RESULTS

Experiment (1): Effect of different concentrations of sodium nitrate (mM) on the growth and lipid content of *Chlorella vulgaris*

Results in Tab. (2) and Fig. (8) revealed that the growth of *Chlorella vulgaris* is directly proportional with increasing concentration of sodium nitrate up to 5mM of $NaNO_3$ more than its normal concentration in BBM (3mM). The maximum growth was indicated at 5 mM of $NaNO_3$ after the elapse of 8 days ($R^{\backslash} = 0.32\ d^{-1}$), the highest growth may be related to the maximum number of cycling (2.53 cycle/d) with the least generation time (3.17 d). On the opposite hand, the rate of growth was subsequently shifted to the lowest one as nitrate deprives to the level 0.10 mM which need more time for regeneration (G =5.9 d) with number of cycling 1.36 cycle/d at 8 days old culture.

Inspection of the biosynthesis and accumulation of lipids of *Chlorella vulgaris* under different concentrations of nitrate was manifested in Tab. (3) and Fig. (9) It was revealed that the biosynthesis and accumulation of lipids of *Chlorella vulgaris* was maximally reached 54.88% as nitrate deprives to the level 0.10 Mm after 12 days of incubation. This value was two folds more than its corresponding control (29.74%). On the other hand, the lipid content was inhibited by 67.2% as *Chlorella vulgaris* treated with 5 mM of $NaNO_3$ after 12 days. The previous results indicate that nitrogen deprivation is a feasible tool for the over production of lipid contents and the relation between lipids and growth was found to be inversely.

Table (2): Effect of different concentrations of sodium nitrate (mM) on the growth parameters of *Chlorella vulgaris*.

Time (days)	0.1mM					1mM					2mM					3mM (control)					5mM				
	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N
0	0.05 ±0.00	-	-	-	-	0.05 ±0.000	-	-	-	-	0.05 ±0.002	-	-	-	-	0.05 ±0.000	-	-	-	-	0.05 ±0.001	-	-	-	-
2	0.06 ±0.005	0.13	0.04	7.72	0.26	0.06 ±0.005	0.13	0.04	7.72	0.26	0.06 ±0.004	0.13	0.04	7.72	0.26	0.07 ±0.002	0.24	0.07	4.12	0.49	0.08 ±0.003	0.29	0.09	3.34	0.60
4	0.07 ±0.002	0.12	0.04	8.36	0.48	0.08 ±0.001	0.17	0.05	5.90	0.68	0.11 ± 0.001	0.28	0.09	3.54	1.13	0.12 ±0.005	0.30	0.09	3.34	1.17	0.13 ±0.001	0.34	0.10	2.92	1.37
6	0.10 ±0.006	0.17	0.05	6.02	0.10	0.12 ±0.001	0.21	0.06	4.78	1.26	0.15 ± 0.005	0.27	0.08	3.72	1.61	0.19 ±0.005	0.32	0.10	3.14	1.91	0.23 ±0.001	0.37	0.11	2.74	2.19
8	0.13 ±0.000	0.17	0.05	5.90	1.36	0.17 ±0.003	0.22	0.07	4.56	1.75	0.2 ± 0.005	0.25	0.07	4.12	1.94	0.24 ±0.00	0.28	0.09	3.54	2.26	0.29 ±0.004	0.32	0.10	3.17	2.53
10	0.10 ±0.000	0.09	0.03	11.15	0.90	0.14 ±0.004	0.14	0.04	7	1.43	0.19 ± 0.001	0.19	0.06	5.19	1.93	0.20 ±0.005	0.20	0.06	5.10	2.00	0.27 ±0.002	0.24	0.08	4.12	2.43
12	0.09 ±0.000	0.06	0.02	15.84	0.76	0.13 ±0.001	0.11	0.03	9.12	1.32	0.18 ±0.003	0.15	0.05	6.54	1.83	0.19 ±0.00	0.16	0.05	6.27	1.91	0.27 ±0.001	0.20	0.061	4.934	2.432

R\ = Mean growth rate
K\= Relative growth rate
G= Generation time
N= Number of recycling

Table (3): Effect of different concentrations of sodium nitrate (mM) on dry. wt (mg/l) and lipid contents (mg lipid /l and % of lipid yield) of *Chlorella vulgaris*.

Time (Day)	0.1mM			1mM			2mM			3mM (control)			5mM		
	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/ l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %
0	122.50	14.47	11.81	122.5	14.47	11.81	120.00	14.178	11.82	122.50	14.47	11.82	120.05	14.18	11.82
2	127.40	15.05	11.81	134.75	15.92	11.81	149.40	17.65	11.82	159.25	18.82	11.82	149.40	17.65	11.82
4	139.00	22.94	16.50	149.45	14.47	9.68	159.20	22.94	14.41	173.90	22.94	13.19	178.80	22.94	12.83
6	147.00	31.60	21.49	159.25	31.59	19.84	168.75	31.60	18.73	183.70	31.60	17.20	200.90	31.60	15.73
8	156.80	48.91	31.19	178.85	48.91	27.35	181.30	40.79	22.49	200.00	40.48	20.24	237.60	31.60	13.30
10	166.00	83.54	50.33	176.40	69.79	39.56	183.75	48.91	26.62	198.45	48.91	24.65	237.60	22.94	9.66
12	168.00	92.20	54.88	176.40	74.89	42.46	181.30	66.25	36.54	193.55	57.56	29.74	235.20	22.94	9.75

