoc piscinate* and *Tolypothrix tenuis.* They observed that *Nostoc piscinate* and *T. fenuis* released phycobilin pigments (phycocyanin and phycoerythrin) in the extracellular medium at salinities of 2.5-3.5%. Zhao *et al*. (2005), indicated that the addition of nitrate could reduce the effect of salt stress on cultivated *Nostoc flagelforme* and enhance its salt resistance. El-Naggar *et al*. (2005) studied the effect of salinity stress (0.3M NaCl) on the N metabolism of cyanobacterium *Anabaena subcylindrica* (Borge) in absence or presence of $CaCl_2$ (0.03 or 0.05). Salinity stress induced reduction in protein content, nitrogenase activity, some amino acids biosynthesis and nucleic acids content. Exogenous addition of $CaCl_2$ to the culture medium alleviated the toxic action induced by salinity.

The aim of this research was to study the effect of low concentrations of $CaCl_2$ on the growth of salinized cultures (0.2M NaCl) of *Anabaena constricta* and *Nostoc linckia*.

Materials and Methods

Organisms

Two algal axenic cultures of filamentous heterocystous *Anabaena constricta* (Geitler) and *Nostoc linckia* (Roth) were isolated from saline alkali soils (pH 9.0), brought from cultivated fields of Sana'a Yemen, (Battah & Khalil, 2008). The organisms were maintained in BG-11 medium (Stainer *et al.,* 1971) at an illumination 3500 lux with regime 16/8 hours light / dark at 27 ^{o}C.

Sodium chloride and calcium chloride treatments

Cultures of *A. constricta* and *N. linckia* (7-9 days old) were inoculated into 0.2 M NaCl parallel with control (0.0 M NaCl). Another set was inoculated into 0.2 M NaCl that also contained two different concentrations of $CaCl_2$ (40 and 60 mM). All flasks

were incubated at a temperature 27±2°C and white light 5000 lux in regime 16/8 hours light/dark.

Growth estimation

The changes in cell number were determined by Haemocytometer cell. The optical density was determined at 750 nm by spectreophotometer (Lefort Tran *et al*., 1988). The dry weight was estimated by Leganes *et al.* (1987). Chlorophyll "a" concentrations in cell were determined by spectrophotometric method of Jeffery & Humphrey (1975). The carotenoids were determined according to Jensen & Liaaen (1959). The phycobiloproteins were determined according to Bennet & Bogorad (1973). The carbohydrate fractions of algal tissues were calculated as mg glucose/100 gm dry weight (Naguib, 1963). The total N content of the algal cultures was estimated by micro Kjeldahl as described by Jacobs (1958). The total soluble proteins were determined quantitavely by Lowery method (Lowery *et al*., 1951).

Gradient gel electrophoresis

Vertical polyacryamide gel electrophoresis (PAGE) was used as described by Laemmli (1970). Gel lanes were analyzed using gel documentation and analysis system consisting of a dark room, a transilluminator, an integrating CD Video camera and image software (AAB software).

Statistical analysis

Data were subjected to the proper statistical analysis according to Snedecor & Cochran (1982).

Results

Addition of 0.2 M NaCl to cultures of *Anabaena constricta* or *Nostoc linckia* caused significant reduction in the cell number with values 34% in case of *A.constricta* and 23% in case of *N. linckia* after 15 days incubation period as shown in Fig. 1,2. Addition of 40 or 60 mM of $CaCl_2$ to salinized cultures caused an increase in the cell number of both organisms, being 1.42 fold and 1.55 fold high for *A. constricta* and *N. linckia*, respectively. The dry weight of both organisms in salinized culture (0.2M NaCl only) decreased in *A. constricta* and *N. linckia* and this decreasing amounted to 42.1% and 40%, respectively . Addition of 40 mM of $CaCl_2$ to salinized cultures caused a significant increase in the dry weight of both organisms as compared with control cultures. This increase in *A.constricta* and *N. linckia* was nearly 1.81 and 1.83 fold, respectively, while addition of 60 mM $CaCl_2$ to salinized culture of both organisms induced an increase of 1.6 and 1.5 fold in *A. constricta* and *N. linckia*, respectively, as compared with salinized culture after 15 days of incubation period (Fig. 1, 2).

