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EFFECT OF DIFFERENT FORMS OF NITROGEN ON GROWTH, NITROGEN FIXATION AND PIGMENT CONTENTS OF CYANOBACTERIA

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ABSTRACT

Growth, nitrogen fixation and pigment contents were followed in four batch cultures of local cyanobacteria strains, namely *Anabaena sp.*, *Nostoc muscorum, Tolypothrix tenuis* and *Calothrix brevissima* supplemented with range of Nitrate-N, urea-N and Ammonium-N. All the nitrogen sources decreased significantly the growth of the tested cyanobacteria strains in the term of dry weight criteria. Cyanobacteria strains exhibited variable nitrogen fixed amounts, chlorophyll a and C-phycocyanin amounts due to the exposure any of Nitrate-N, urea-N and Ammonium-N However, the ability of these cyanobacteria strains to assimilate the nitrogen source differs from each other. This could be explained by giving the assimilation order of the tested nitrogen sources as NO_3.N > urea-N > ammonium-N for all cyanobacteria strains.

INTRODUCTION

Diazotrophic cyanobacteria are a major component of microbial flora in rice paddy fields, and contribute to the fertility of such ecosystems (Roger and Kulasooriya, 1980;Ghazal, 1988; Whitton and Roger, 1989). Research on the practical utilization of cyanobacteria in rice fields has been focused mainly on inoculation with cyanobacterial inocula produced in soil (Venkataraman, 1981). Inoculation techniques are usually based on utilization of foreign strains, which are presumably better adapted to local environmental conditions. This implies that the number and contribution of inoculated strains to total nitrogen fixation are normally lower than that of indigenous strains (Reddy and Roger, 1988). Agricultural practices that enhance the growth of local strains for producing inocula have been proposed as a better way to improve the use of cyanobacteria as biofertilizers (Roger, 1989). The selection of strains of inoculation should take account of the response of their nitrogen fixing capacity to situations that can limit it, such as carbon limitation, Presence of combined nitrogen and changes of light intensity and pH (Prosperi *et al.*, 1992 and Mussa *et al.*, 2003).

This work deals with the growth of four cyanobacteria isolated from the Egyptian rice fields, their nitrogen fixing capacity as well as their chlorophyll a and C-phycocyanin contents in relation to the presence of different combined nitrogen sources (NO3-N, urea-N and ammonium-N) commonly used as nitrogen fertilizers in rice fields.

MATERIALS AND METHODS

Cyanobacterial strains, namely *Anabaena sp.*, *Nostoc muscorum*, *Tolypothrix tenuis* and *Calothrix brevissima* isolated from the Egyptian rice fields (Ghazal, 1988). They were then grown on modified Watanabe medium (EL-Nawawy *et al.*, 1958) under continuous illumination (5000 Lux) at temperature of 28 – 32 ^{0} C up to their appropriate logarithmic growth phase

The developed cyanobacteria growth was then inoculated (10 mL homogenized culture) into 500 mL flasks containing 100 mL sterilized modified Watanabe medium previously supplied with 0, 25, 50 and 100 ppm (NO_3–N), 0, 25, 50 and 100 ppm (urea–N) and 0, 25, 50 and 100 ppm (NH_4–N) each individually. Each treatment was repeated 3 times and arranged in complete randomized design (Gomez and Gomez, 1984). Flasks were then incubated for intervals of 0, 7, 15 and 21 days under continuous white light (3000 Lux) at 28-32 °C. At the end of each incubation period, the growth of each cyanobacterium strain was harvested by filtration and subjected to determine the cyanobacteria dry weight (g L^{-1} medium), extra and intracellular -N (mg L^{-1} medium), total fixed–N (mg L^{-1} medium) chlorophyll a (µg mL^{-1} cyanobacteria suspension), C-phycocyanin (µg mL^{-1}cyanobacteria suspension) and C-phycocyanin / chlorophyll a ratio.

Total nitrogen in cyanobacteria filaments and /or extra-cellular nitrogen filtrate was determined using the micro-Kjeldahl method according to Jackson (1973).

Chlorophyll a content of the filamentous cyanobacteria was estimated according to the method described by Metzener et al. (1965) and Chlorophyll a concentration was then calculated according the following equation:

Chlorophyll a concentration = $10.3E_{663} - 0.918E_{644}$ = µg. Chl. mL^{-1}

C-Phycocyanin pigment was extracted by the method of Chapman (1988).C- phycocyanin concentration was then calculated according to the formula of Bryant et al. (1979) as the following:

$$\text{Concentration of C- phycocyanin (µg mL}^{-1}) = \frac{E\,(620nm) - 0.72\,E\,(650nm)}{6.29}$$

RESULTS AND DISCUSSION

Data in Tables 1,2, &3 and Figures 1, 2 &3 indicate the effect of three different nitrogen sources on the growth and pigments contents of four cyanobacteria strains namely *Anabaena sp.*, *Nostoc muscorum, Tolypothrix tenuis* and *Calothrix brevissima*. Nitrogen sources and the cyanobacteria strains are commonly utilized as mineral fertilizers and biofertilizers in rice fields, respectively.

Nitrate nitrogen:

Incorporation of NO_3-N into culture medium had significantly adversed biomass production of all cyanobacteria strains (Table 1).

The depression in dry weight yield considerably varied depending upon cyanobacteria strains, culture age and NO_3-N level.

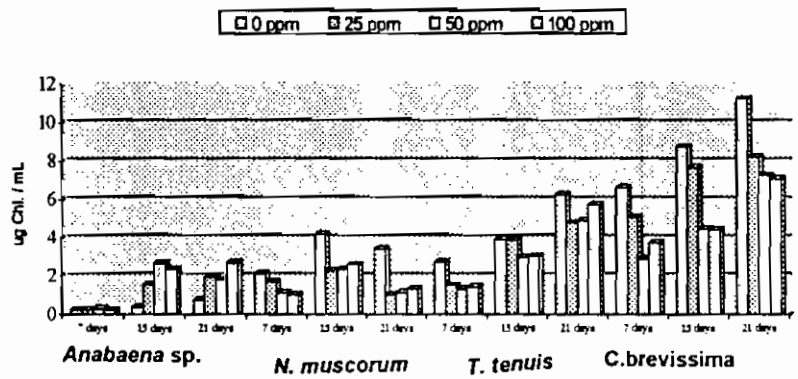

Fig. (1a): Effect of different concentrations of NO_3-N on chlorophyll a in cyanobacterial suspension.

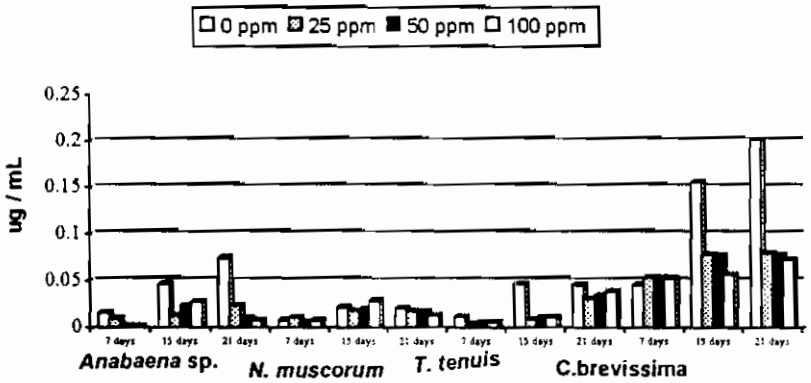

Fig. (1b): Effect of different concentrations of NO_3-N on C-phycocyanin in cyanobacterial suspension.