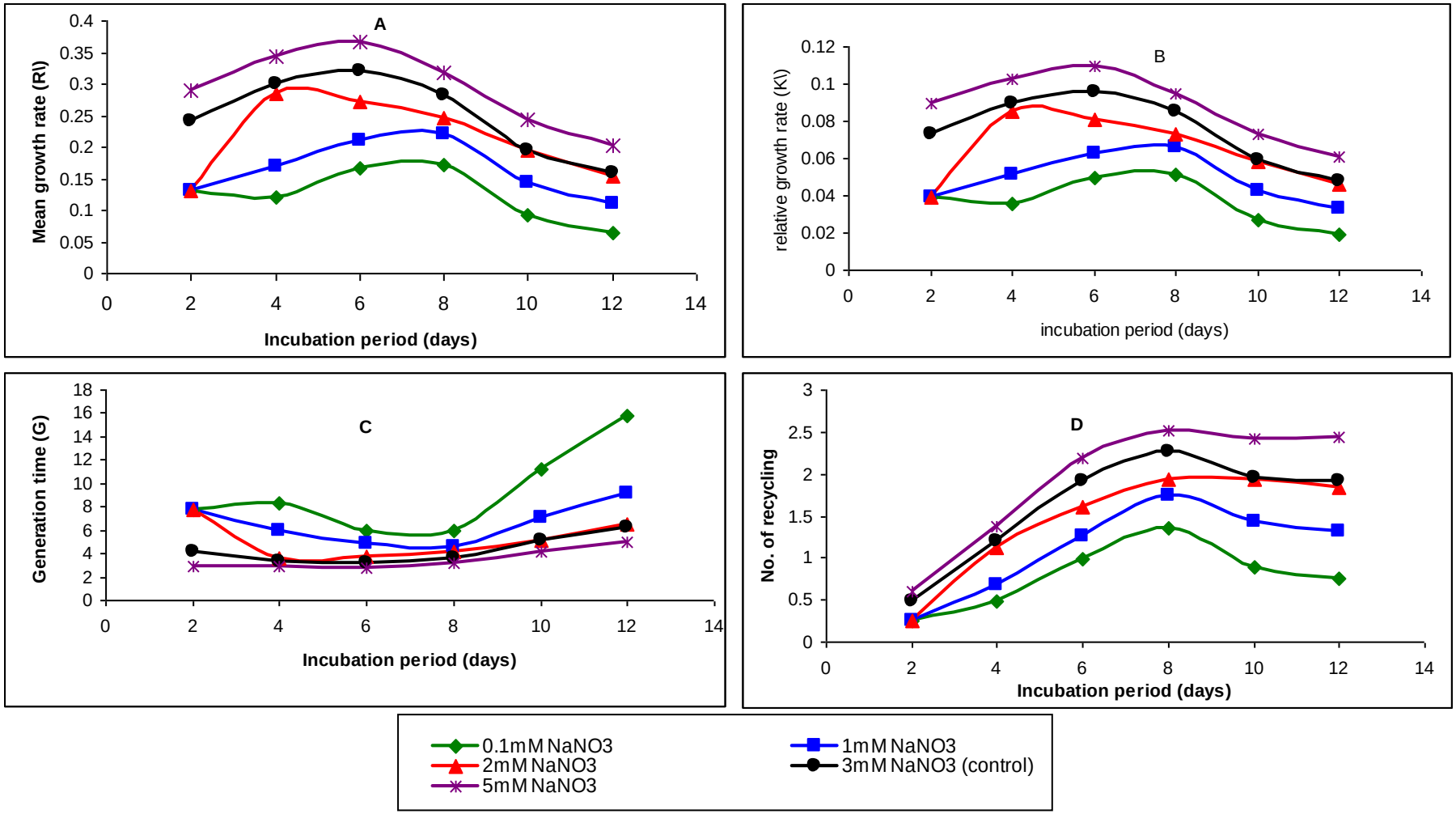

Fig.8. Effect of different concentrations of sodium nitrate (mM) on growth parameters of *Chlorella vulgaris* measured as: **A**-Mean growth rate (R\) **B**-Relative growth rate (K\) **C**-Generation time (G) **D**-Number of recycling.

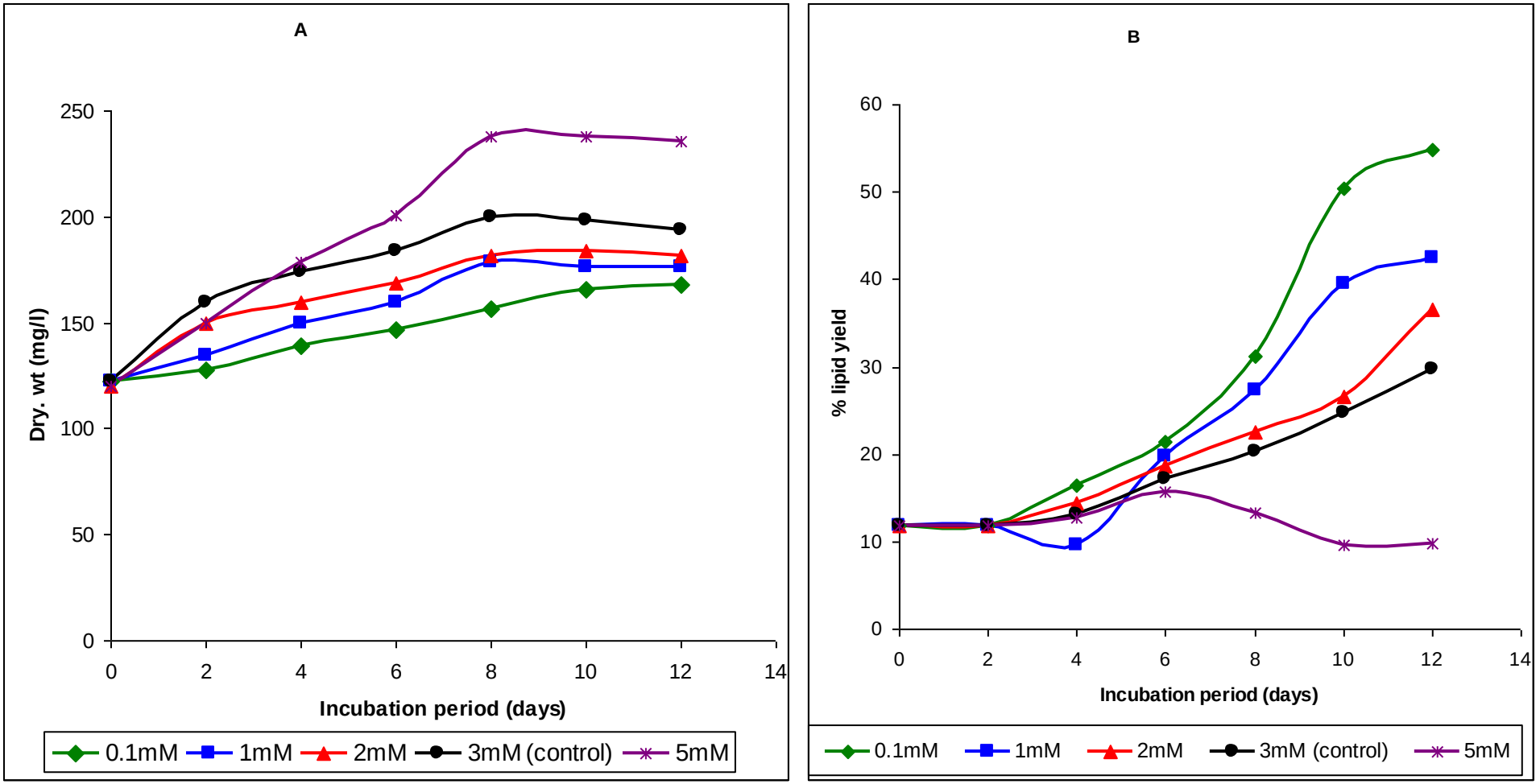

Fig.9. Effect of different concentrations of sodium nitrate (mM) on *Chlorella vulgaris*:

A- Dry wt. (mg/l). **B-** % of Lipid yield.

Experiment (2): Effect of different concentrations of sodium chloride (mM) on the growth and lipid content of *Chlorella vulgaris*

The mean growth rates and cell productivity of *Chlorella vulgaris* grown at different salinities (0.3 mM, 0.4 mM, 0.42 mM and 0.45 mM NaCl) were shown in Tab. (4) and Fig. (10). The results indicated that the mean growth rates for *Chlorella vulgaris* was subsequently decreased with increasing the level of salinity. Where as treating the alga with 0.45 mM of NaCl resulted in growth inhibition with 26.5% from the corresponding control after 6 days old culture, with the least generation time (4.01 d), through which the number of cycles was 1.5 cycle/day. Mean while these parameters were maximally attained at 6 days old culture in untreated control (0.4mM NaCl) where, the mean growth rate was recorded 0.34 d^{-1} with generation time 2.95 days, during this time the alga recycled itself 2.03 times.