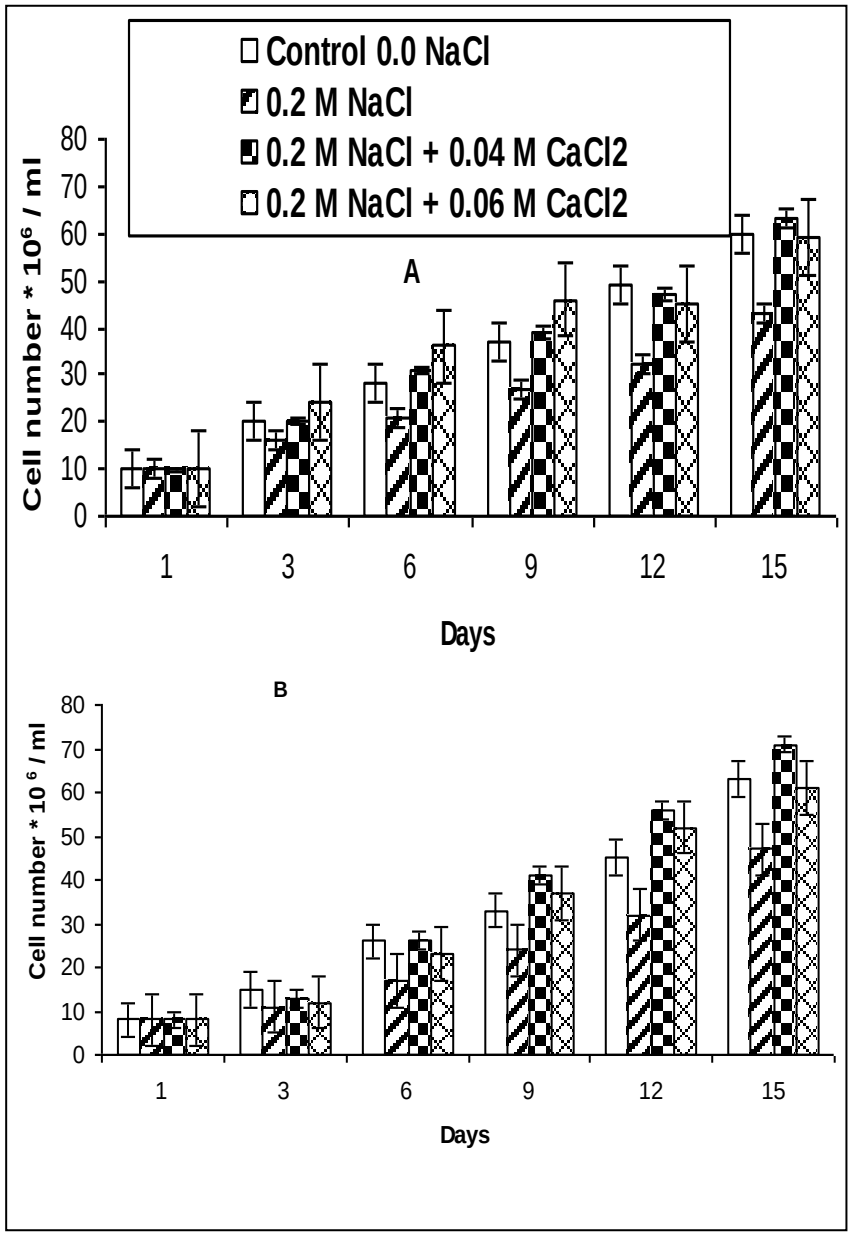

Fig. 1. Effect of two concentrations of $CaCl_2$ on salinized culture of: A- *A. constricta* B- *N. linckia* (Cell number x 10 ^{6} /ml).