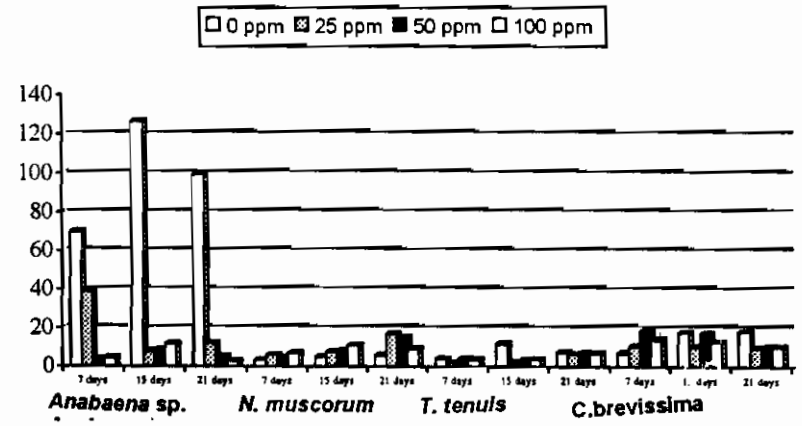

Fig. (1c): Effect of different concentrations of NO_3-N on C-phycocyanin/Chlorophyl a ratio in cyanobacterial suspension.

Table (1): Sodium nitrate influence on dry weight, Extracellular-N Intracellular-N and Total fixed-N of different cyanobacterial strains

strains	*Anabaena sp.*			*Nostoc muscorum*			*Tolypothrix tenuis*			*Calothrix brevissima*		
NO_3-N	period days											
ppm	7	15	21	7	15	21	7	15	21	7	15	21
	Dry weight (gl^{-1} medium)											
0	0.780	1.090	1.390	0.570	1.310	1.400	0.770	1.390	2.380	1.400	1.540	1.970
25	0.420	0.830	1.190	0.540	1.040	1.670	1.020	1.200	1.870	1.040	1.350	2.580
50	0.130	0.490	1.080	0.440	1.010	1.320	0.810	0.960	1.850	1.020	1.310	2.260
100	0.110	0.480	1.060	0.430	0.920	0.730	0.400	0.820	1.780	0.850	1 010	1.980
	Extracellular-N (mg l^{-1} medium)											
0	19.000	14.000	7.000	13.000	14.000	7.000	12.000	17.000	7.000	12.000	17.000	9.000
25	17.000	14.000	5.000	15.000	21.000	10.000	14.000	16.000	8.000	11.000	17.000	6.000
50	14.000	16.000	9.000	14.090	17.000	12.000	15.000	13.000	10.000	9.000	18.000	8.000
100	16.000	19.000	12.000	12.000	15.000	16.000	19.000	21.000	10.970	10.000	16.000	9.000
	Intracellular-N (mg l^{-1} medium)											
0	12.600	52.100	63.240	22.230	65.500	70.700	46.500	72.600	87.420	57.700	78.120	135.800
25	22.300	44.400	57.070	29.100	59.900	63.200	21.900	51.510	65.200	47.900	54.600	130.500
50	14.600	25.900	48.200	27.900	46.900	58.500	27.200	51.710	74.200	39.400	82.500	125.600
100	12.200	19.200	43.800	21.000	40.590	29.300	18.300	48.900	63.000	37.600	70.800	119.040
	Total fixed-N (mg l^{-1} medium)											
0	36.930	66.100	70.240	35.230	79.500	77.700	58.500	89.600	94.420	69.700	95.120	144.800
25	39.300	58.400	62.040	44.100	80.900	73.200	35.900	67.510	73.200	58.900	71.600	136.500
50	28.600	41.900	57.530	41.990	63.900	70.500	42.200	64.710	84.200	48.400	100.500	133.600
100	28.200	38.200	55.800	33.000	55.590	45.300	37.300	69.900	73.970	47.600	86.800	128.040

LSD values for the aforementioned parameters.

	dry weight		Extracellular-N		Intracellular-N		Total fixed-N	
	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
A	0.028	0.037	0.760	1.000	2.130	2.830	1.050	1.370
B	0.028	0.037	0.760	1.000	2.130	2.830	1.050	1.370
C	0.032	0.042	0.870	1.160	2.460	3.260	1.200	1.590
AB	0.014	0.018	0.380	0.500	1.060	1.410	0.520	0.690

Table (3): Effect of different concentrations of ammonium sulphate on dry weight, Extracellular- N,Intracellular-N and Total fixed-N of different cyanobacterial strains

strains	*Anabaena sp.*			*Nostoc muscorum*			*Tolypothrix tenuis*			*Calothrix brevissima*		
NH4-N ppm	period days											
	7	15	21	7	15	21	7	15	21	7	15	21
	Dry weight (g L^{-1} medium)											
0	0.780	1.090	1.190	0.570	1.310	1.400	0.770	1.380	2.380	1.400	1.540	1.970
25	0.240	0.280	0.380	0.520	0.540	0.610	0.440	0.410	0.350	0.410	0.430	0.470
50	0.220	0.270	0.320	0.480	0.400	0.360	0.350	0.300	0.280	0.370	0.400	0.420
100	0.100	0.130	0.150	0.420	0.380	0.280	0.310	0.250	0.180	0.330	0.350	0.380
	Extracellular-N (mg L^{-1}medium)											
0	19.000	14.000	8.000	13.000	14.000	7.000	15.000	17.000	7.000	12.000	17.000	9.000
25	70.000	80.000	87.000	60.000	82.000	65.000	59.000	85.000	55.000	50.000	91.000	61.000
50	104.000	139.000	144.000	109.000	180.000	126.000	113.000	160.000	111.000	97.000	178.000	111.000
100	196.000	230.000	215.000	142.000	217.000	205.000	201.000	270.000	245.000	182.000	280.000	240.000
	Intracellu ar-N (mg L^{-1}medium)											
0	18.600	52.100	63.240	15.630	65.500	70.700	46.500	72.600	87.420	57.700	78.120	135.800
25	8.100	7.440	14.900	7.440	6.400	5.600	9.300	7.400	14.000	11.900	11.500	11.400
50	7.460	14.900	19.500	20.500	8.400	0.000	14.900	14.800	13.100	9.300	7.440	7.740
100	13.100	18.600	11.200	16.200	14.900	0.000	18.600	12.100	11.500	39.100	17.300	8.100
	Total fixed-N (mg L^{-1}medium)											
0	37.600	66.100	71.240	28.630	79.500	77.700	61.500	89.600	94.420	69.700	95.120	144.800
25	78.100	87.440	101.900	67.440	88.400	70.800	68.300	92.400	69.000	61.900	102.500	72.400
50	111.460	153.900	163.500	129.500	188.400	126.000	127.900	174.800	124.100	106.300	185.440	118.740
100	209.100	248.600	226.200	158.200	231.900	205.000	219.600	282.100	256.500	221.100	297.300	248.100