Inspection of the biosynthesis and accumulation of lipids of *Chlorella vulgaris* under the stress of salinity were recorded in Tab. (5) and Fig. (11), where the over production of lipids was directly increased with increasing the level of salinity. The maximum percent of yield was recorded (43.15%) following the treatment of *Chlorella vulgaris* with 0.45mM NaCl at 12 days old culture. Where as in control samples the percent of accumulation of lipids was (29.47%). The previous results indicate that salinity is a feasible tool for the over production of lipid contents and the relation between lipids and growth was found to be inversely.

Table (4): Effect of variable concentrations of sodium chloride (mM) on the growth parameters of *Chlorella vulgaris*.

Time (days)	0.30 mM					0.40 mM (control)					0.42mM					0.45mM				
	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N
0	0.07 ±0.003	-	-	-	-	0.07 ±0.003	-	-	-	-	0.07 ±0.003	-	-	-	-	0.07 ±0.001	-	-	-	-
2	0.09 ±0.001	0.28	0.07	4.43	0.45	0.10 ±0.002	0.26	0.07	4.30	0.47	0.10 ±0.000	0.26	0.07	4.56	0.44	0.09 ±0.001	0.18	0.06	5.47	0.37
4	0.14 ±5x10^{-4}	0.25	0.08	4.01	0.99	0.16 ±0.001	0.30	0.09	3.34	1.20	0.15 ±0.001	0.27	0.08	3.67	1.09	0.13 ±0.001	0.23	0.07	4.36	0.92
6	0.25 ±0.005	0.31	0.09	3.27	1.83	0.29 ±0.001	0.34	0.10	2.95	2.03	0.27 ±0.001	0.32	0.10	3.10	1.93	0.2 ±0.001	0.25	0.08	4.01	1.50
8	0.32 ±0.026	0.27	0.08	3.70	2.18	0.35 ±0.003	0.29	0.09	3.46	2.31	0.33 ±0.011	0.28	0.08	3.63	2.21	0.24 ±0.001	0.22	0.07	4.49	1.78
10	0.29 ±0.02	0.21	0.06	4.93	2.03	0.30 ±0.005	0.21	0.06	4.78	2.09	0.30 ±0.01	0.21	0.06	4.78	2.09	0.24 ±0.005	0.18	0.05	5.68	1.76
12	0.26 ±0.002	0.16	0.05	6.40	1.87	0.27 ±0.002	0.16	0.05	6.27	1.91	0.27 ±0.005	0.16	0.05	6.27	1.91	0.23 ±0.004	0.14	0.04	7.00	1.71

R\ = Mean growth rate
K\= Relative growth rate
G= Generation time
N= Number of recycling

Table (5): Effect of different concentrations of sodium chloride (mM) on dry. wt (mg/l) and lipid contents (mg lipid /l and % of lipid yield) of *Chlorella vulgaris*.

Time (Day)	0.3mM			0.4mM (control)			0.42mM			0.45mM		
	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %
0	122.50	14.47	11.82	122.50	14.47	11.82	122.5	14.47	11.82	122.5	14.47	11.82
2	143.70	16.98	11.82	159.25	18.82	11.82	142.10	16.79	11.82	137.20	16.22	11.82
4	160.00	31.60	19.75	173.90	22.94	13.19	156.80	31.60	20.15	156.80	31.60	20.15
6	179.00	40.78	22.61	183.70	31.60	17.20	186.20	40.48	21.74	176.40	40.48	22.95
8	191.10	48.91	25.59	200.00	40.48	20.24	196.00	57.57	29.37	191.10	57.57	30.13
10	194.30	57.57	29.63	198.45	48.91	24.65	200.90	66.23	32.97	186.20	66.23	35.57
12	191.10	66.23	34.66	193.55	57.56	29.74	191.10	66.23	34.66	183.75	79.30	43.15