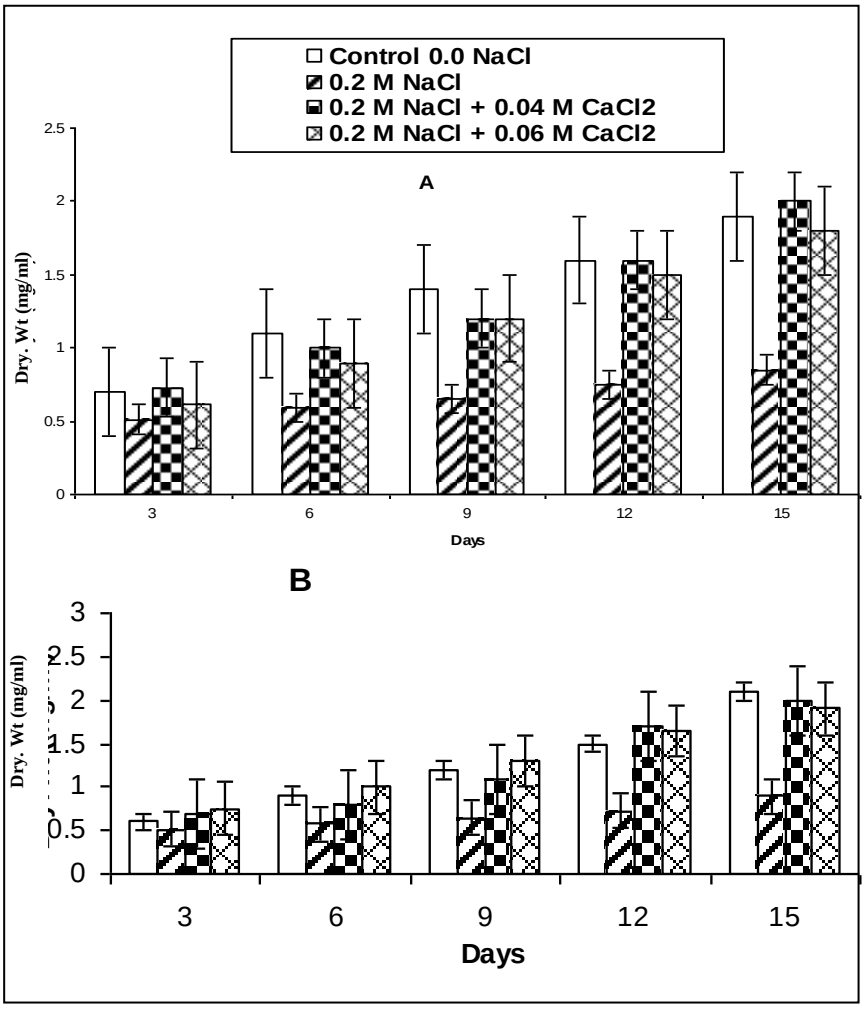

Fig. 2. Effect of two concentrations of $CaCl_2$ on salinized culture of: A- *A. constricta* B- *N. linckia* (Dry.wt. mg/ml).

Application of 0.2 M NaCl only for both organisms resulted in sharp decrease in chl"a" content nearly to the control (Table 1). Addition of 40 or 60 mM $CaCl_2$ to salinized culture of both organisms caused an increase in chl"a" and carotenoid contents as compared to salinized culture. The presence of 0.2 M NaCl in the culture medium caused a significant decrease in metabolic activities of *A. constricta* and *N. linckia* (Tables 2,3). Addition of 40 or 60 mM $CaCl_2$ to salinized culture of both organisms induced significant increases in metabolic activities as glucose, N and protein contents. The effect of 40 mM $CaCl_2$ was more prominent than the effect of 60 mM $CaCl_2$.

TABLE 1. Effect of two concentrations of $CaCl_2$ on chlorophyll a and carotein contents of salinized cultures of different ages of *A. constricta* and *N. linckia*.

