Effects of Cyanobacteria crude extracts on growth and related physiological activities of *Chlorococcum humicola and Chlorella vulgaris*

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ABSTRACT

This, investigation was concerned with studying the effects of the crude extract of four cyanobacteria on the growth and some physiological activities of two unicellular green algae (Chlorella vulgaris and Chlorococcum humicola). Two of these cyanobacteria (Microcystis aeruginosa and Nodularia harveyana) are known to produce toxin, while the other two cyanobacteria (Nostoc linckia and Lyngbya valderianum) are known to produce auxin-like substances. The obtained results indicated, in general, that growth and the studied physiological activities, except amino acids biosynthesis, of both Chlorococcum and Chlorella were inhibited by crude extracts of the two cyanobacteria species Microcystis and Nodularia. The increase in amino acid (free amino acids & proline) biosynthesis recorded for both experimented algae treated by crude extracts of M. aeruginosa and N. harveyana might be attributed to certain defense mechanisms in response to toxin stress. The obtained data concerned with the effects of crude extracts of the two cyanobacteria Nostoc and Lyngbya on the algal species under investigation, revealed that the crude extracts in concentrations 5-15 µg/ml, had stimulatory effects on growth and physiological activities of both algae. The highest concentrations, 20 µg/ml, were inhibitory to growth and all physiological activities except for amino acids which were stimulated.

Key words: *Auxin-like substances, Chlorella vulgaris, Chlorococcum humicola, Cyanobacteria, toxins.*

INTRODUCTION

Several cyanobacteria genera and species that form water blooms in fresh and brackish water bodies, produce potent hepatotoxic and / or neurotoxic compounds. These are responsible for repeated cases of animal and human poisoning in many areas of the world (Carmichael *et al.,* 1988; Sivonen *et al.,* 1990; Lawton and Codd, 1991; Azevedo *et al.,* 1994).The colonial species of *Microcystis aeruginosa* and the filamentous species *Nodularia harveyana* are common hepatotoxic cyanobacteria in eutrophic freshwaters (Carmichael, 1992; Beattie *et al.,* 2000). *Microcystis aeruginosa* can produce peptide-toxins (Microcystins) which are responsible for death of livestock, birds and fish, as well as for gastrointestinal illness in humans (Birk *et al.,* 1989). *Nodularia harveyana* had been also shown to produce a peptide-toxin (Nodularin) with a similar hepatotoxic activities; this peptide was shown to be smaller than that of Microcystin, however, with similar chemical

composition and causes the same liver damage for animals (Carmichael, 1997). A limited literature was published on the effect of cyanobacteria toxins on plants (Abe *et al.,* 1996; Kurki-Helasmo and Meriluoto, 1998; Codd *et al.,* 1999; ; Saker *et al.,* 2000; McElhiney *et al.,* 2001).

On the other hand, the occurrence of biologically active substances which promote the growth of algae and other plant organisms had been reported by many investigators (Gupta and Gupta, 1970; Abdel Wahab and Kobbia, 1976; Jeannin *et al.,* 1991; Cho *et al.*, 1997 & 1999; Hong *et al*., 1997; Shanab, 2001). Recently the crude extract of five freshwater cyanobacteria were found to have auxin-like activity on potato tissue cultures (Shanab *et al.,* 2003).

This investigation was carried out to study the effects of two toxin producing cyanobacteria species (*Microcystis aeruginosa* and *Nodularia harveyana*) and two cyanobacteria species producing auxin-like substances (*Nostoc linckia* and *Lyngbya valderianum*) on the growth and some physiological activities of two unicellular green algae (*Chlorella vulgaris* and *Chlorococcum humicola*).

MATERIALS AND METHODS

Test organisms

Cultures of *Chlorella vulgaris* Beij. and *Chlorococcum humicola* (Näg.) Rab. (two unicellular, non-motile, green algae) were isolated from soil sites of El-Fayoum (Egypt), where they flourish nearly all the year round, they were prepared in unialgal Axenic cultures (Abdel-Rahman *et al.*, 2004 a).