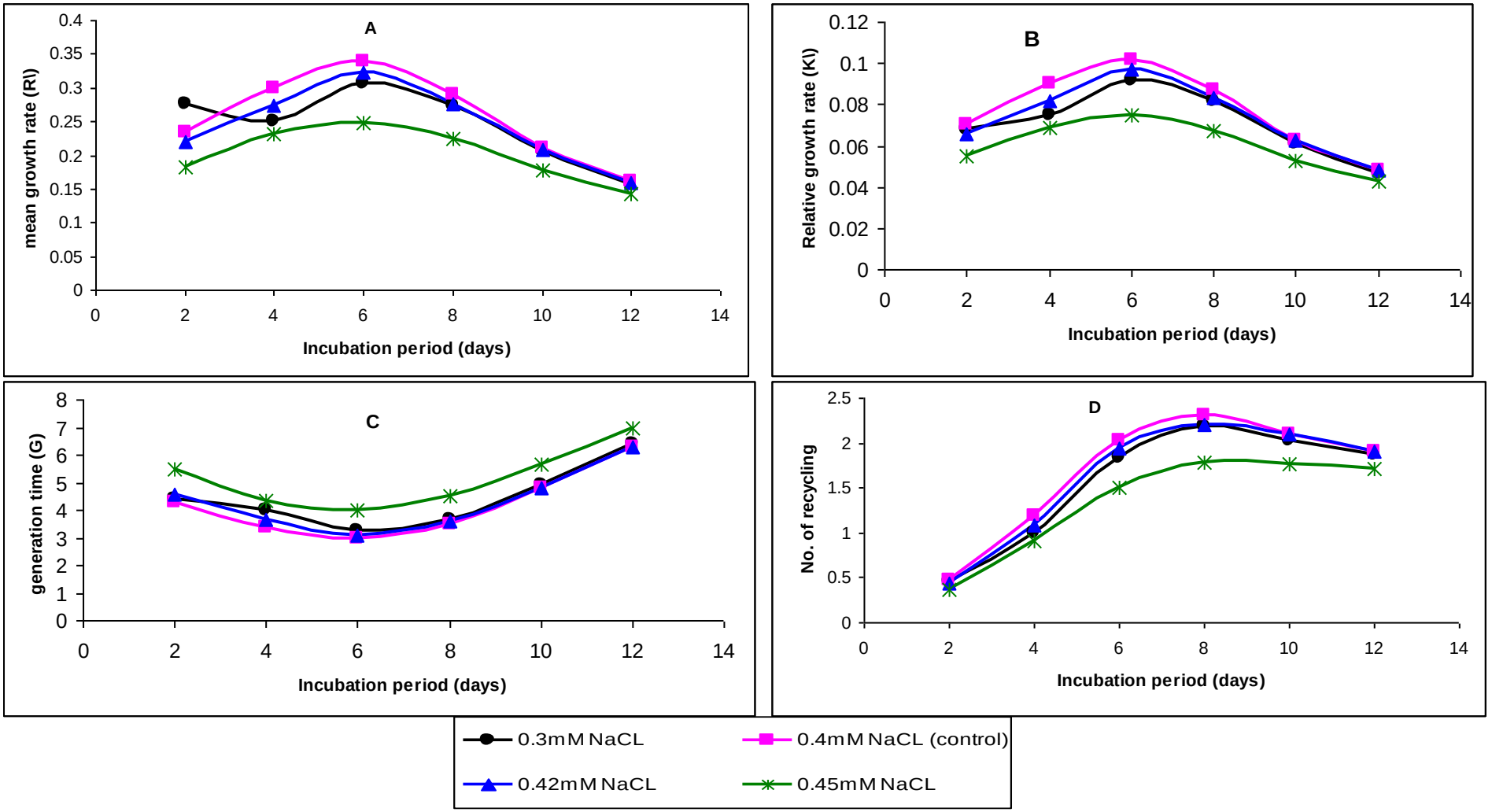

Fig.10. Effect of different concentrations of sodium chloride (mM) on growth parameters of *Chlorella vulgaris* measured as: **A**-Mean growth rate (R\) **B**-Relative growth rate (K\) **C**-Generation time (G) **D**-Number of recycling.

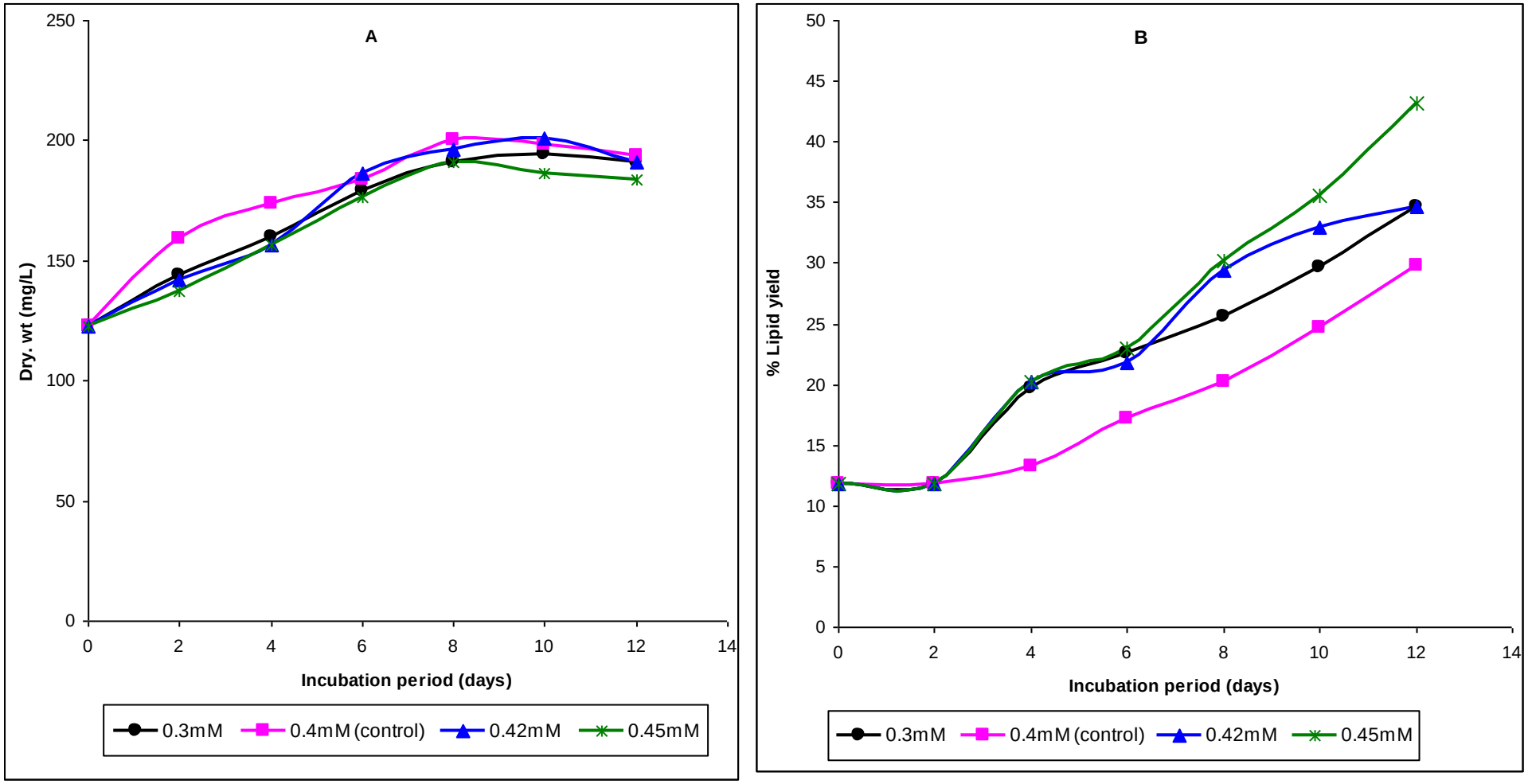

Fig.11. Effect of different concentrations of sodium chloride (mM) on *Chlorella vulgaris*:

A- Dry wt. (mg/l). **B-** % of Lipid yield.

Experiment (3): Effect of different concentrations of ferrous sulphate (µM) on the growth and lipid content of *Chlorella vulgaris*

Results in Tab. (6) and Fig. (12) revealed that there was a variation in the growth of *Chlorella vulgaris* under the effect of different concentrations of ferrous sulphate (µM). Where after 6 days of culturing *Chlorella vulgaris* with 26.43 µM and 35.25 µM of ferrous sulphate, the mean growth rate increased with 10.34% and 6.89% respectively more than their corresponding control. On the other hand, after 6 days of culturing the alga in 44.06 µM of ferrous sulphate the mean growth rate of *Chlorella vulgaris* was inhibited by 51.72% from its corresponding control. The data also revealed that after 6 days of culturing the alga with 26.43µM of ferrous sulphate the mean growth rate reached its maximum value (0.32 d^{-1}) parallel with the minimum generation time value (3.14 d), during this period the alga recycled itself 1.91 times per day.

The biosynthesis and accumulation of lipids of *Chlorella vulgaris* under the stress of ferrous sulphate was recorded in Tab. (7) and Fig. (13). Results revealed that the over production of lipids was subsequently increased slightly with increasing the level of ferrous sulphate up to 35.25 µM. But, any further excess more than 35.25 µM resulted in an inhibition in the lipid accumulation as the lipid content was decreased by 28.94% from the corresponding control after 12 days of culturing *Chlorella vulgaris* in 44.06 µM of ferrous sulphate. Moreover the maximum accumulation of lipid reached 34.6% after 12 days old culture treated with 35.25 µM of ferrous sulphate.

Table (6): Effect of different concentrations of ferrous sulphate (µM) on the growth parameters of *Chlorella vulgaris*.