LSD values for the aforementioned parameters.

	dry weight		Extracellular-N		Intracellular-N		Total fixed-N	
	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
A	0.023	0.031	4.900	6.500	2.430	3.220	3.170	4.200
B	0.023	0.031	4.900	6.500	2.430	3.220	3.170	4.200
C	0.027	0.035	5.660	7.510	2.800	3.720	3.660	4.850
AB	0.012	0.015	2.450	3.250	1.210	1.610	1.580	2.100

Table (2): Effect of different concentrations of Urea on dry weight, Extracellular-N, Intracellular-N and Total fixed-N of different cyanobacterial strains

Urea-N	period days											
ppm	7	15	21	7	15	21	7	15	21	7	15	21
	Dry weight (g l^{-1} medium)											
0	0.780	1.090	1.190	0.570	1.310	1.400	0.770	1.390	2.380	1.400	1.540	1.970
25	0.390	0.420	0.550	0.340	0.970	1.090	0.590	0.890	1.790	0.680	0.700	0.890
50	0.330	0.390	0.430	0.290	0.410	0.450	0.310	0.640	1.160	0.580	0.640	0.460
100	0.200	0.330	0.400	0.250	0.310	0.340	0.240	0.260	0.580	0.340	0.400	0.430
					Extracellular-N (mg L^{-1} medium)							
0	19.000	14.000	7.000	13.000	14.000	7.000	15.000	14.000	7.000	19.000	17.000	9.000
25	39.000	27.000	26.000	41.000	16.000	9.000	48.000	19.000	10.000	46.000	19.000	12.000
50	34.000	26.000	23.000	61.000	36.000	28.000	49.000	50.000	15.000	48.000	46.000	28.000
100	27.000	19.000	20.000	66.000	41.000	31.000	52.000	48.000	31.000	88.000	48.000	30.000
					Intracellular-N (mg L^{-1} medium)							
0	38.600	52.100	63.240	22.300	65.500	73.700	46.500	72.600	87.420	41.030	78.120	135.800
25	8.100	26.100	29.500	14.800	48.400	22.900	37.800	137.900	106.500	17.900	63.240	112.800
50	7.900	23.120	33.720	11.200	31.600	15.440	8.200	9.200	34.720	7.440	7.440	5.300
100	7.500	9.320	11.100	9.300	21.100	13.600	7.200	7.400	9.400	11.200	11.100	4.600
					Total fixed-N (mg L^{-1} medium)							
0	57.600	66.100	70.240	35.300	79.500	80.700	61.500	86.600	94.420	60.030	95.120	144.800
25	47.100	53.100	55.500	55.800	64.400	31.900	85.800	156.900	116.500	63.900	82.240	124.800
50	41.900	49.120	56.720	72.200	67.600	43.440	57.200	59.200	49.720	55.440	53.440	33.300
100	24.500	28.320	31.100	75.300	71.100	44.600	59.200	55.400	40.400	99.200	54.100	34.600

LSD values for the aforementioned parameters.

	dry weight		Extracellular-N		Intracellular-N		Total fixed-N	
	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
A	0.025	0.033	4.230	5.470	5.890	7.820	2.650	3.520
B	0.025	0.033	4.230	5.470	5.890	7.820	2.650	3.520
C	0.028	0.038	4.890	6.480	6.810	9.040	3.060	4.060
AB	0.012	0.016	2.120	2.810	2.950	3.910	1.330	1.760

A, Strain; B, concentration and C,period

Increasing NO_3-N concentration in the cyanobacteria culture media proportionally decreases the cyanobacterial biomass. For instance, decreases of 83.30, 55.05 and 22.30% less than control were recorded for 50 ppm-supplied *Anabaena sp.* at 7-, 15 - and 21-day old culture, respectively. The corresponding percentage decreases for *N. muscorum* were 22.8, 22.9 and 5.7%, while *T. tenuis* scored respective decreases of 5.20, 30.94 and 22.27% against relative decreases for *C. brevissima* of 57.14, 14.94 and 14.72%. As the level of sodium nitrate had increased, the growth of respective cyanobacteria candidates continuing to decrease and recording the least dry weight of 0.11g L^{-1} medium (*Anabaena sp.*), 0.43 g L^{-1} medium (*N. muscorum*), 0.40 g L^{-1} medium (*T. tenuis*) and 0.85 g L^{-1} medium (*C. brevissima*) at 7-day incubation period. Despite of NO_3-N showed an adverse effect on the cyanobacteria growth, it was obvious that increasing time of incubation up to 21-day increased the dry weight yield but the increases were lower than those recorded by the control treatment at same incubation period. Irrespective of NO_3-N amount and culture age, C. *brevissima* exhibited the highest biomass yield (2.58 gL^{-1} medium) followed by *T. tenuis* (2.38 g L^{-1} medium), *N. muscorum* (1.67 g L^{-1} medium) and *Anabaena sp* (1.39 g L^{-1} medium).

The presence of NO_3-N in culture medium exerted variable effects on extra- and intracellular nitrogen accumulated in the tested cyanobacteria strains (Table 1). Increasing incubation periods and NO_3-N concentration decreased the extracellular and intracellular nitrogen amount accumulated by *Anabaena sp.* The corresponding highest extracellular nitrogen value was 19.0 mg L^{-1} medium at 15-day incubation period against 43.8 mg L^{-1} medium for intracellular nitrogen at 21-day old period; both values were recorded with the use of 100 ppm NO_3-N in the culture medium. *Nostoc muscorum* had recorded its lowest extracellular nitrogen at 21-day old despite the increases noticed over the control treatment along with rising NO_3-N concentration from 25 up to 100 ppm. The corresponding extracellular nitrogen values were 7.00, 10.00, 12.00 and 16.00 mg L^{-1} medium for 25. 50 and 100 ppm NO_3.N, respectively. The highest amount of extracellular nitrogen recorded by *N. muscorum* was 21.00 mg L^{-1} medium at 15-day old.

On the contrary, the inclusion of NO_3-N in the culture medium of *N. muscorum* increased the intracellular nitrogen amount with increasing the NO_3-N level but the values recorded under the effect of NO_3-N were significantly different and less than those of the control treatments at all incubation periods. Neglecting both cyanobacteria culture age and /or salt level, the highest intracellular nitrogen value for *N. muscorum* was 70.70 mg l^{-1} medium against the lowest value of 21.00 mg L^{-1} medium. *T. tenuis* exhibited different responses for NO_3-N towards any of extra- or intracellular nitrogen.

Addition of NO_3-N to the cyanobacterial culture medium increased significantly the secreted nitrogen than that of the control treatment at all incubation periods. The highest extracellular nitrogen content of *T. tenuis* was 21.00 mg L^{-1} medium (15-day old) against the lowest one of 7.00 mg L^{-1} medium (21-day old).

For intracellular nitrogen, sodium nitrate at all levels reduced significantly intracellular nitrogen content for *T. tenuis* than those recorded by the control treatment at all incubation periods. The highest value was 87.00 mg L^{-1} medium and the lowest one was 18.30 mg L^{-1} medium.