Culture and crude extract of Cyanobacteria

Axenic unialgal cultures of *Microcystis aeruginosa* PCC 7806 and *Nodularia harvenya* PCC 7804 were kindly supplied by the Pasteur Culture Collection (PCC), Paris, France. The two species *Nostoc linckia* and *Lyngbya valderianum* were isolated from soil and water of Ain Helwan (Egypt), purified and prepared in Axenic unialgal cultures by Shanab *et al.* (2003). The four cyanobacteria species were cultured in 1 litre conical flasks containing 400 ml BG-11 medium (Hughes *et al.*, 1958) supplemented with sterile compressed air (by passing through a series of "Wolf" bottles containing disinfecting solutions of $CuSO_4$, $HgCl_2$ and ended with sterilized water) and kept in controlled conditions of light (80 µmol $m^{-2}s^{-1}$, 16hr / 8hr light / dark regime) and temperature (25± 1ºC). Algal cells were harvested, by centrifugation, at the end of exponential growth phase, lyophilized and kept in deep freeze (-20ºC). The crude extracts were prepared by suspending 5 mg of lyophilized cells in 50 ml Bold's basal medium (Bischoff and Bold,1963), sonnicated for 10 min. at 90 K cycles (Fisher Sonic Dismembrator Model 150), then filtered through Watmann No.1 filter paper.

Treatments

All experiments were carried out in 250 ml conical flasks, containing 100 ml Bold's basal medium which was adjusted to contain 0 (control), 5, 10, 15 or 20 µg/ml of lyophilized cell extract of the 4 previously mentioned cyanobacteria species. Culture conditions were the same as previously mentioned. The starting cultures were adjusted to contain 0.055 x 10^6 cells / ml for both *Chlorococcum humicola* and *Chlorella vulgaris*. The experimental cultures were harvested at the beginning of the stationary phase, 7 days old, for the determination of growth and some physiological activities of the two experimented algae (Abdel-Rahman *et al.*, 2004 b).

Measurements

Growth parameters were mainly determined by cell counts (using haemocytometer slide) and dry weight (filtration through weighed glass filter and dried overnight at 105 ºC). Pigments (Chlorophylls a and b, carotenoids) were determined (homogenized algal cells kept in 85% acetone overnight at 4 ºC and spectrophotometrically measured at 542, 644 and 663 nm) from equations proposed by Metzner *et al.*, 1965. Carbohydrates (total, water soluble and insoluble) were determined by anthrone-sulfuric acid method (Fales, 1951; Schlegel, 1956). Protein (total, water soluble and insoluble), free amino acids and proline were measured according to the methods proposed by Lowry *et al.* (1951), Moore and Stein (1948) and Bates *et al.* (1973) respectively. The obtained data were statistically analyzed using the least significant difference test (L.S.D) at 1% and 5% levels of probability and presented in Tables (1-4); results of free amino acids, proline and total amino acids illusterated in Figures (1 and 2) as % of the control.

RESULTS

Data presented in Table (1) summarize the effect of different concentrations of 4 cyanobacteria crude extracts on growth (Cell No.) and pigment content of *Chlorococcum humicola.* It is clear that all crude extract concentrations of *Microcystis* and *Nodularia* had significant inhibitory effects on growth, pigments, carbohydrates (all fractions) and proteins (all fractions) Table (2). Carotenoids, however, were slightly stimulated at low concentrations (5 and 10 µg/ml) of the two previous cyanobacteria extracts. On the contrary, amino acid biosynthesis, especially proline and free amino acids (Fig. 1), were significantly stimulated by all concentrations of extracts used.

On the other hand, the crude extracts of *Nostoc* and *Lyngbya* at concentrations from 5 to 15 µg/ml, had a general stimulatory effect on growth, pigments (Table 1), carbohydrates, proteins (Table 2) and amino acid biosynthesis (Fig.1). The highest concentration of extracts used (20 µg/ml), however, had inhibitory effects on growth and all physiological activities studied, except the biosynthesis of amino acids, especially free amino acids and proline, which were significantly stimulated.

The effects of crude extracts of *Microcystis* and *Nodularia* on *Chlorella vulgaris* were similar to a great extent to those observed for *Chlorococcum humicola*, where inhibition was observed for growth, pigments (Table 3), carbohydrate and protein biosynthesis (Table 4), while a stimulation was recorded for amino acids biosynthesis (Fig. 2).