Time (days)	17.62 µM (control)					26.43µM					35.25µM					44.06µM				
	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N
0	0.08 ±0.002	-	-	-	-	0.08 ±0.001	-	-	-	-	0.08 ±0.001	-	-	-	-	0.08 ±0.00	-	-	-	-
2	0.10 ±0.002	0.16	0.05	6.14	0.33	0.11 ±0.005	0.22	0.07	4.56	0.44	0.11 ±0.011	0.21	0.06	4.78	0.42	0.09 ±0.001	0.10	0.03	10.75	0.19
4	0.15 ±0.002	0.24	0.07	4.18	0.96	0.17 ±0.002	0.28	0.08	3.67	1.09	0.16 ±0.001	0.26	0.08	3.81	1.05	0.12 ±0.001	0.15	0.05	6.57	0.61
6	0.27 ±0.001	0.29	0.09	3.38	1.77	0.30 ±0.005	0.32	0.10	3.14	1.91	0.28 ±0.005	0.31	0.09	3.24	1.85	0.14 ±0.003	0.14	0.04	7.34	0.82
8	0.3 5±0.002	0.27	0.08	3.72	2.15	0.35 ±0.002	0.27	0.08	3.72	2.15	0.3 8±0.002	0.28	0.08	3.58	2.23	0.16 ±0.00	0.13	0.04	7.71	1.04
10	0.30 ±0.008	0.19	0.06	5.19	1.93	0.32 ±0.005	0.21	0.06	4.93	2.03	0.32 ±0.005	0.20	0.06	5.02	1.99	0.16 ±0.002	0.10	0.03	10.38	0.96
12	0.28 ±0.005	0.15	0.05	6.54	1.83	0.28 ±0.002	0.15	0.05	6.69	1.79	0.30 ±0.007	0.16	0.05	6.40	1.87	0.15 ±0.00	0.08	0.02	13.08	0.92

R\ = Mean growth rate
K\= Relative growth rate
G= Generation time
N= Number of recycling

Table (7): Effect of different concentrations of ferrous sulphate (µM) on dry. wt (mg/l) and lipid contents (mg lipid /l and % of lipid yield) of *Chlorella vulgaris*.

Time (Day)	17.62 µM (Control)			26.43 µM			35.25 µM			44.06 µM		
	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %
0	122.50	14.47	11.82	122.5	14.47	11.82	122.50	14.78	11.82	122.50	14.47	11.82
2	159.25	18.82	11.82	161.70	191.12	11.82	158.27	18.71	11.82	137.20	16.22	11.82
4	173.90	22.94	13.19	176.40	31.20	17.68	181.13	31.19	17.22	142.10	14.10	9.92
6	183.70	31.60	17.20	183.75	31.20	16.98	193.55	33.74	17.43	156.80	26.50	16.89
8	200.00	40.48	20.24	210.70	34.55	16.39	200.90	48.29	24.04	166.60	29.53	17.73
10	198.45	48.91	24.65	205.80	56.84	27.62	200.90	56.84	28.29	166.60	31.20	18.73
12	193.55	57.56	29.74	191.10	56.84	29.74	188.65	65.38	34.6	161.70	33.74	20.87

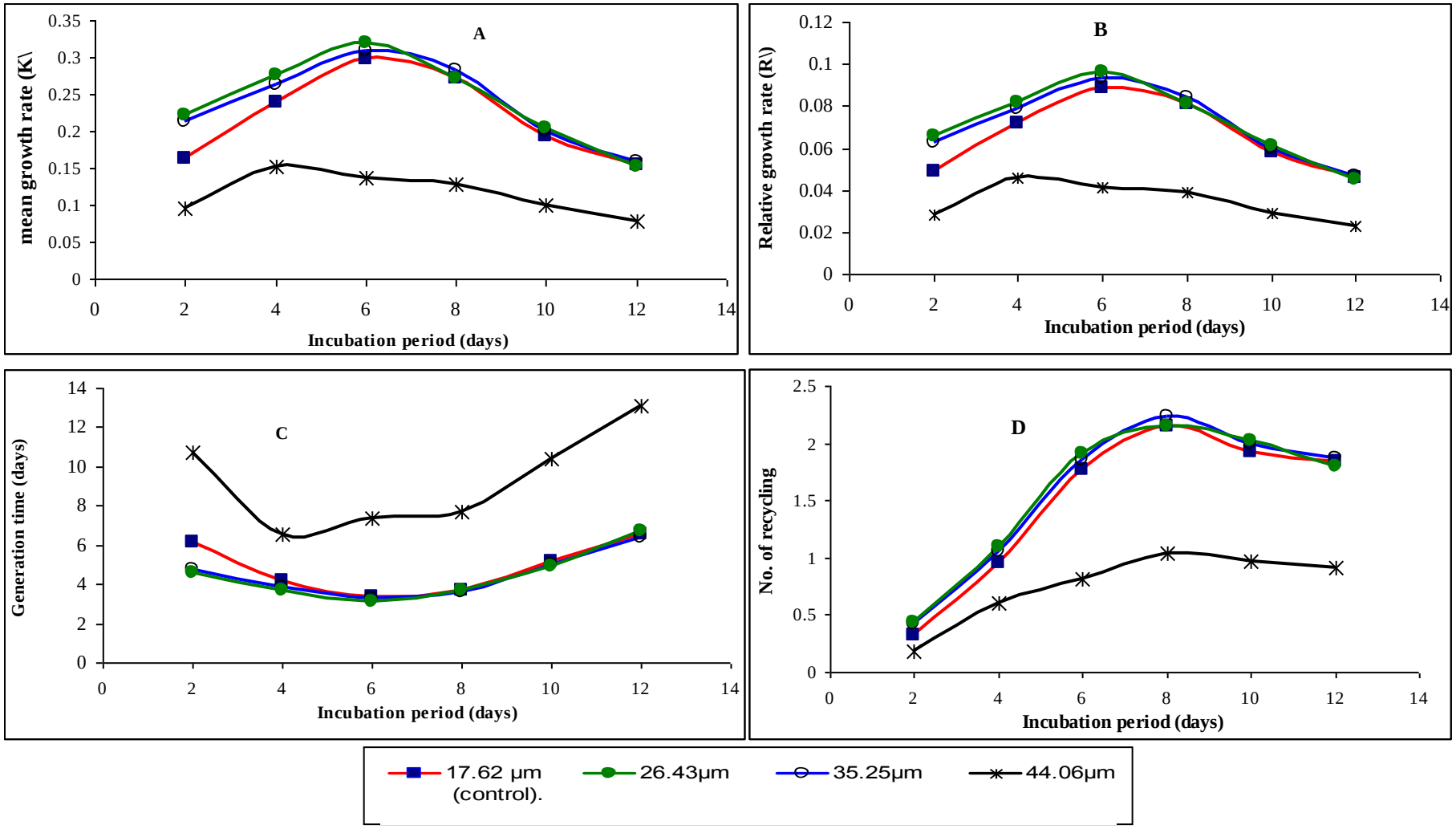

Fig.12. Effect of different concentrations of ferrous sulphate (µM) on growth parameters of *Chlorella vulgaris* measured as: **A**-Mean growth rate (R\) **B**-Relative growth rate (K\) **C**-Generation time (G) **D**-Number of recycling.