Calothrix brevissima, when treated with NO_3-N exhibited different behaviors towards the extracellular nitrogen secreted into the culture media The highest values were obtained only after two weeks (15-day old) of incubation with all NO_3-N levels. The respective values were 17.00, 17.00 18.00 and 16.00 mg L^{-1} medium for 0, 25, 50 and 100 ppm NO_3-N respectively. The lowest values were recorded at 21-day old incubation period in respective to 9.00, 6.00, 8.00 and 9.00 mg L^{-1} medium, which corresponding to 0, 25, 50 and 100 ppm NO_3-N.

Regarding the intracellular nitrogen for *C. brevissima*, the application of different levels of NO_3-N of 25, 50 and 100 ppm in the culture medium reduced significantly the amount of the intracellular nitrogen than those of the control treatment without salt at all examined incubation periods. The highest intracellular amount of 135.80 mg L^{-1} medium was recorded by the control treatment at 21-day old, while the lowest one (37.60 mg L^{-1} medium) was obtained with the use of 100 ppm NO_3-N at 7-day old incubation age.

Nitrate nitrogen decreased significantly the amount of total nitrogen uptake due to all tested cyanobacteria strains at all incubation periods than those of the control treatment. The percentage reduction in the total nitrogen uptake due to NO_3-N treatments considerably varied depending upon cyanobacteria strain, culture age and NO_3-N level. In respect to the latter, raising its level to 100 ppm resulted for instance in reduction percentages of 23.16, 42.10 and 20.56 for *Anabaena sp.* at 7-, 15- and 21-day age old. The corresponded reduction percentage for *N. muscorum*, *T. tenuis* and *C. brevissima* were (6.31, 26.3, 41.7), (36.24, 22.00, 31.20) and 31.71, 19.26, 11.57) due to 7-, 15- and 21-day age old.

However, irrespective of NO_3-N level and the culture age, C. *brevissima* had recorded the highest total nitrogen uptake amount of 144.8 mg l^{-1} medium followed by *M. tenera* (94.42 mgL^{-1} medium), *N. muscorum* (80.90 mg L^{-1} medium) and finally *Anabaena sp.* (70.24 mg L^{-1} medium), while *Anabaena sp.* recorded the lowest total nitrogen uptake amount of 28.20 mg L^{-1} medium.

The application of NO_3-N at rates of 25, 50 and 100 ppm in the cyanobacterial culture media showed different responses towards chlorophyll a and C-Phycocyanin concentrations (Figs.1a&b) as well as the C-Phycocyanin /chlorophyll a ratio (Fig.1c). These responses depend on time of exposure and the cyanobacterial strains.

Increasing the exposure period up to 21-day under the effect of NO_3-N increased gradually the amount of chlorophyll a content for *Anabaena sp.*, *T. tenuis* and *C. brevissima*. The corresponding highest chlorophyll a amounts of 2.716, 5.726 and 8.240 μg mL^{-1} cyanobacterial suspension were due to 21-day incubation period at 100, 50 and 25 ppm NO_3-N, respectively. This trend had achieved with *N. muscorum* up to 15-day incubation period only, after which the amount of chlorophyll a started to decline up to 21- day.

The respective highest chlorophyll a value recorded by *N. muscorum* under the effect of NO_3-N was 2.578 μg mL^{-1}cyanobacterial suspension at 100 ppm NO_3-N level. It is also noticed that the inclusion of NO_3-N into the cyanobacterial culture had hindered the production of chlorophyll a content for all tested cyanobacterial strains and would be given in the sequence of 25 ppm < 50 ppm < 100 ppm NO_3-N.

Concerning C-phycocyanin, it was detected that the use of NO_3-N with different levels had exhibited different dramatic influences varied from cyanobacterial strain to the other. C- phycocyanin content for both *T. tenuis* and *C. brevissima* had significantly increased with increasing the incubation period up to 21-day old as the level of NO_3-N raised from 25 to 50 and 100 ppm. These increases were significantly lower than those of the control treatments at all incubation periods.

The highest C- phycocyanin content of 0.038 and 0.079 μg mL^{-1} were corresponded to *T. tenuis* and *C. brevissima* at 21-day old for both cyanobacterial strains, respectively. The respective NO_3-N levels were 100 ppm and 25 ppm.

However, the adverse influence of NO_3-N on C- phycocyanin contents for both *Anabaena sp.* and *N. muscorum* would be given in the sequence of 25 ppm < 50 ppm < 100 ppm. This drastic effect of NO_3-N on C-phycocyanin content led its values to be significantly less than those of the control treatments. The highest C-phycocyanin values recorded by any of *Anabaena sp.* and *N. muscorum* were 0.027 and 0.028 μg mL^{-1}cyanobacterial suspension at 15-day incubation period for both cyanobacterial strains.

The C- phycocyanin ratio under the effect of NO_3-N had also drastically injured to be significantly less than those of the control treatment with no salts at all incubation periods for the tested cyanobacterial strains. The highest ratioes recorded under the effect NO_3-N were 38.793, 16.932, 6.926 and 18.314 respective to 25, 25, 50 and 50 ppm *for Anabaena sp., N. muscorum*, T. *tenuis*, and *C. brevissima*, respectively.

Irrespective of salt level and the incubation period, the highest ratioes were 125.000, 16.932, 11.598 and 18.245 relative to *A. oryza, N. muscorum, T. tenuis*, and *C. brevissima*.

Urea nitrogen:

Biomass yield of the tested cyanobacterial strains, whether treated with urea-N or not, steadily increased with prolonging incubation period up to 21-day old (Table 2). In tha absence of urea-N, *T. tenuis* developed in faster rates compared to those observed with other cyanobacteria members. A unique pattern of growth was detected in cultures supplemented with urea. Urea-N addition led in general to decrease significantly the biomass production compared with those without urea. Increasing urea-N level from 25 to 50 and 100 ppm had decreased significantly the dry weight for all tested cyanobacterial strains at all incubation periods in comparison with the treatments received no urea-N.

The adverse influence of urea-N on the growth of the tested cyanobacterial strains would be given in the sequence of 25ppm < 50ppm< and 100 ppm urea. The highest dry weight yields under the effect of urea-N were obtained with 21-day old culture of *T. tenuis* (1.79 gL^{-1} medium) followed by, *N. muscorum* (1.09 gL^{-1} medium), *C. brevissima* (0.89 gL^{-1} medium) and finally 0.55 gL-1 medium, while the lowest ones were 0.20, .024, .025 and .034 gL^{-1} medium in respective to *Anabaena sp., T. tenuis, N. muscorum* and *C. brevissima* at 7-day old cultures. Irrespective of urea-N amount and culture age, *T. tenuis* exhibited the highest biomass yield (2.380 gL^{-1} medium) while the lowest (0.20 gL^{-1} medium) was produced by *Anabaena sp.*.