Age (days)	Treatments	*A. constricta*		*N. linckia*	
		Chl"a"	"Car"	Chl"a"	"Car"
3	Control	0.220 ± 0.006	0.280 ± 0.006	0.150 ± 0.006	0.320 ± 0.006
	0.2 M NaCl	0.060 ± 0.006	0.058 ± 0.006	0.013 ± 0.003	0.020± 0.006
	0.2 M NaCl + 40m M $CaCl_2$	0.230 ± 0.006	0.230 ± 0.006	0.180 ± 0.006	0.400 ± 0.006
	0.2 M NaCl + 60 mM $NaCl_2$	0.220 ± 0.012	0.220 ± 0.012	0.160 ± 0.006	0.380± 0.003
6	Control	0.307 ± 0.012	0.390 ± 0.006	0.200 ± 0.006	0.390± 0.006
	0.2 M NaCl	0.120 ± 0.058	0.130 ± 0.006	0.130 ± 0.006	0.070± 0.006
	M NaCl + 40 m M $CaCl_2$	0.353 ± 0.088	0.45 ± 0.006	0.250 ± 0.006	0.480± 0.003
	0.2 M NaCl + 60m M $NaCl_2$	0.320 ± 0.058	0.400 ± 0.006	0.210 ± 0.006	0.450± 0.003
9	Control	0.303 ± 0.013	0.570 ± 0.006	0.290 ± 0.006	0.480± 0.006
	0.2 M NaCl	0.200 ± 0.058	0.250 ± 0.006	0.190 ± 0.006	0.116 ± 0.009
	0.2 M NaCl + 40m M $CaCl_2$	0.486 ± 0.033	0.680 ± 0.006	0.310 ± 0.006	0.540± 0.006
	0.2 M NaCl + 60m M $NaCl_2$	0.460 ± 0.058	0.620 ± 0.006	0.260 ± 0.006	0.520± 0.006
12	Control	0.456 ± 0.008	0.690 ± 0.006	0.37 ± 0.006	0.590 ± 0.06
	0.2 M NaCl	0.276 ± 0.008	0.250 ± 0.006	0.313 ± 0.003	0.180± 0.06
	0.2 M NaCl + 40m M $CaCl_2$	0.573 ± 0.012	0.683 ± 0.009	0.420 ± 0.006	0.630± 0.03
	0.2 M NaCl + 60m M $NaCl_2$	0.510 ± 0.006	0.623 ± 0.007	0.373 ± 0.007	0.570± 0.06
15	Control	0.570 ± 0.006	0.756 ± 0.007	0.490 ± 0.006	0.670± 0.06
	0.2 M NaCl	0.353 ± 0.007	0.320 ± 0.006	0.356 ± 0.003	0.240± 0.06
	0.2 M NaCl + 40m M $CaCl_2$	0.0680 ± 0.006	0.74 ± 0.006	0.820 ± 0.006	0.770± 0.03
	0.2 M NaCl + 60m M $NaCl_2$	0.0646 ± 0.012	0.680 ± 0.006	0.740 ± 0.006	0.710± 0.06
Significance		**	**	**	**

** = Significant difference at $P \leq 0.01$ according to F-test. Chlorophyll "a" = Chl"a" and Carotein = car.

TABLE 2. Effect of two concentrations of $CaCl_2$ on some metabolites of salinized culture of different ages of *A. constricta*.

Age (days)	Treatments	Glucose ug/ml	Nitrogen mg N/100 ml	Protein mg/100 ml
3	Control	23 ±0.250	0.57 ± 0.006	7.09 ± 0.012
	0.2 M NaCl	28 ± 0.180	0.35 ± 0.006	5.68 ± 0.046
	0.2 M NaCl 40m M $CaCl_2$	35.8 ± 0.320	0.78 ± 0.006	7.68 ± 0.063
	0.2 M NaCl + 60m M $NaCl_2$	35.2 ± 0.610	0.60 ± 0.006	7.08 ± 0.004
6	Control	44.8 ± 0.810	1.20 ± 0.060	8.29 ± 0.006
	0.2 M NaCl	36 ± 0.580	0.75 ± 0.06	6.74 ± 0.063
	0.2 M NaCl + 40m M $CaCl_2$	55 ± 0.580	1.52 ± 0.060	10.34 ± 0.063
	0.2 M NaCl + 60mM $NaCl_2$	47 ± 0.580	1.30 ± 0.003	12.2 ± 0.115
9	Control	62.4 ± 0.660	1.91 ± 0.006	8.5 ± 0.057
	0.2 M NaCl	57 ± 0.580	1.20 ± 0.110	13.2 ± 0.058
	0.2 M NaCl + 40mM $CaCl_2$	88.6 ± 0.330	2.11 ± 0.009	11.61 ± 0.063
	0.2 M NaCl + 60m M $NaCl_2$	84 ± 0.580	1.97 ± 0.006	15.4 ± 0.067
12	Control	94.6 ± 0.580	2.42 ± 0.006	10.0 ± 0.577
	0.2 M NaCl	84 ± 0.580	1.51 ± 0.020	16.4 ± 0.067
	0.2 M NaCl + 40m M $CaCl_2$	128 ± 0.580	2.80 ± 0.060	13.18 ± 0.091
	0.2 M NaCl + 60m M $NaCl_2$	119 ± 0.580	2.50 ± 0.115	17.21 ± 0.063
15	Control	120.4 ± 0.580	3.10 ± 0.115	14.4 ± 0.067
	0.2 M NaCl	130 ± 0.580	1.70 ± 0.060	18.2 ± 0.067
	0.2 M NaCl + 40m M $CaCl_2$	160 ± 0.580	3.68 ± 0.060	16.6 ± 6.057
	0.2 M NaCl + 60m M $NaCl_2$	151 ± 0.580	3.22 ± 0.060	11.44 ± 0.122
Significance		**	**	**

** = Significant difference at $P \leq 0.01$ according to F-test.

TABLE 3. Effect of two concentrations of $CaCl_2$ on some metabolites activities of salinized cultures of different ages of *N. linckia*.