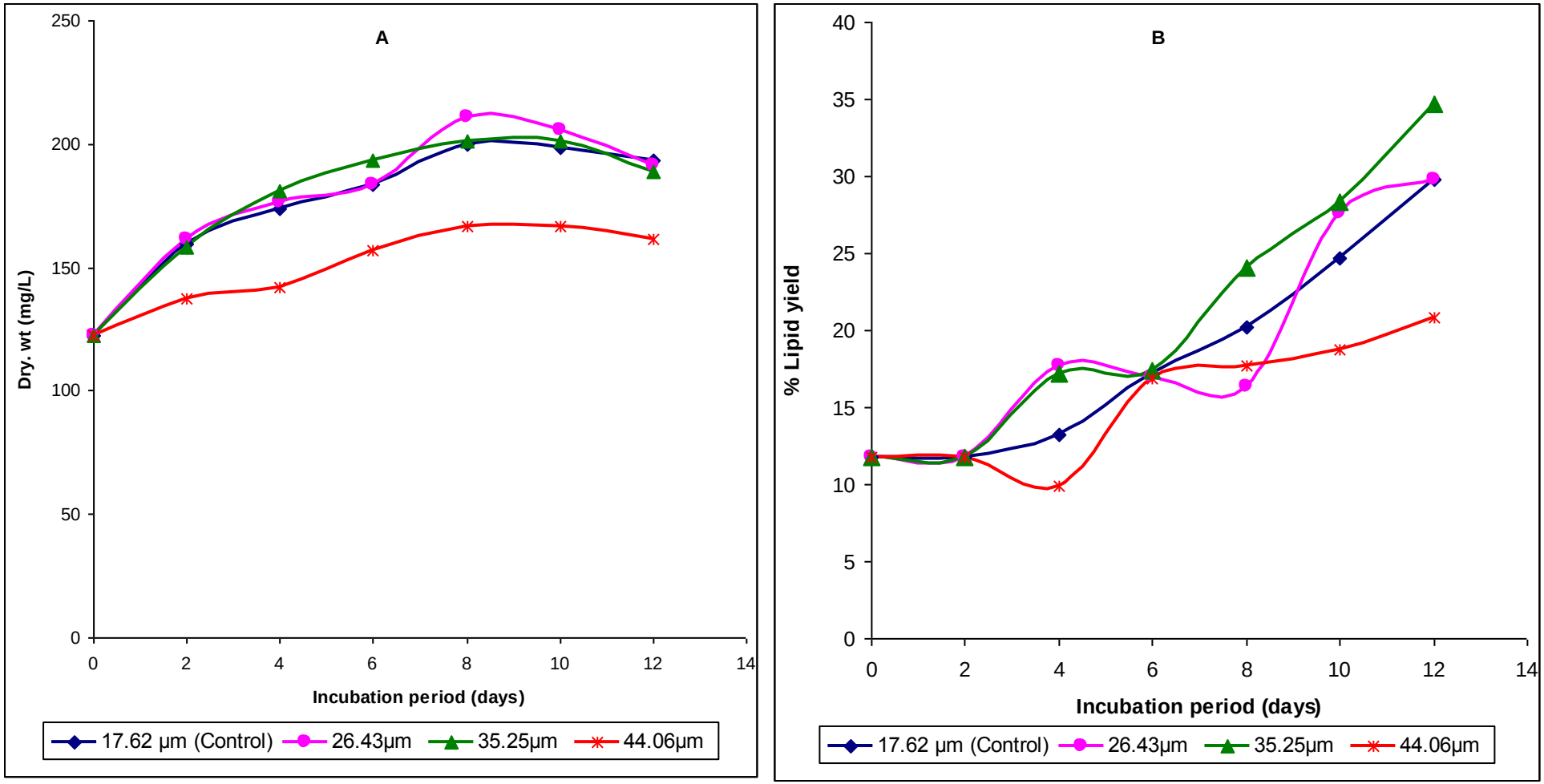

Fig.13. Effect of different concentrations of ferrous sulphate (µM) on *Chlorella vulgaris*:

A- Dry wt. (mg/l). **B-** % of Lipid yield.

Experiment (4): Effect of different concentrations of manganese chloride (µM) on the growth and lipid content of *Chlorella vulgaris*

The mean growth rates and cell productivity of *Chlorella vulgaris* grown at different concentrations of manganese chloride were shown in Tab. (8) and Fig. (14). It was revealed that the growth of *Chlorella vulgaris* was directly proportional with increasing the concentrations of manganese chloride more than its concentration in BBM (7.3 µM). Where as treating the alga with 10 µM and 12 µM of manganese chloride led to increase the mean growth rate with 6 % and 9 % respectively after 6 days old culture. On the other hand treating the alga with 2 µM and 4 µM of manganese chloride resulted on a noticeable inhibition in the mean growth rate by the value 15.15% and 9.09% respectively less than the corresponding control after 6 days old culture. Data also showed that the maximum mean growth rate was recorded 0.36 d^{-1} in cultures treated with 12 µM of manganese chloride, parallel with the minimum generation time value (2.74 d), during this period the alga recycled itself 2.19 times per day.

Results in Tab. (9) and Fig. (15) revealed that the biosynthesis and accumulation of lipids in *Chlorella vulgaris* was slightly affected under different concentrations of manganese chloride (µM). Where the data indicated that after 12 days old cultures treated with 2 µM, 4 µM and 10 µM of manganese chloride the lipid content was slightly shifted from their corresponding control. The maximum accumulation of lipid was attained (33.36%) after 12 days of seeding the culture medium with 12 µM of manganese chloride with increasing percentage 13.59 % from its corresponding control.

Table (8): Effect of different concentrations of manganese chloride (µM) on the growth parameters of *Chlorella vulgaris*.

Time (days)	2.00µM					4.00µM					7.30 µM (control)					10.00 µM					12.00 µM				
	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N
0	0.05 ±0.000	-	-	-	-	0.05 ±0.000	-	-	-	-	0.05 ±0.000	-	-	-	-	0.05 ±0.000	-	-	-	-	0.05 ±0.000	-	-	-	-
2	0.06 ±0.001	0.13	0.04	7.50	0.27	0.07 ±0.002	0.19	0.06	5.02	0.40	0.07 ±0.007	0.24	0.07	4.3	0.47	0.08 ±0.005	0.29	0.09	3.34	0.60	0.08 ±0.005	0.29	0.09	3.34	0.60
4	0.09 ±0.002	0.21	0.06	5.02	0.80	0.10 ±0.001	0.25	0.08	3.76	1.06	0.13 ±0.005	0.34	0.10	3.01	1.33	0.13 ±0.001	0.34	0.10	3.01	1.33	0.10 ±0.001	0.34	0.10	3.01	1.33
6	0.16 ±0.001	0.28	0.08	3.76	1.60	0.17 ±0.006	0.30	0.09	3.34	1.80	0.20 ±0.005	0.33	0.10	3.01	2.00	0.21 ±0.002	0.35	0.10	3.01	2.00	0.22 ±0.005	0.36	0.11	2.74	2.19
8	0.20 ±0.007	0.25	0.08	3.76	2.13	0.21 ±0.01	0.26	0.08	3.76	2.13	0.24 ±0.005	0.28	0.08	3.76	2.13	0.26 ±0.005	0.30	0.09	3.34	2.40	0.27 ±0.002	0.30	0.09	3.34	2.40
10	0.18 ±0.002	0.18	0.05	6.02	1.66	0.19 ±0.002	0.19	0.06	5.02	2.00	0.20 ±0.002	0.20	0.06	5.02	2.00	0.23 ±0.005	0.22	0.07	4.3	2.33	0.25 ±0.004	0.23	0.07	4.30	2.33
12	0.17 ±0.002	0.15	0.04	7.53	1.60	0.18 ±0.001	0.15	0.05	6.02	2.00	0.19 ±0.002	0.16	0.05	6.02	2.00	0.20 ±0.005	0.17	0.05	6.02	2.00	0.23 ±0.002	0.18	0.06	5.02	2.39