Crude extracts of *Nostoc* and *Lyngbya* at concentrations 5-15 µg/ml (Fig. 2) showed, in general, very high significant stimulation of growth and all physiological activities investigated, expect amino acid biosynthesis which was either non significantly changed or slightly changed in some cases (i.e. slightly stimulated or inhibited). The higher concentration of crude extracts of these two cyanobacteria (20 µg/ml) caused significant inhibition of growth and all studied physiological activities, except for proline which was significantly stimulated.

It was clear that the stimulation of growth and physiological activities by the crude extracts of *Nostoc* and *Lyngbya* was more pronounced for *Chlorella* than for *Chlorococcum.* On the contrary these extracts stimulated the production of more proline in *Chlorococcum* than in *Chlorella.*

Table (1): Effect of different concentrations of cyanobacteria crude extract on growth (cell No.) and pigment contents of Chlorococcum humicola.

Treatments Algal extract (µg / ml)	[X]Cell No. x10^6 / ml	[X]Pigments (µg / ml)		
		Chlorophylls (a + b)	Carotenoids	Total
Control	**0.991± 0.003**	**2. 219 ± 0.030**	**1.060 ± 0.020**	**3.279 ± 0.053**
Microcystis				
5	0.871^S ± 0.003	1.808 ^{N} ± 0.092	1.179 ^{N} ± 0.079	2.987 ^{N}± 0.053
10	0.770 ^{S} ± 0.010	1.632 ^{S} ± 0.044	1.214 ^{S} ± 0.033	2.846 ^{S} ± 0.079
15	0.760 ^{S} ± 0.005	1.518 ^{S} ± 0.030	0.810 ^{S} ± 0.026	2.328 ^{S} ± 0.050
20	0.392 ^{S} ± 0.003	0.792 ^{S} ± 0.018	0.431 ^{S} ± 0.053	1.223 ^{S} ± 0.022
Nodularia				
5	0.984 ^{N} ± 0.043	1.342 ^{S} ± 0.020	0.911 ^{S} ± 0.005	2.253 ^{S} ± 0.003
10	0.871 ^{S} ± 0.003	1.809 ^{S} ± 0.092	1.144 ^{N} ± 0.026	2.953 ^{S} ± 0.040
15	0.770 ^{S} ± 0.009	1.874 ^{S} ± 0.015	0.933 ^{S} ± 0.015	2.807 ^{N} ± 0.030
20	0.536 ^{S} ± 0.003	1.333 ^{S} ± 0.007	0.546 ^{S} ± 0.053	1.879 ^{S} ± 0.062
Nostoc				
5	1.575 ^{S} ± 0.001	2.249 ^{N} ± 0.003	1.285 ^{S} ± 0.005	3.534 ^{S} ± 0.004
10	1.924 ^{S} ± 0.026	2.482 ^{S} ± 0.057	1.259 ^{S} ± 0.028	3.741 ^{S} ± 0.084
15	1.663 ^{S} ± 0.004	2.345 ^{S} ± 0.018	0.942 ^{S} ± 0.036	3.287 ^{N} ± 0.053
20	0.903 ^{S} ± 0.005	1.602 ^{S} ± 0.038	0.449 ^{S} ± 0.005	2.051 ^{S} ± 0.044
Lyngbya				
5	1.512 ^{S} ± 0.006	2.486 ^{S} ± 0.038	1.593 ^{S} ± 0.079	4.079 ^{S} ± 0.119
10	1.734 ^{S} ± 0.008	2.332 ^{S} ± 0.010	1.162 ^{S} ± 0.015	3.494 ^{S} ± o.o26
15	1.639 ^{S} ± 0.003	2.301 ^{N} ± 0.050	1.166 ^{S} ± 0.013	3.467 ^{N} ± 0.066
20	0.898 ^{S} ± 0.005	1.558 ^{S} ± 0.062	0.546 ^{S} ± 0.015	2.096 ^{S} ± 0.075

[X] Letters beside data indicate the statistical analysis where: N = Non significant and S = significant at least at P > 0.05.

Table (2): Effect of different concentrations of cyanobacteria crude extract on carbohydrate and protein contents of Chlorococcum humicola.