Extracellular nitrogen production by the tested cyanobacterial strains either treated with urea-N or not, steadily decreased with increasing incubation period. In the absence of urea-N, the extracellular nitrogen for *Anabaena sp.* decreased faster to reach the percentage decrease rate of 63.16% when incubated from 7 to 21-day, while the respective decreases percentages of 53.13, 52.63 and 45.15 were recorded by *T. tenuis, C. brevissima* and *N. muscorum*, respectively in comparison with the control treatment. In cyanobacterial cultures supplemented with urea-N, prolonging the incubation period had decreased the extracellular nitrogen amount. *Anabaena sp.* had responded positively to urea-N addition up to 50 ppm level at all incubation periods. Raising urea-N level to 100 ppm had decreased sharply the extracellular nitrogen production also in *Anabaena sp.*. The respective extracellular amount recorded by *Anabaena sp.* averaged 24.00, 28.30 and 22.00 mg N L^{-1} medium for the use of 25, 50 and 100 ppm urea-N, respectively. Same behavior observed with *Anabaena sp.* had detected with the other tested cyanobacterial strains in response to urea-N application, as *C. brevissima* exhibited the highest amount of extracellular nitrogen of 88 mg N L^{-1} medium (100 ppm urea & 7-day old) followed by, *N. muscorum* (66.00 mg N L^{-1} medium) at 7-day old and 100 ppm urea-N, *T. tenuis* (50.00 mg N L^{-1} medium) at 15-day old and 50 ppm urea-N and finally *Anabaena sp.* 39.00 mg N L^{-1} medium at 7-day old and 25 ppm urea-N.

However, to more or less, the application of urea-N in the culture media of the tested cyanobacteria strains had enhanced the secretion of nitrogen from the cyanobacterial cells into the culture medium.

Regardless, the intracellular nitrogen, it was obvious that prolonging the incubation period had increased the intracellular nitrogen for cyanobacteria strains, while addition of urea-N led to decrease significantly the intracellular nitrogen amount for all cyanobacteria candidates at all incubation periods. As the intracellular nitrogen correlated positively with time of incubation, it is worthy to state that the amounts of intracellular nitrogen obtained due to the culture treated with urea-N were significantly less than those with no urea. It is also important to note that 25 ppm urea-N had the least harmful effect towards the intracellular nitrogen for all cyanobacterial strains compared with the control treatment. The highest intracellular nitrogen amounts recorded under urea-N influence were 137.90, 112.80, 48.40 and 33.72 mg N L^{-1} medium for *T. tenuis* (15-day old), *C. brevissima* (21-day old), *N. muscorum* (15-day old) and *Anabaena sp.* (21-day old), respectively. Irrespective of urea-N level and culture age, *C. brevlissima* gave the highest

intracellular nitrogen amount (135.80 mg N L^{-1} medium) while the lowest was produced by *C. brevissima* (4.60 mg N L^{-1} medium) also.

The total nitrogen uptake for non- urea-N affected cyanobacterial strains had increased significantly with increasing the culture age up to 21-day old. The respective highest value was 144.80 mg N L^{-1} medium for *C. brevissima* at 21-day incubation period, while the lowest one (35.30 mg N L^{-1} medium) was for *N. muscorum* at 7-day incubation period. When the tested cyanobacterial strains exposed to different level of urea, both *Anabaena sp.* and *C. brevissima* showed steadily increases in the total nitrogen uptake amounts as the culture age had prolonged. However, these increases were significantly less than those of non-urea treated cultures. On the other hand, urea-treated cultures of, *N. muscorum* and *T. tenuis* exhibited gradual increases in the total nitrogen uptake amount with increasing time of incubation up to15-day only and thereafter started to decline.

Neverthless, when the cyanobacterial strains affected with urea-N, *T. tenuis* can withstand 25 ppm urea-N and gave its highest total nitrogen uptake record of 156.90 mg N L^{-1} medium at 15-day old followed by 124.80 mg N L^{-1} medium (21-day old) for *C. brevissima* 75.30 mg N L^{-1} medium (7-day old) for *N. muscorum* and 56.7 mg N L^{-1} medium (21-day old) for *Anabaena sp.*. The corresponding urea-N levels were 25 ppm, 100 ppm and 50 ppm, respectively. While the lowest total nitrogen uptake amounts for cyanobacteria strains affected with urea-N were 43.44, 40.40, 33.30 and 24.50 mg N L^{-1} medium due to *N. muscorum T. tenuis, C. brevissima* and *Anabaena sp.,* respectively. The corresponding incubation periods and urea levels were 21-day & 50 ppm urea, 21-day & 100 ppm urea-N, 21-day and 50 ppm urea-N and finally 7-day old and 100 ppm urea-N. Inferior of culture age and urea-N level, *T. tenuis* gave the highest nitrogen uptake amount of 156.90 mg N L^{-1} medium against 24.50 mg N L^{-1} medium the lowest value for *Anabaena sp.*

In the absence of urea-N, chlorophyll a and/or C- phycocyanin contents had significantly increased with increasing the cyanobacteria culture age up to 21-day old (Figs. 2 a, b & c). Application of 25 ppm urea-N had positively increased both chlorophyll a and C- phycocyanin in respective to increasing incubation time for all tested cyanobacterial strains up to 21-day old, although the values were significantly different and less than those received no urea. The corresponding highest chlorophyll a values of 2.403, 4.144, 6.937 and 4.120 μg mL^{-1} cyanobacterial suspension were recorded at 21-day old for *Anabaena sp., N. muscorum, T. tenuis* and *C. brevissima,* respectively, while, the highest recorded values of C- phycocyanin were 0.033, 0.022, 0.057 and 0.11 μg mL^{-1} cyanobacterial suspension for *Anabaena sp., N. muscorum, T. tenuis* and *C. brevissima*, respectively. These superior C- phycocyanin values were also μg mL^{-1} obtained at the culture age of 21-day old. The inclusion of more than 25 ppm urea-N into the tested cyanobacterial culture media had adversed both chlorophyll a and C- phycocyanin production. However, this adverse effect had continued to cease completely the production of chlorophyll a and C- phycocyanin for the tested cyanobacterial strains at all incubation periods.

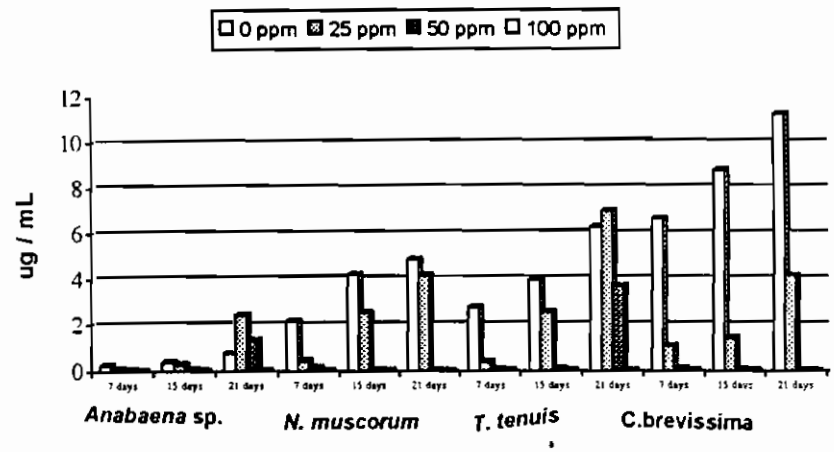

Fig. (2a): Effect of different concentrations of urea-N on chlorophyll a in cyanobacterial suspension.