R\ = Mean growth rate
K\= Relative growth rate
G= Generation time
N= Number of recycling

Table (9): Effect of different concentrations of manganese chloride (µM) on dry. wt (mg/l) and lipid contents (mg lipid /l and % of lipid yield) of *Chlorella vulgaris*.

Time (days)	2µM			4µM			7.3 µM (control)			10 µM			12 µM		
	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %
0	122.50	14.47	11.82	122.50	14.47	11.82	122.50	14.47	11.82	122.50	14.47	11.82	122.50	14.47	11.82
2	139.65	16.51	11.82	154.35	18.24	11.82	159.25	18.82	11.82	159.25	18.82	11.82	159.25	18.82	11.82
4	151.90	22.65	14.91	171.50	22.65	13.21	173.90	22.94	13.19	181.30	31.20	17.21	183.75	31.20	16.98
6	159.25	31.20	19.59	181.30	31.20	17.21	183.70	31.60	17.20	191.10	33.74	17.66	200.90	33.74	16.80
8	173.95	33.74	19.40	196.00	33.74	17.22	200.00	40.48	20.24	205.80	48.30	23.47	208.25	48.29	23.19
10	166.60	48.30	28.99	191.10	48.29	25.27	198.45	48.91	24.65	196.00	56.84	28.90	200.90	56.84	28.29
12	164.15	48.26	29.40	188.65	48.29	25.60	193.55	57.56	29.74	193.55	56.84	29.37	196.00	65.40	33.36

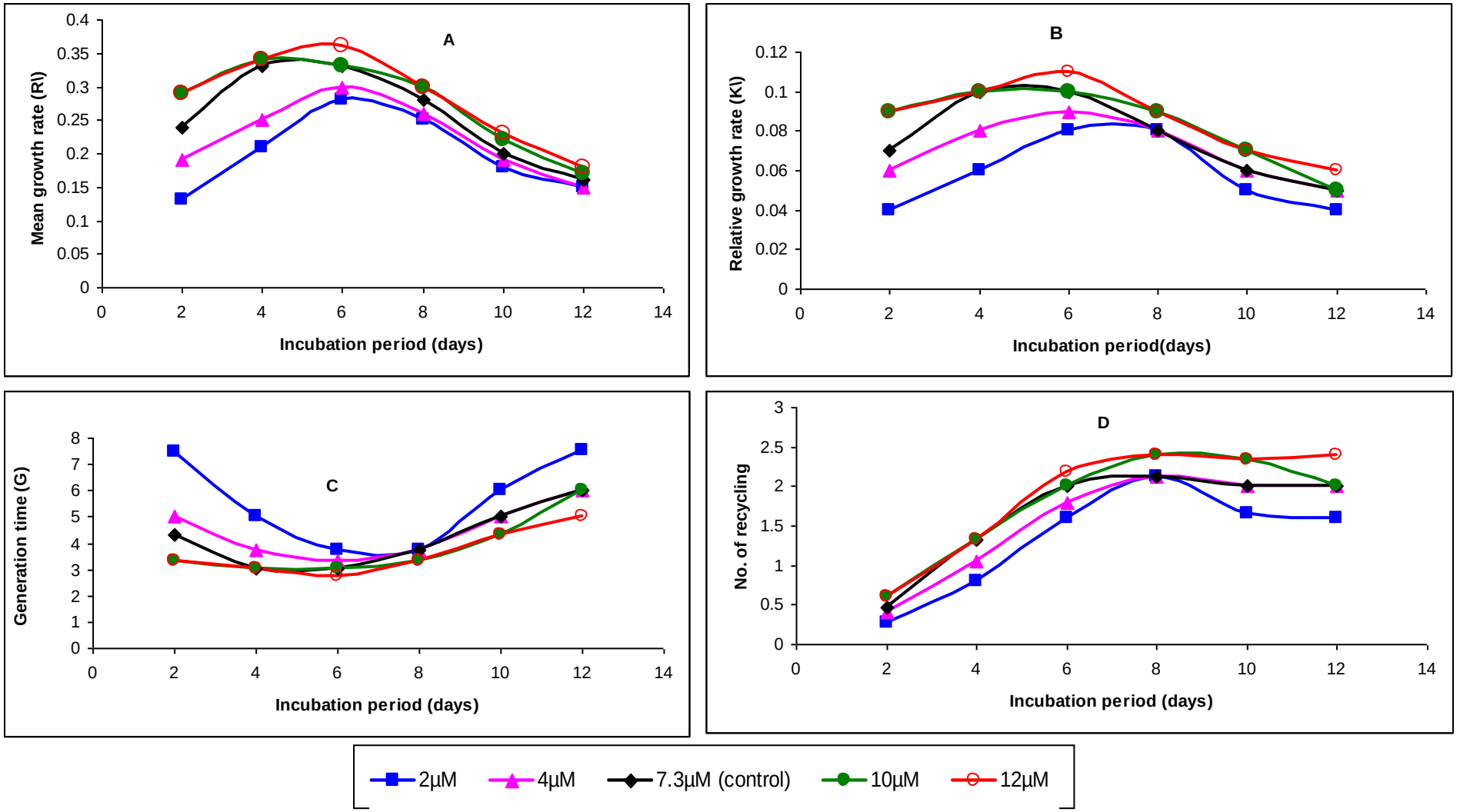

Fig.14. Effect of different concentrations of manganese chloride (µM) on growth parameters of *Chlorella vulgaris* measured as: **A**-Mean growth rate (R$^{\backslash}$) **B**-Relative growth rate (K$^{\backslash}$) **C**-Generation time (G) **D**-Number of recycling.