Treatments Algal extract (µg / ml)	[X]Carbohydrate (µg / mg Dry Wt.)			[X]Protein (µg / mg Dry Wt.)		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Control	**42.87 ± 0.64**	**182.64 ± 0.58**	**225.51 ± 0.64**	**22.17 ± 0.59**	**64.88 ± 0.07**	**87.05 ± 0.58**
Microcystis						
5	38.06^S ± 0.06	160.30^S ± 0.75	198.36^S ± 0.69	16.57^S ± 0.58	50.39^S ± 0.58	66.96^S ± 1.10
10	40.48^N ± 0.20	139.05^S ± 0.29	179.53^S ± 0.58	16.12^S ± 0.64	46.43^S ± 0.62	62.55^S ± 1.2
15	40.30^S ± 0.15	100.31^S ± 0.12	140.61^S ± 0.17	12.08^S ± 0.53	36.52^S ± 0.06	48.60^S ± 0.64
20	20.40^S ± 0.69	45.06^S ± 0.58	65.46^S ± 0.60	5.38^S ± 0.01	18.09^S ± 0.12	23.47^S ± 1.2
Nodularia						
5	34.59^S ± 0.06	102.12^S ± 0.59	136.71^S ± 0.65	17.04^S ± 0.61	60.90^N ± 2.90	77.94^S ± 0.58
10	38.06^S ± 0.06	160.30^S ± 0.57	198.36^S ± 0.69	16.57^S ± 0.58	50.39^S ± 0.58	66.96^S ± 1.10
15	31.58^S ± 0.07	78.29^S ± 0.58	109.87^S ± 0.59	22.24^N ± 1.2	64.17^N ± 0.59	86.41^N ± 0.63
20	30.51^S ± 0.60	75.03^S ± 0.06	105.54^S ± 0.58	7.59^S ± 0.12	29.46^S ± 0.02	37.05^S ± 0.64
Nostoc						
5	40.84^S ± 0.06	184.62^N ± 1.2	225.46^N ± 0.6	30.69^S ± 0.58	108.35^S±0.66	138.04^S ± 1.2
10	50.70^S ± 0.61	176.15^S ± 1.2	226.85^N ± 1.2	27.54^S ± 1.40	101.70^S ± 1.2	129.24^S ± 0.58
15	50.36^S ± 0.69	154.71^S ± 0.64	205.07^S ± 0.58	24.80^S ± 0.58	98.38^S ± 0.58	123.18^S ± 1.2
20	30.12^S ± 0.06	80.31^S ± 0.12	110.43^S ± 0.68	16.12^S ± 0.64	60.12^S ± 0.58	76.24^S ± 0.58
Lyngbya						
5	42.07^N ± 0.64	180.53^S ± 0.12	222.60^N ± 1.1	30.12^S ± 0.06	96.07^S ± 0.58	126.19^S ± 0.06
10	51.31^S ± 0.74	162.61^S ± 0.53	213.92^S ± 1.1	27.00^S ± 0.04	92.48^S ± 0.59	119.44^S ± 0.33
15	50.63^S ± 1.70	160.52^S ± 0.69	211.15^S ± 0.57	22.28^N ± 1.2	90.16^S ± 0.02	112.44^S ± 1.2
20	31.27^S ± 0.59	82.26^S ± 0.02	113.53^S ± 1.2	12.20^S ± 0.06	60.01^S ± 0.58	72.21^S ± 0.46

[X] Letters beside data indicate the statistical analysis where: N = Non significant and S = significant at least at P > 0.05.

Table (3): Effect of different concentrations of cyanobacteria crude extract on growth (cell No.) and pigment contents of Chlorella vulgaris.