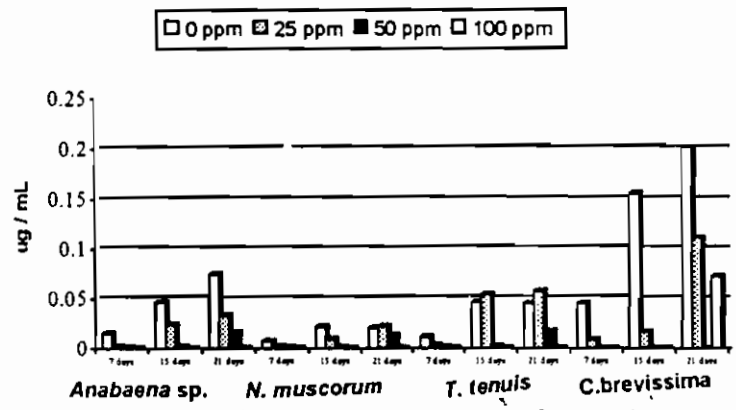

Fig. (2b): Effect of different concentrations of urea-N on C-phycocyanin in cyanobacterial suspension.

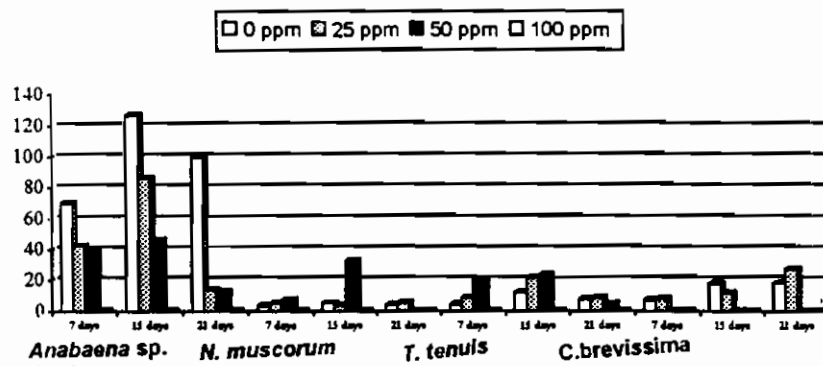

Fig. (2c): Effect of different concentrations of urea-N on C-phycocyanin / Chlorophyl a ratio in cyanobacterial suspension.

Consequently, the changes in C- phycocyanin / chlorophyll a ratio had also influenced by the inclusion of urea into the cyanobacterial culture media. The corresponding highest ratioes were 85.714, 32.258, 22.989 and 26.942 for *Anabaena sp., N. muscorum, T. tenuis* and *C. brevissima,* in respective to culture age of 15-day, 15-day, 15-day and 21-day, as the corresponding urea level for these values were 25, 50, 50 and 25 ppm urea-N.

It is also worthy to note that 50 ppm urea had also entirely damaged the chlorophyll a and C-phycocyanin production as well as the C- phycocyanin / chlorophyll a ratio at 21-day culture age.

Ammonia nitrogen:

The cyanobacterial strains that received no NH_4-N had produced biomass amount significantly increased with increasing their culture age up to 21-day (Table 3). The respective biomass dry weight values were 0.78, 1.09 and 1.19 g L^{-1} medium (*Anabaena sp.*); 0.57, 1.31 and 1.40 g L^{-1}mediun(*N. muscorum*); 0.77, 1.38 and 2.38 g L^{-1}medium (*T. tenuis*) and 1.40, 1.54 and 1.97 g L^{-1} medium (*C. brevissima*) at 7, 15, and 21-day old, respectively. Incorporation into culture medium of NH_4-N significantly diminished the biomass production of all cyanobacterial strains compared with the control treatment. The loss of the dry weight increased as the level of NH_4-N in the culture medium had augmented, although the dry weight yield increased with increasing exposure time to NH_4-N. At any of the tested incubation period, the increase of NH_4-N level gradually from 25 to 50 and then 100 ppm had decreased significantly the biomass yield of any tested cyanobacterial strains. At 7-day incubation period, *Anabaena sp.* produced biomass yield of 0.24, 0.22 and 0.10 g L^{-1} medium when NH_4-N were applied at levels of 25, 50 and 100 ppm, respectively. The corresponding dry weights of the other cyanobacterial strains at 7-day old period were 0.52, 0.48 and 0.42 g L^{-1}medium (*N. muscorum*); 0.44, 0.35 and 0.31 g L^{-1}medium (*T. tenuis*) and 0.41, 0.37 and 0.33 g L^{-1}medium (*C. brevissima*). These obtained dry weight values at 7-day had increased with increasing the incubation up to 21-day and NH_4-N level up to 50 ppm for *Anabaena sp.* and *C. brevissima*, while this trend was true with *N. muscorum* only with use of 25 ppm NH_4-N in the culture media after the which the dry weight yield started to decrease dramatically with increasing NH_4-N in the culture media up to 50 and 100 ppm with increasing the incubation period up to 21-day.

However, the growth of *T. tenuis* had been adversed by the use of 25 ppm NH_4-N with increasing the culture age gradually from 7 to 15 and then 21-day. The most harmful influence resulted in NH_4-N incorporation into the culture media of the tested cyanobacterial strains had been observed with 100 ppm (NH_4-N level application. To indicate this behavior of NH_4-N it is worthy to state that the percentage decreases happened along with the use of this NH_4-N (100 ppm) up to 21-day were 87.39% (*Anabaena sp.*), 94.44% (*T. tenuis*), 80.71% (*C. brevissima*) and 80.00% (*N. muscorum*) in comparison with the control treatment without NH_4-N. Irrespective of NH_4-N level and culture age, *T. tenuis* recorded the highest biomass yield of 2.38 g L^{-1} medium followed by 1.97, 1.40 and 1.19 g L^{-1} medium for *C. brevissima, N. muscorum* and *Anabaena sp.*, respectively.

The addition of NH_4-N to the cyanobacteria culture media supported significantly the production of extracellular nitrogen production in comparison with the control treatment without NH_4-N. The highest extracellular nitrogen amount recorded without the use of NH_4-N was 19.00 mg N L^{-1} medium for *Anabaena sp.* (7-day old) and the lowest one of 7.00 mg N L^{-1} medium for *N. muscorum* (21-day old). Except for *Anabaena sp.* the addition of NH_4-N into the cyanobacterial culture media had significantly enhanced the production of the extracellular nitrogen up to 15-day old incubation period at all NH_4-N used levels, then started to decrease to lesser amounts of extracellular nitrogen than those achieved by the other two culture incubation periods of 7 or 21-day old. While *Anabaena sp.* when exposed to different levels of NH_4-N along with increasing the incubation period time up 21-day had increased the amount of extracellular nitrogen.

However, under the effect of NH_4-N when applied to the tested cyanobacterial strains, *C. brevissima* gained the highest extracellular nitrogen amount of 280.00 mg N L^{-1} medium followed by 270.00, 230.00 and 217.00 mg N L^{-1} medium for *T. tenuis, Anabaena sp.* and *N. muscorum* at the age of 15-day old, respectively.

Concerning the intracellular nitrogen determined in the cultural cells of the tested cyanobacterial strains under the effect of different levels of NH_4-N incubated for different time of 7, 15 and 21-day, it could be noted that cyanobacteria strains received no NH_4-N had achieved higher significant amounts more than those received NH_4-N.