Age (days)	Treatments	Total glucose ug/ml	Total nitrogen mg N/100 ml	Total protein mg/100 ml
3	Control	32.63 ± 0.66	0.56 ± 0.06	3.8 ± 0.17
	0.2 M NaCl	29 ± 0.58	0.34 ± 0.06	4.2 ± 0.12
	0.2 M NaCl + 40m M $CaCl_2$	52.6 ± 1.20	0.6 ± 0.06	4 ± 0.06
	0.2 M NaCl + 60mM $NaCl_2$	43 ± 0.58	0.54 ± 0.06	4 ± 0.12
6	Control	40.53 ± 0.57	0.72 ± 0.006	4 ± 0.058
	0.2 M NaCl	43 ± 0.58	0.63 ± 0.006	4.2 ± 0.115
	0.2 M NaCl + 40m M $CaCl_2$	61 ± 0.58	0.96 ± 0.006	3.21 ± 0.121
	0.2 M NaCl + 60mM $NaCl_2$	50 ± 0.58	0.84 ± 0.006	5.4 ± 0.230
9	Control	65.3 ± 0.124	1.12 ± 0.023	6.45 ± 0.030
	0.2 M NaCl	51.67 ± 0.667	0.6 ± 0.251	4.93 ± 0.38
	0.2 M NaCl + 40m M $CaCl_2$	95 ± 0.577	1.52 ± 0.058	9.6 ± 0.036
	0.2 M NaCl + 60m M $NaCl_2$	86 ± 0.577	4.31 ± 3.24	7.8 ± 0.058
12	Control	95.4 ± 0.230	1.5 ± 0.058	9.4 ± 0.00
	0.2 M NaCl	81 ± 0.577	1.14 ± 0.058	6.4 ± 0.230
	0.2 M NaCl + 40m M $CaCl_2$	125 ± 0.577	1.8 ± 0.058	12.13 ± 0.075
	0.2 M NaCl +60m M $NaCl_2$	101 ± 0.577	1.43 ± 0.044	10.16 ± 0.08
15	Control	126.33 ± 0.190	2 ± 0.077	13.6 ± 0.346
	0.2 M NaCl	116.55 ± 0.293	1.4 ± 0.058	9.11 ± 0.063
	0.2 M NaCl + 40m M $CaCl_2$	146 ± 0.577	2.32 ± 0.058	16.13 ± 0.075
	0.2 M NaCl + 60m M $NaCl_2$	129.33 ± 1.201	2.1 ± 0.058	15.6 ± 0.057
Significance		**	N.S	**

** = Significant difference at $P \leq 0.01$ and N.S. =non significant according to F-test

Protein electrophoresis pattern of A. constricta

As shown in Fig. 3 and Table 4 the structural pattern of the 0.2 M NaCl treated culture after 15 days showed the disappearance of a protein with an apparent molecular weight of 77 KDa that had been present in the control track (Fig. 3, Table 4). At the same time two proteins at 171 and 70 KDa appeared. Addition of 0.04M $CaCl_2$ to salinized culture (with 0.2 M NaCl) of *A. constricta* showed the disappearance of one protein at 77 KDa and the appearance of three protein bands at 17, 61 and 162 KDa. The treatment with 60 mM $CaCl_2$ produced three protein bands with an apparent 18,58 and 138 KDa as compared with salinized culture alone after 15 days old.

Protein electrophoresis pattern of N. linckia

The 0.2 M NaCl treated culture showed no major changes of protein patterns as compared with control (Fig. 4 and Table 4). The culture of 0.2 M NaCl produced two proteins with apparent protein profiles with 44 and 6 KDa that also, was evidenced in control track, but the difference between them was in the percentage of intensity. Addition of 40 or 60 mM $CaCl_2$ to salinized culture of *N. linckia* also produced the same proteins with an apparent 43 and 6 KDa that differed only in the percentage of intensity.