Treatments Algal extract (µg / ml)	XCell No. X 106 / ml	XPigments (µg / ml)		
		Chlorophylls (a + b)	Carotenoids	Total
Control	**1.358 ± 0.003**	**2.147 ± 0.053**	**1.016 ± 0.010**	**3.163 ± 0.132**
Microcystis				
5	1.387 N ± 0.011	1.971 S ± 0.030	1.118 S ± 0.026	3.089 N ± 0.057
10	1.578 S ± 0.026	1.870 S ± 0.036	1.250 S ± 0.026	3.120 N ± 0.062
15	1.163 S ± 0.031	1.434 S ± 0.030	1.179 S ± 0.028	2.613 S ± 0.057
20	0.542 S ± 0.035	0.792 S ± 0.007	0.823 N ± 0.028	1.615 S ± 0.290
Nodularia				
5	1.133 S ± 0.005	2.222 N ± 0.038	0.972 S ± 0.003	3.194 N ± 0.062
10	1.387 N ± 0.011	1.971 S ± 0.003	0.972 S ± 0.003	2.943 N ± 0.150
15	1.057 S ± 0.001	1.870 S ± 0.036	1.025 N ± 0.010	2.895 N ± 0.062
20	0.787 S ± 0.006	1.091 S ± 0.018	0.946 S ± 0.013	2.037 S ± 0.030
Nostoc				
5	3.762 S ± 0.003	2.895 S ± 0.028	1.522 S ± 0.026	4.417 S ± 0.053
10	4.086 S ± 0.004	3.186 S ± 0.038	1.716 S ± 0.026	4.902 S ± 0.062
15	2.938 S ± 0.008	2.680 S ± 0.013	1.540 S ± 0.026	4.220 S ± 0.038
20	1.265 S ± 0.005	1.628 S ± 0.026	0.810 S ± 0.010	2.438 S ± 0.356
Lyngbya				
5	3.250 S ± 0.003	3.018 S ± 0.015	1.553 S ± 0.018	4.571 S ± 0.033
10	3.172 S ± 0.001	2.702 S ± 0.036	1.716 S ± 0.010	4.418 S ± 0.048
15	2.632 S ± 0.003	2.578 S ± 0.006	1.505 S ± 0.026	4.083 S ± 0.084
20	1.788 S ± 0.002	2.108 N ± 0.106	1.060 S ± 0.003	3.168 N ± 0.110

[X] Letters beside data indicate the statistical analysis where: N = Non significant and S = significant at least at P > 0.05.

Table (4): Effect of different concentrations of cyanobacteria crude extract on carbohydrate and protein contents of Chlorella vulgaris.

Treatments Algal extract (µg / ml)	[X]Carbohydrate (µg / mg Dry Wt.)			[X]Protein (µg / mg Dry Wt.)		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Control	**35.20 ± 0.81**	**160.65 ± 0.12**	**195.85 ± 0.35**	**25.54 ± 1.2**	**75.31 ± 1.2**	**100.85 ± 2.3**
Microcystis						
5	34.72^{N} ± 0.58	128.46^{S} ± 0.58	163.18^{S} ± 1.2	20.17^{S} ± 1.2	64.51^{S} ± 1.1	84.68^{S} ± 1.3
10	32.06^{S} ± 0.58	105.08^{S} ± 0.12	137.14^{S} ± 0.69	19.43^{S} ± 0.85	54.59^{S} ± 1.7	74.02^{S} ± 1.3
15	30.91^{S} ± 0.58	83.29^{S} ± 1.20	114.20^{S} ± 1.8	17.22^{S} ± 0.7	41.91^{S} ± 1.2	59.13^{S} ± 1.9
20	21.49^{S} ± 0.26	42.12^{S} ± 0.63	63.61^{S} ± 0.89	7.53^{S} ± 0.62	25.59^{S} ± 1.2	33.12^{S} ± 1.8
Nodularia						
5	35.75^{N} ± 0.66	169.87^{S} ± 0.08	205.62^{S} ± 0.74	20.84^{S} ± 1.1	53.85^{S} ± 1.2	74.69^{S} ± 1.3
10	34.72^{N} ± 0.58	128.46^{S} ± 0.58	163.18^{S} ± 1.2	20.17^{S} ± 1.2	64.51^{S} ± 0.97	84.68^{S} ± 1.3
15	35.57^{N} ± 0.10	128.01^{S} ± 0.58	163.58^{S} ± 1.2	22.52^{N} ± 1.1	54.59^{S} ± 1.7	77.11^{S} ± 1.3
20	28.42^{S} ± 0.58	65.36^{S} ± 0.58	93.77^{S} ± 1.2	10.47^{S} ± 1.2	34.04^{S} ± 0.58	44.51^{S} ± 1.7
Nostoc						
5	45.48^{S} ± 0.52	233.79^{S} ± 1.1	279.27^{S} ± 1.6	39.42^{S} ± 0.68	98.92^{S} ± 1.1	38.34^{S} ± 1.7
10	44.88^{S} ± 0.52	225.71^{S} ± 0.59	270.59^{S} ± 1.2	36.60^{S} ± 0.6	96.02^{S} ± 1.2	132.62^{S} ± 1.8
15	41.93^{S} ± 0.59	212.56^{S} ± 1.2	254.49^{S} ± 1.7	30.82^{S} ± 1.3	82.91^{S} ± 0.57	113.73^{S} ± 1.9
20	36.05^{N} ± 0.58	156.34^{S} ± 0.55	192.39^{S} ± 1.1	16.61^{S} ± 1.2	38.89^{S} ± 0.58	54.90^{S} ± 1.7
Lyngbya						
5	46.59^{S} ± 0.12	236.71^{S} ± 0.46	283.3^{S} ± 0.58	32.52^{S} ± 1.2	96.63^{S} ± 0.58	129.15^{S} ± 1.7
10	45.85^{S} ± 0.64	231.24^{S} ± 0.64	277.09^{S} ± 1.3	30.45^{S} ± 1.2	94.19^{S} ± 1.2	124.64^{S} ± 2.3
15	40.78^{S} ± 1.0	206.46^{S} ± 1.2	247.24^{S} ± 2.2	28.64^{N} ± 1.2	80.28^{N} ± 1.9	108.92^{N} ± 2.1
20	35.27^{N} ± 0.17	155.69^{S} ± 0.69	190.96^{S} ± 0.87	12.05^{S} ± 0.64	30.20^{S} ± 1.9	42.25^{S} ± 1.5