Consequently, the highest intracellular nitrogen yields were obtained by *C. brevissima* followed by *T. tenuis, N. muscorum* and *Anabaena sp.* at 21-day old and corresponding to 135.80, 87.42, 70.70 and 63.24 mg N L^{-1} medium. The inclusion of NH_4-N in the cultural media had hindered significantly the production of the intracellular nitrogen at all levels and incubation periods. However, the highest intracellular nitrogen amount recorded and the effect of NH_4-N could be explained as 39.10 mg N L^{-1} medium at 7-day old and 100 ppm NH_4-N (*C. brevissima*), 20.5 mg N L^{-1} medium at 7-day old and 50 ppm NH_4-N (*N. muscorum*), 19.5 mg N L^{-1} medium at 21-day old and 50 ppm NH_4-N and 18.60 mg N L^{-1} medium at 7-day old and 100 ppm NH_4-N *(T. tenuis)* against the lowest values of 00.0, 7.44, 7.40 and 7.46 mg N L^{-1} medium in respective to *N. muscorum* (50 ppm NH_4-N at 21-day old*), C. brevissima* (50 ppm NH_4-N at 15-day old), *T. tenuis* (25 ppm NH_4-N at 15-day old) and *Anabaena sp.* (50 ppm NH_4-N at 7-day old).

The presence of NH_4-N t in the culture media exerted variable effects on the accumulation of fixed nitrogen amount. In NH_4-N free medium, *C. brevissima* fixed more N_2 (144.80 mg N L^{-1} medium) than did *T. tenuis* (94.42 mg NL^{-1} medium), *N. muscorum* (79.50 mg N L^{-1} medium) or *Anabaena sp.*(71.24 mg NL^{-1} medium), such estimates are irrespective of culture age (Table, 3). Addition of low quantity of NH_4-N (25 ppm) to growth medium had slightly increased the total fixed nitrogen with respective values of 31.90, 8.90, 2.80 and 7.38 mg N L^{-1} medium for *Anabaena sp., N. muscorum, T. tenuis* and *C. brevissima,* respectively in comparison with the control treatment. The cyanobacteria strains variably behaved as NH_4-N level

increased. While increases up to ca. 60.54% were recorded as a result of raising NH_4-N concentration in *Anabaena sp.*culture medium, more than 78.00% and 79.00% N were taken up by *N. muscorum* and *T. tenuis* against 64.01% N for *C. brevissima.* As expected, accumulation of N in cyanobacterial strains tissues progressively increased with culture age. Irrespective of the culture age and the ammonium salt level, *C. brevissima* gave the superior *N accumulation* amount of 297.30 mg N L^{-1} medium followed by 282.10, 244.60 and 231.90 mg N L^{-1} medium for *T. tenuis, Anabaena sp.* and *N. muscorum,* respectively. It could be noticed that the culture age of 15-day old is the splendid for nitrogen accumulation for both *T. tenuis* and *C. brevissima.*

Nitrogen free cyanobacterial culture deemed to be the superior for both chlorophyll a and C-phycocyanin production (Figs. 3a& b). Chlorophyll a amounts yielded 0.744, 3.431, 6.266 and 11.224 μg mL^{-1} cyanobacterial suspension for the control treatment of *Anabaena sp., N. muscorum, T. tenuis* and *C. brevissima* respectively. However, the corresponding C-phycocyanin amounts were 0.074, 0.020, 0.45 and 0.201 μg mL^{-} cyanobacterial suspension for *Anabaena sp., N. muscorum, T. tenuis* and *C. brevissima,* respectively. Addition of low quantity of ammonium salt to the cyanobacterial culture media resulted in sharp decreases in both chlorophyll a and C- phycocyanin amounts to the limit that those achieved values could be neglected and may throw out of consideration.

Raising the level of ammonium salt to 50 and/or 100 ppm led to approximately complete damage of both chlorophyll a or C- phycocyanin production, that is finally destroyed the C- phycocyanin /chlorophyll a ratio that became with no value when cyanobacterial culture strains were exposed to NH_4-N.

Combined nitrogen adjacent to the cyanobacteria in the culture medium had hindered the growth and N_2 .fixation either for NO_3-N, NH_4-N or urea-N. The developing the cyanobacteria strains in presence of NO_3-N had decreased the biomass production compared with the control treatment. With respective decreases for instance 83.30, 55.05 and 22.30% for *Anabaena sp.* through out the incubation periods. The corresponding total nitrogen decrease percentages for *Anabaena sp.* also were 23.61, 42.1 and 20.56 throughout the incubation period.

In case of urea, the presence of urea in the culture media of the tested cyanobacteria strains seemed to be suppressive to biomass production and nitrogen fixation of these cyanobacteria strains despite, the cyanobacteria treated with urea had steadily increased with prolongation of the incubation period up to 21- day, but these increases were significantly less than those of the control treatment.

Same trend was observed when NH_4-N had supplemented to the tested cyanobacterial culture, however, the ability of the tested cyanobacteria strains to assimilate the nitrogen source differ from each other. This could be explained by giving the assimilation order of the tested nitrogen sources by each strain as NO_3-N > urea-N > ammonium-N for all cyanobacteria strains.

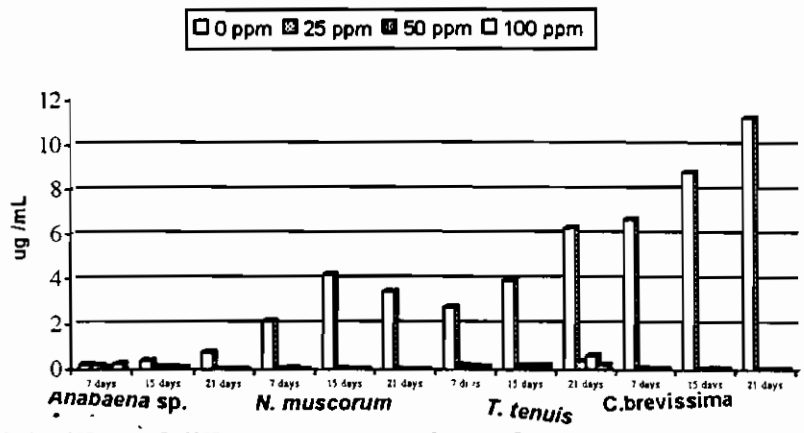

Fig. (3a): Effect of different concentrations of NH_4-N on chlorophyll a in cyanobacterial suspension.

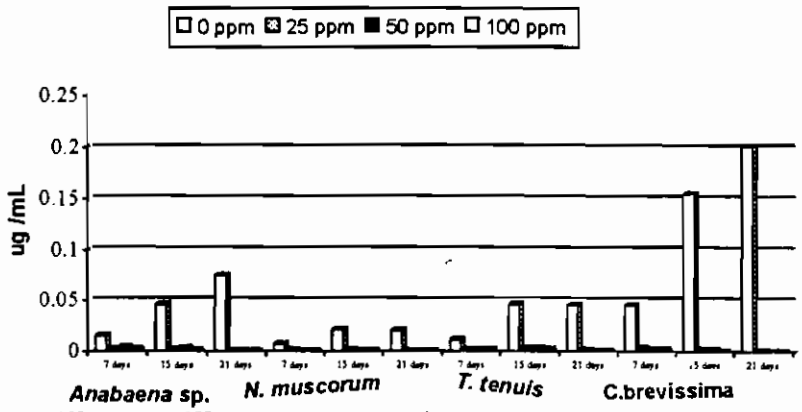

Fig. (3b): Effect of different concentrations of NH_4-N on C-phycocyanin in cyanobacterial suspension.