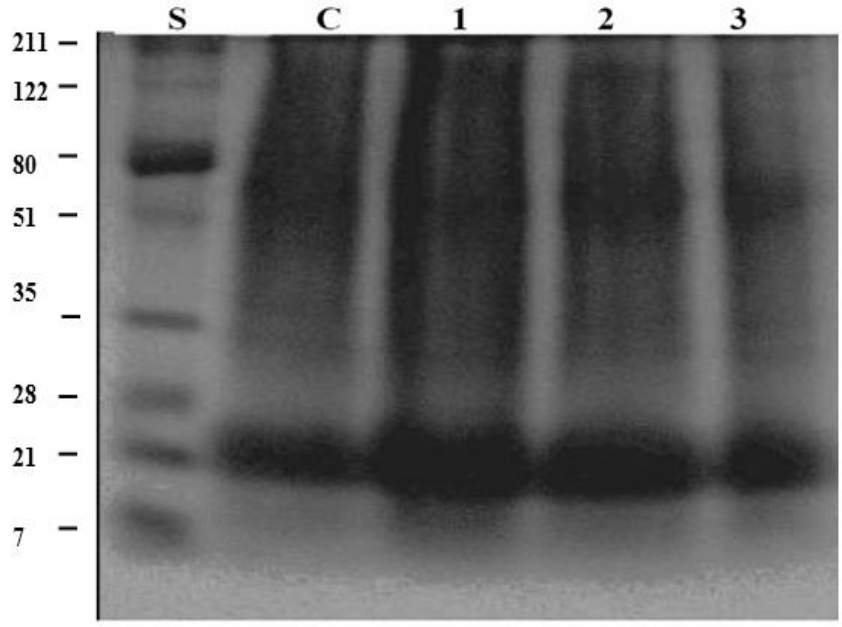

Fig. 3. Photographic picture of the gel electrophoresis of protein in *A. constricta*. [Lane S=Standard, C=Control , Lane 1=0.2 M NaCl , Lane 2= 0.2 M NaCL+40 mM $CaCL_2$ and Lane 3= 0.2 M NaCL+60 mM $CaCL_2$].

TABLE 4. The relative intensity (percent) of molecular weights (M. wt.) representing protein bands for *A. constricta* and *N. linckia* after 15 days incubation.

	Bands	Control		0.2 M NaCl		0.2 M NaCl + 40mM $CaCl_2$		0.2 M NaCl + 60 mM $CaCl_2$		Standard M. wt (KDa)
		AMT%	M. wt	AMT %	M. wt	AMT %	M. wt	AMT%	M. wt	
A. constricta	1	46.98	77	4.03	171	4.74	162	14.06	138	211
	2	4.22	37	65.84	70	4.1	70	1.95	70	122
	3	3.19	33	5.79	36	16.01	61	17.51	58	80
	4	45.61	19	3.2	32	1.77	36	1.1	36	51
	5			21.11	19	1.56	33	1.42	33	35
	6					71.82	17	63.97	18	28
N. linckia	1	32.44	44	16.34	44	68.61	43	39.82	43	211
	2	67.56	6	83.66	6	31.39	6	60.18	6	122