[X] Letters beside data indicate the statistical analysis where: N = Non significant and S = significant at least at P > 0.05

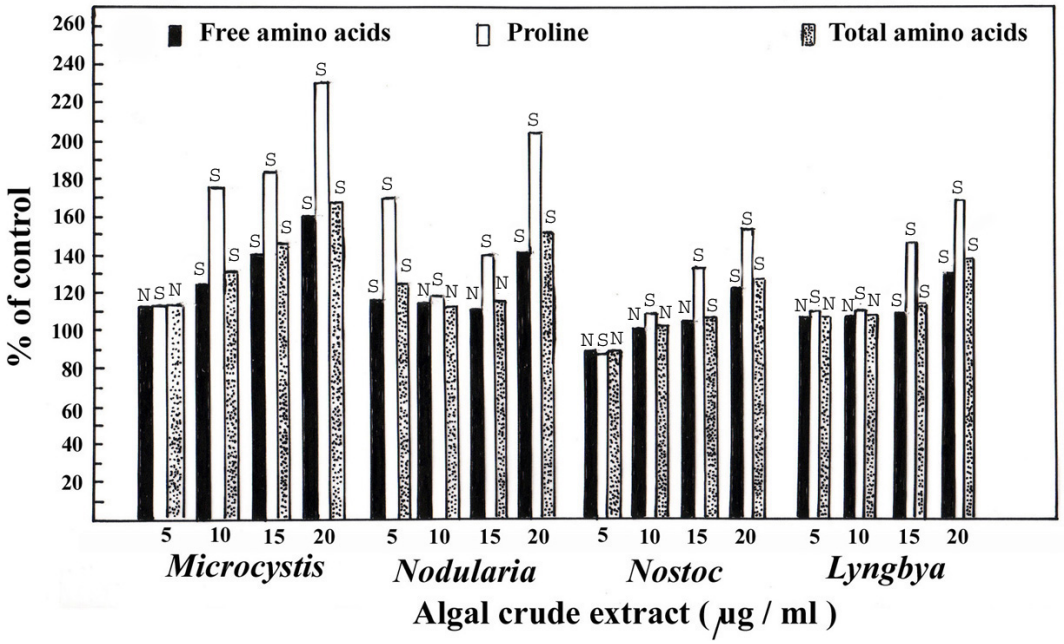

Fig. (1): Effect of different concentrations of Cyanobacteria crude extract on free amino acids, proline and total amino acids of Chlorococcum humicola. Results are presented as % of control. Letters on the columns indicate the statistical analysis of the original data, where: N = Non significant and S = significant at least at P > 0.05.