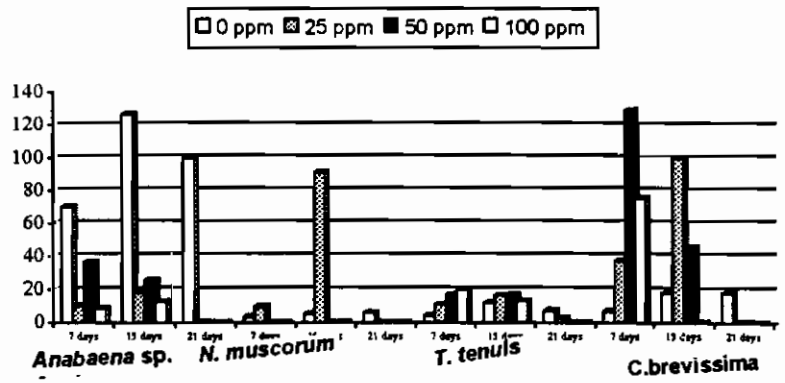

Fig. (3c): Effect of different concentrations of NH_4-N on C-phycocyanin/Chlorophyl a ratio in cyanobacterial suspension.

Thus up to less extent, cyanobacteria can grow using urea-N, NH_4-N and NO_3-N as a sole source of nitrogen since low N_2-fixing activities were detected (Aref, 2001).

Stewart (1980) described nitrogen, which could be assimilated for cyanobacteria growth as they depend on type, may assimilate nitrogen source ranging from N_2 to amino acids, amides and peptides. Those cyanobacteria that utilize N_2 and inorganic nitrogen ions can use these as sole nitrogen source for growth. Most cyanobacteria utilize NO_3^- or NH_4^+ equally well as sole nitrogen source for growth. When provided with NO_3^- and NH_4^+ simultaneously most cyanobacteria assimilate NH_4^+ more rapidly, and in the longer term they assimilate NH_4^+ rather than NO_3^- because NH_4^+ represses synthesis of nitrate reductase. He also explained that uptake of NO_3^- results in a pH increase in the medium; uptake of NH_4^+ results in pH drop and this in our case may explain why is the growth is dropped and decreased when the cyanobacterial culture treated with any of NO_3^- or NH_4^+ salts.

Cyanobacteria grow using urea as sol source of nitrogen has the ability to degrade urea and urease seems to be solely responsible for urea breakdown. As mentioned by Ge *et al.* (1990), cyanobacterial urease synthesis is constitutive, although ammonia-grown cells appeared to have somewhat higher level of enzyme activity than those grown with urea or N_2. Obviously, the enzyme is indispensable for utilization of exogenous urea as nitrogen source.

However, the natural environments of most cyanobacteria are not likely contain high levels of urea. This, plus the fact that urease is constitutive, suggests that the enzyme may have other function as well. It is supported that urease also serves to scavenge amino nitrogen from urea produced internally as a result of various metabolic activities. The two major sources of urea in cellular nitrogen metabolism are degradation of arginine and purines.

Cyanobacteria can store large amounts of arginine in the nitrogenous reserve material cyanophycin, an arginine-as partate polymer, which can be mobilized during periods of nitrogen depletion (Allen, 1984). Cyanophycin may also serve as a short-term amino nitrogen "buffer" during nitrogen fixation (Gupta and Carr 1981a). During cyanophycin mobilization, arginine is released for further metabolism, and urea is a product in some pathways of arginine degradation. Several cyanobacteria have arginase activity, which indicated that urea- producing degradation pathway is present (Gupta and Carr, 1981b).

Thus, urease would be important for prevcnting the loss of amino groups in urea.

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تأثير صور مختلفة من النيتروجين على النمو وتثبيت النيتروجين ومحتوى الصبغات للسيانوبكتريا

السيدة على حسن
قسم بحوث الميكروبيولوجيا الزراعية – معهد بحوث الأراضي والمياه والبيئة مركز البحوث الزراعية – الجيزة – مصر

في هذه الدراسة أجريت تجربة في المعمل باستخدام أربعة سلالات من السيانوبكتريا كل على حده وكانت هذه السلالات هي:
Anabaena sp, Nostoc muscorum, Tolypothrix tenuis, and Calothrix brevissima
حيث نميت هذة السلالات على بيئة Watanabe المعدلة عند درجة حرارة من ٢٨ – ٣٢°م تحت الإضاءة المستمرة ثم بعد ذلك تم اضافة مصادر مختلفة من النيتروجين حيث أضيفت كل من نترات الصوديوم بتركيزات (صفر، ٢٥، ١٠٠،٥٠جزء في المليون نيتروجين). أو اليوريا (صفر، ٢٥، ٥٠، ١٠٠ جزء المليون نيتروجين) أو كبريتات الأمونيوم (صفر ، ٢٥ ، ٥٠، ١٠٠ جزء المليون نيتروجين). ثم بعد ذلك حضنت لمدة ٢١ يوم حيث أخذت بعد ذلك عينات من السياتوبكتريا النامية أسبوعيا لتقدير الوزن الجاف وكمية النيتروجين المثبت والمحتوى الكلورفيللى وتركيز الفيكوسيانين. ويمكن تلخيص النتائج فيما يلى:

١- لقد أدت إضافة نترات الصوديوم كمصدر وحيد للنيتروجين إلى بيئة تنمية الطحالب بصفة عامة إلى نقص معنوي في محصول الوزن الجاف لهذه الطحالب وكذا كمية النيتروجين المثبت.
٢- أدى استخدام نترات الصوديوم كمصدر وحيد للنيتروجين في بيئة تنمية السياتوبكتريا إلى نقص معنوي في محتواها من الفيكوسيانين.
٣- أدى تعرض سلالات السياتوبكتريا إلى أي من تركيزات نيتروجين اليوريا إلى نقص معنوي في وزنها الجاف بالمقارنة بمعاملة الكنترول.
٤- زيادة تركيز نيتروجين اليوريا في البيئة لأكثر من ٢٥ جزء في المليون أدى إلى تدهور محتوى السياتوبكتريا تحت الاختبار من الكلوروفيل والفيكوسيانين.
٥- أدى وجود نيتروجين كبريتات الأمونيوم في بيئة تنمية السياتوبكتريا إلى نقص وزنها الجاف بالمقارنة مع معاملة الكنترول.
٦- لقد أدى إضافة ٢٥ جزء في المليون من نيتروجين كبريتات الأمونيوم في بيئة تنمية السياتوبكتريا إلى زيادة طفيفة في كمية النتيروجين المثبت بها.
٧- أدى نيتروجين كبريتات الأمونيوم بكل التركيزات إلى انخفاض حاد في محتوى السياتوبكتريا من الكلوروفيل أ والفيكوسيانين عند كل فترات التحضين.