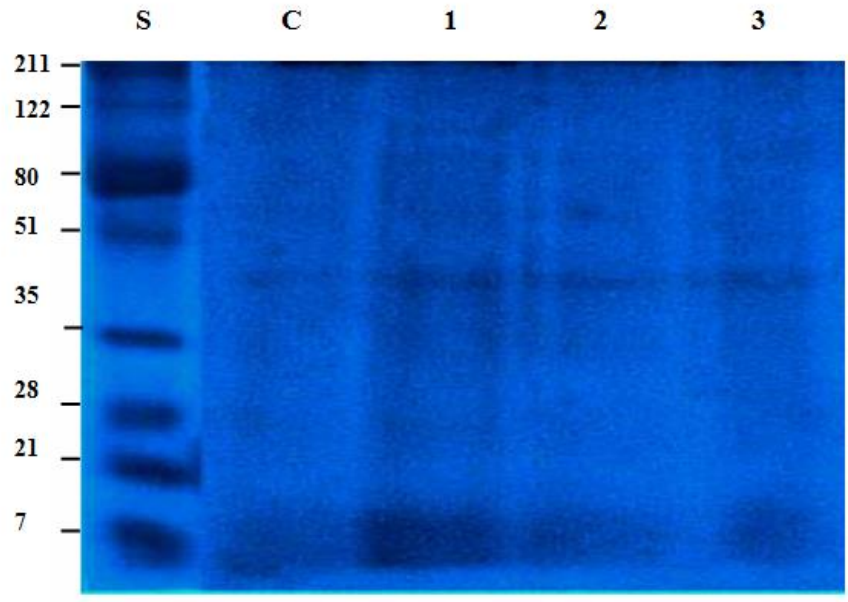

Fig. 4. Photographic picture of the gel electrophoresis of protein in *N. linckia.*[Lane S=Standard, C=Control, Lane 1=0.2 M NaCl, Lane 2= 0.2 M NaCL+40 mM $CaCL_2$ and Lane 3= 0.2M NaCL+60 mM $CaCL_2$].

Discussion

The addition of 0.2 M NaCl to culture media of *A. constricta* and *N. linckia* caused a significant decrease in cell number, dry weight, optical density and different pigments. The results obtained agreed with Blumwald & Tel-or (1984), who observed that, the chlorophyll contents of *Synechococcus* 6311 was essentially stable through the process of salt adaption, with an observed enhancement in the synthesis of biloprotein pigments (phycocyanin and phycoerythrin). There was an enhancement in the synthesis of salt adapted cells of *synechococcus* 6311, while they indicated that the synthesis of both pigments in the heterocystous *N. muscorum* was slightly lower at high salt concentration of NaCl. The growth rate of *Porphyridium cruentum* was influenced by NaCl. Optimum growth was found with salinities ranging between 0.45 M and 0.8M NaCl. A further increase in salinity to 1.5 M NaCl resulted in a drastic drop in algal growth (Lee *et al*., 1989).

The combination of low concentrations of $CaCl_2$ (40 or 60 mM) with salinized culture (0.2 M NaCl) of *A constricta* and *N. linckia*, caused a significant increase in the growth parameters and metabolic activity as compared with control (0.2M NaCl only). In accordance with the present results, Ahmed *et al*. (1989) found that the growth of *Chlorella vulgaris* was markedly inhibited with the rise of NaCl level. However, a marked growth stimulation was observed under certain combinations of NaCl and $CaCl_2$.

Calcium chloride at (0.2 g/l) favoured germination process and a zero concentration of $CaCl_2$ hindered germination of *Anabaena* sp. (Shivaprakash *et al*., 2004). Biomass through the sporulation and germination cycle was 50 times more than 20 times increases in continuous vegetative growth.

The mechanism of Ca in stressed plants could be activated through membrane stability (Munns *et al*., 1983). Also low Ca increases membrane permeability at high external NaCl (Greenway & Munns, 1980). Leopold & Wilting (1984) found that Ca served partially to protect tissues from NaCl damage and lessens the leakiness of organic metabolites. Therefore, it could be generalized that Ca relief occurs in the following sequence: Stabilization and repair of NaCl damaged membrane including thylakoids, less uptake of Na^+ (less toxicity) and preservation of cell metabolites from leakiness. The carotenoid and polysaccharides content were increased to eliminate free radical and regulate osmotic pressure (Bi Yonghong *et al*., 2005).

Many organisms are respond to shock treatment by synthesizing a new set of proteins (Bhagwat & Apte, 1989; Schubert *et al.*,1993; Thomas *et al.,* 1990 and Rajeshwar & Donat, 1996). The synthesis of cellular metabolites in response to salt stress by halotolerant and halosensitive *N. muscorum* were grown at varying levels of NaCl in liquid medium were studied by Shobhana & Kaushik (2002). Also, they stated that protein synthesis was stimulated up to 0.05 M NaCl only in the halotolerant strain. Qualitative changes in protein showed the presence of salt

sensitive protein (37 kDa) and emergence of 42.5, 27 and 72 kDa proteins that provide tolerance to the halotolerant strain. Our results indicate that the cyanobacteria *A. constricta* and *N. linckia* responded to shock treatments be producing electrophoresis pattern of both organisms under shock of 0.2 M NaCl alone or with addition of 40 or 60 mM $CaCl_2$ provided major changes (appearance disappearance) of protein patterns. Our results are similar to those of El-Naggar *et al*. (2005) who found disappearance of some protein bands (76, 42 and 39 KDa) for *A. subcylindica* as compared with the control. Addition of $CaCl_2$ to the salinized culture caused the reappearance of these bands. The 40 KDa proteins appeared in both salt and salt-calcium treated cells.