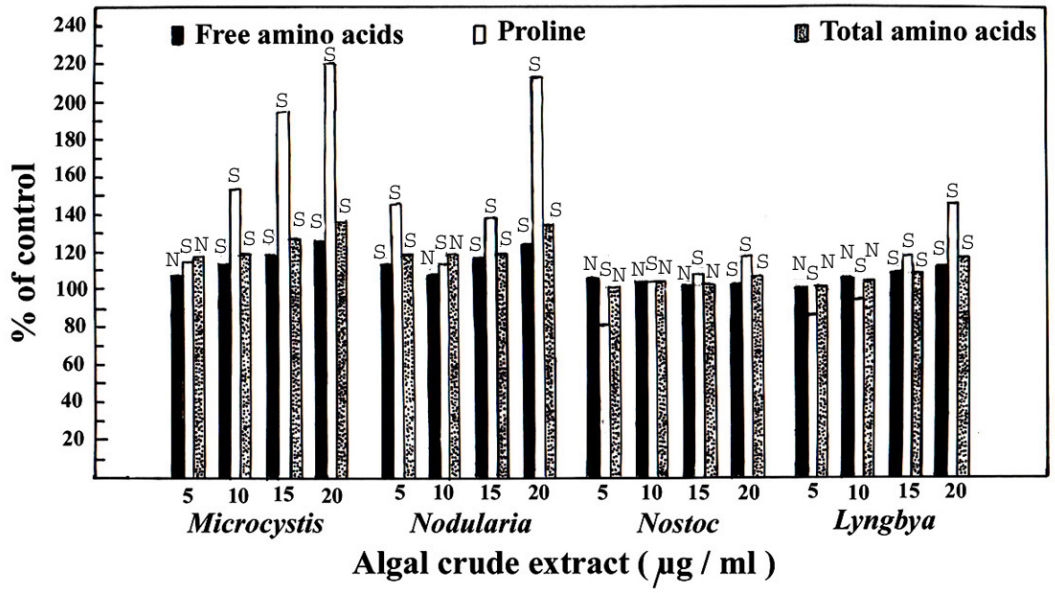

Fig. (2): Effect of different concentrations of Cyanobacteria crude extract on free amino acids, proline and total amino acids of Chlorella vulgaris. Results are presented as % of control. Letters on the columns indicate the statistical analysis of the original data, where: N = Non significant and S = significant at least at P > 0.05.

DISCUSSION

The obtained results in this work revealed, in general, that growth and the studied physiological activities of both *Chlorococcum* and *Chlorella* were inhibited by crude extracts of the two cyanobacteria *Microcystis aeruginosa* and *Nodularia harveyana*. Similar results were recorded in higher plants, where microcystin-LR (0.005 and 0.05 µ g/ml) was found to inhibit the growth and chlorophyll biosynthesis in tissue cultures of *Solanum tuberosum* and microcystin-LR, -RR and –LA (1.6, 1.9 and 7.7 µ g/ml) were found to inhibit the growth of *Sinapsis alba* seedling (McElhiney *et al.,* 2001). Abe *et al.*(1996) had reported that the rate of photosynthesis of the primary leaves of *Phaseolus vulgaris* was reduced by 50% within 8hr of dipping leaves in microcystin; they proposed that relatively low concentrations of microcystin-LR caused damage to the photosynthetic apparatus of *P. vulgaris*. Other authors (Kurki-Helasmo and Meriluoto, 1998) cultivated *Sinapsis alba* (cv BOR 351) seeds for 7 days on a solid nutrient medium supplemented with 0-40 µg/ml microcystin-RR. They recorded that the growth of the resulting seedling was affected at 0.8 µg/ml microcystin-RR and malformed plants were observed at 5.0 µ g/ml microcystin; they also found that the inhibition of protein phosphatase 1 and 2A activity was correlated with growth inhibition.

Although, no investigations were reported for the effect of Nodularin on plants, yet inhibition of growth and metabolism in plants is expected due to the similarity in chemical composition of nodularin and microcystin, as found with results in this work.

The increase in amino acid (free amino acids & proline) biosynthesis recorded for both experimented algae treated by crude extracts of *M. aeruginosa* and *N. harveyana* might be attributed to certain defense mechanisms in response to toxin stress; a similar behavior was observed for these two algae when they were under salinity stress (Abdel-Rahman *et al.,* 2004 b). The effect of the crude extracts of *M. aeruginosa* and *N. harveyana* or the purified microcystin and nodularin on the different enzymes of *Chlorococcum* or Chlorella, such as protein phosphatase, Δ-pyrroline-5-carboxylate synthetase and proline dehydrogenase, under toxin stress are waiting for future investigations.