Any substantial increase in salt stress in nature will affect the ecological and economically important cyanobacterial communities. These communities will be in turn affect the productivity of higher plants. Where cyanobacteria are being considered as an alternate natural source of nitrogenous fertilizers for rice paddies and other crops. Finally to keep salt levels in water not rise too high, we must add Ca^{+2} to water to antagonize the harmful effects of salt on cyanobacterial communities.

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التأثيرات الايجابيه لكلوريد الكالسيوم على نوعين من الطحالب الخضراء المزرقة تحت إجهاد الملوحه

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تهدف الدراسة إلى توضيح تأثير تركيز 0,04 أو 06, 0 جزيئ (مللى مول) من كلوريد الكالسيوم على نوعين من الطحالب الخضراء المزرقة هما *أنابينا كونستركتا* و*نوستوك لينكيا* المزروعين في وسط غذائي ملحي بتركيز 0,2 جزئى (مللى مول) من كلوريد الصوديوم .

أوضحت النتائج أن 0,2 جزئ من كلوريد الصوديوم يحدث تأثير معنويا بنقص النمو وذلك بقياس العدد الخلوي والوزن الجاف والطيف الضوئي و نقص محتوى كلوروفيل "أ" والكاروتينات . ونقص في محتوى الجلوكوز والبروتين لكلا من الطحلبين . عند إضافة تركيز 0,04 أو 06, 0 جزئي من كلوريد الكالسيوم إلى الطحلبين والمزروعين في وسط غذائي به 0,2 جزىء من كلوريد الصوديوم حدثت زيادة معنوية في معدلات النمو المختلفة والنشاطات الفسيولوجية لكلا من الطحلبين .

أظهرت نتائج تحليل البروتين لطحلب *أنابينا كونستركتا* والذي تمت معاملته بتركيز 0,2 جزىء من كلوريد الصوديوم باختفاء احد أنواع البروتينات عند الوزن الجزئى 77 كيلو دالتون وظهور نوعين من البروتينات عند الأوزان الجزيئية 70 ،171 كيلو دالتون . وعند إضافة تركيز 0,04 جزئى من كلوريد الكالسيوم إلى طحلب *أنابينا كونستركتا* المزروع في وسط غذائي ملحي (0,2 جزىء من كلوريد الصوديوم) لوحظ اختفاء احد البروتينات عند الوزن الجزئى 77 كيلو دالتون وظهور ثلاث أنواع أخرى من البروتينات عند الأوزان الجزيئية 17 و 61 و 162 كيلو دالتون وذلك مقارنة بالمزرعة المضاف إليها ملح الصوديوم (0,2 جزىء من كلوريد الصوديوم فقط). وبإضافة 06, 0 جزيء من كلوريد الكالسيوم إلي طحلب *أنابينا كونستركتا* المزروع في وسط غذائي يحتوى على 0,2 جزىء من كلوريد الصوديوم لوحظ اختفاء احد البروتينات عند الوزن الجزئى 77 كيلو دالتون وظهور ثلاث أنواع من البروتينات عند الأوزان الجزيئية 18 ، 58 و 138 كيلو دالتون وذلك مقارنة بالمزرعة المضاف إليه ملح الصوديوم (0,2 جزىء من كلوريد الصوديوم فقط) .

وبدراسة التحليل الكيفي للبروتين لطحلب *نوستوك لينكيا* المزروع في وسط غذائي ملحي (0,2 جزىء من كلوريد الصوديوم) لم تظهر تغيرات في أنواع البروتين مع اختلاف في النسبة المئوية لكثافة البروتين مقارنة بالتجربة الضابطة (بدون 0,2 جزىء من كلوريد الصوديوم) وبإضافة تركيز0,04 أو 06, 0 جزئي من كلوريد الكالسيوم إلى طحلب *نوستوك لينكيا* المزروع في وسط غذائى به 0,2 جزىء من كلوريد الصوديوم لم تحدث أى تغيرات في أنواع البروتين ولكن فقط في النسبة المئوية لكثافة البروتين وذلك مقارنة بالمزرعة المضاف إليها ملح الصوديوم (0,2 جزىء من كلوريد الصوديوم فقط).