The obtained data concerned with the effects of crude extracts of the two cyanobacteria *Nostoc* and *Lyngbya* on the experimented algae, revealed that the concentrations of crude extracts, 5-15 µg/ml, had stimulatory effects on growth and physiological activities of both experimented algae. Higher concentrations of crude extracts, 20 µg/ml, were inhibitory to growth and all physiological activities except for amino acids which were stimulated. The same cyanobacteria species (*Nostoc linckia* and *Lyngbya valderianum*) were used in one of our recent publications (Shanab *et al.,* 2003), where it was found that both of them had an auxin-like activity, stimulated the proliferation of healthy strong potato plantlets (6.9 cm shoot length, 4 cm root length and 4 leaves per shoot) from nodal explants (1 cm). Therefore it is not surprising to observe such stimulatory effect of the crude extract of these two cyanobacteria. Water extracts of marine algae were found, by some authors, to activate the growth of micro-algae (Cho *et al.,* 1999). The inhibitory effect of high concentrations of the crude extract of *Nostoc* and *Lyngbya* could be better explained, in future studies, when endogenous content of auxin, as well as perhaps exogenously secreted ones, are

measured for the experimental algae under normal and toxin-stress conditions.

Finally, cyanobacteria were used, for a long time, in many parts of the world in agricultural practice in Paddy fields (Watanabe, 1962; El Nawawy, 1972). The increase in crop yields as a result of algal inoculation can not only be attributed to nitrogen-fixing property of cyanobacteria, but may be largely due to the growth regulating substances endogenously produced by these algae. This suggestion is greatly supported by the fact that non-nitrogen fixing species as *Phormidium* spp. and *Oscillatoria* spp. (Gupta and Shukla, 1967; Gupta and Gupta, 1970) stimulated the growth of rice. It is hoped that unicellular green algae would give solutions for the toxic and stimulatory mechanisms involved in such effects of cyanobacteria on plants.

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الملخص العربي

تأثير مستخلصات السيانوبكتريا على النمو و الانشطة الفسيولوجية لطحلبي كلوروكوكم هيوميكولا و كلوريلا فولجاريس

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أهتم هذا البحث بدراسة تأثير المستخلصات الخام لأربعة من السيانوبكتيريا على النمو وبعض الانشطة الفسيولوجية لطحلبين من الطحالب الخضراء وحيدة الخلية (كلوروكوكم هيوميكولا و كلوريلا فولجاريس). ولقد دلت الابحاث السابقة على ان نوعين من السيانوبكتيريا المستخدمة (ميكروسيستس ايروجينوزا و نوديولاريا هارفيانا) تنتج سموما ، بينما النوعين الاخرين المستخدمين (نوستوك لينكيا و لينبيا فالدريانم) تنتج مواد شبيهة بالاوكسينات الموجودة في النباتات الراقية.

دلت النتائج ، بوجه عام ، ان جميع تركيزات المستخلصات الخام لطحلبي ميكروسيستس و نوديولاريا تسببت في تثبيط كبير لنمو وللانشطة الفسيولوجية لك ل من طحلبي كلوروكوكم و كلوريلا. و لقد اقترح ان يكون الاجهاد الناتج عن المواد السامة مشابه للاجهاد الملحي الذي سبق دراسته معمليا لهذين الطحلبين حيث انتجا في كلا الحالتين ، الاجهاد الملحي و الاجهاد السمي ، كمية كبيرة من البرولين و الاحماض الامينية الحرة ، الا ان ذلك يحتاج الى دراسات مستقبلية للانزيمات و التي عرف عنها التدخل لمواجهة الاجهاد الملحي. و على النقيض مما سبق ، تسببت التركيزات ٥-١٥ ميكروجرام / مل من المستخلصات الخام لطحلبي نوستوك و لينبيا في تحفيز معنوي للنمو و للانشطة الفسيولوجية لكل من الطحلبين المستخدمين في هذه الدراسة (كلوروكوكم و كلوريلا)، الا ان التركيز المرتفع (٢٠ ميكروجرام / مل) من المستخلصات تسبب في تثبيط النمو وجميع الانشطة الفسيولوجية فيما عدا تخليق الاحماض الامينية و الذي حفز بهذا التركيز. ويوضح البحث صعوبة تفسير ذلك دون معرفة محتوى الاوكسينات النباتية الداخلية او المحتمل افرازها للخارج في طحالب التجارب في الظروف العادية اوفي ظروف الاجهاد السمي.