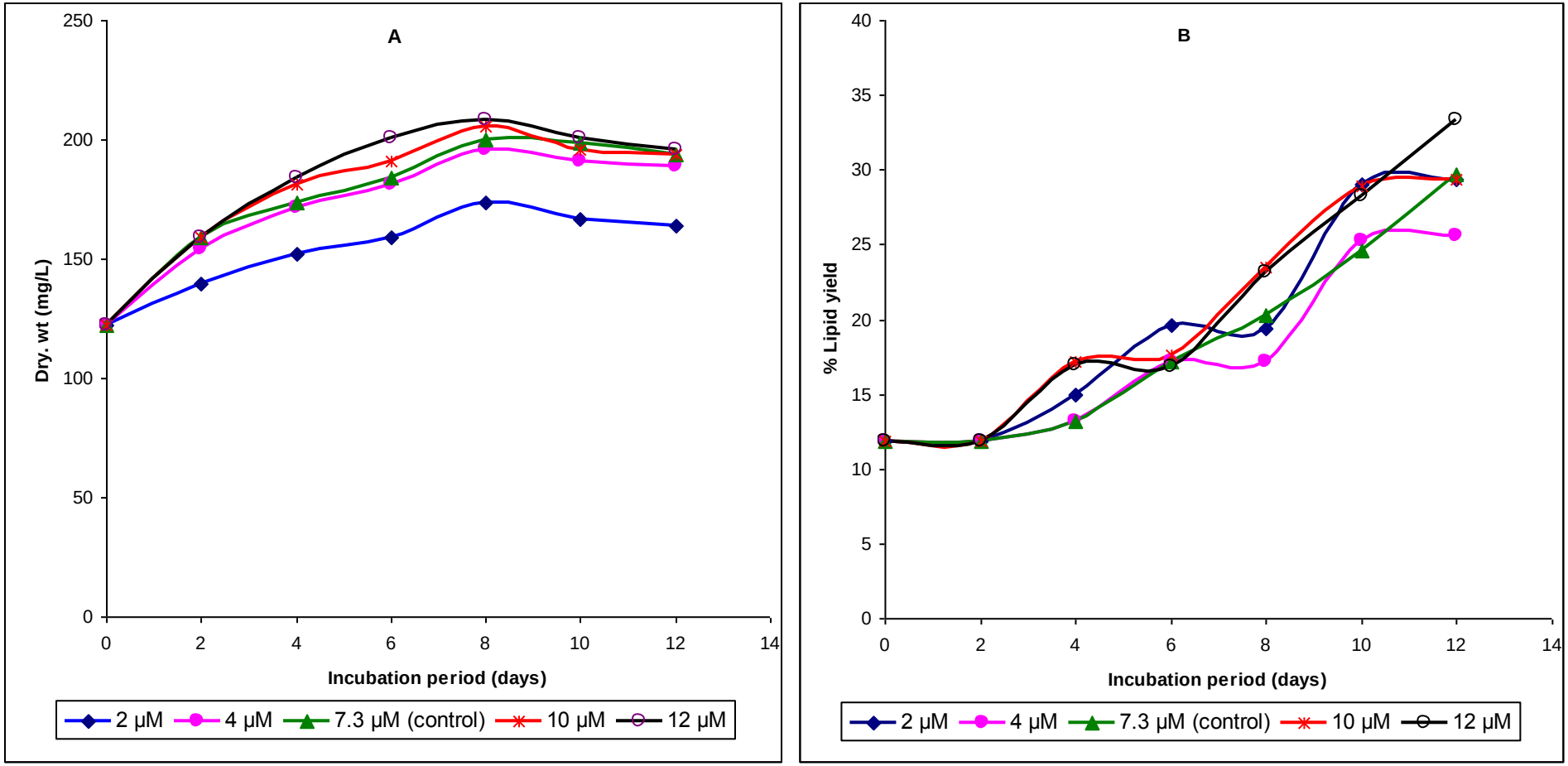

Fig.15. Effect of variable concentrations of manganese chloride (µM) on *Chlorella vulgaris*:

A- Dry wt. (mg/l). **B-** % of Lipid yield.

Experiment (5): Effect of different concentrations of cobalt nitrate (µM) on the growth and lipid content of *Chlorella vulgaris*

The mean growth rates and cell productivity of *Chlorella vulgaris* grown at different concentrations of cobalt nitrate (µM) are shown in Tab. (10) and Fig. (16). It was observed that the growth of *Chlorella vulgaris* was slightly affected under different cobalt nitrate concentrations. The growth was more or less similar than its concentration in BBM (1.68 µM). Treating the alga with 1µM and 2.5 µM of cobalt nitrate resulted in inhibition in the mean growth rate by 8.6 % and 11.4 % respectively from their corresponding control after 6 days old culture. Data also showed that treating *Chlorella vulgaris* with 2 µM of cobalt nitrate didn't cause any noticeable changes in the growth of *Chlorella vulgaris* from the corresponding control throughout the different incubation period.

Results in Tab. (11) and Fig. (17) manifested that the biosynthesis and accumulation of lipids in *Chlorella vulgaris* was slightly affected with the variable concentrations of cobalt nitrate (µM). Where the data obtained after 12 days old cultures at 2 µM and 2.5 µM of cobalt nitrate revealed that the lipid content in *Chlorella vulgaris* increased by 15 % and 2.6 % respectively more than their corresponding control. While after 12 days of culturing the alga with 1 µM the lipid content decreased by 7.96 % less than the corresponding control. The maximum accumulation of lipid was recorded (34.21 %) after 12 days old cultures treated with 2 µM more than its corresponding control.

Table (10): Effect of different concentrations of cobalt nitrate (µM) on the growth parameters of *Chlorella vulgaris*.

Time (days)	1.00 µM					1.68 µM (control)					2.00 µM					2.50 µM				
	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N
0	0.05±0.000	-	-	-	-	0.05±0.000	-	-	-	-	0.05±0.000	-	-	-	-	0.05±0.000	-	-	-	-
2	0.06±0.003	0.13	0.04	7.52	0.27	0.07±0.003	0.24	0.07	4.30	0.47	0.07±0.006	0.24	0.07	4.30	0.47	0.06±0.006	0.13	0.04	7.52	0.27
4	0.11±0.006	0.28	0.09	3.34	1.20	0.13±0.003	0.34	0.10	3.01	1.33	0.13±0.003	0.34	0.10	3.01	1.33	0.11±0.006	0.28	0.09	3.34	1.20
6	0.19±0.055	0.32	0.10	3.01	1.99	0.21±0.006	0.35	0.10	3.01	1.99	0.21±0.006	0.35	0.10	3.01	1.99	0.18±0.006	0.31	0.09	3.34	1.78
8	0.22±0.006	0.27	0.08	3.76	2.13	0.24±0.006	0.28	0.09	3.34	2.40	0.25±0.006	0.29	0.09	3.34	2.40	0.23±0.006	0.28	0.08	3.76	2.13
10	0.19±0.006	0.19	0.06	5.02	1.99	0.22±0.020	0.21	0.06	5.02	1.99	0.22±0.006	0.21	0.06	5.02	1.99	0.20±0.005	0.20	0.06	5.02	1.99
12	0.18±0.003	0.15	0.05	6.02	1.99	0.20±0.006	0.17	0.05	6.02	1.99	0.20±0.006	0.17	0.05	6.02	1.99	0.19±0.006	0.16	0.05	6.02	1.99

R\ = Mean growth rate
K\= Relative growth rate
G= Generation time
N= Number of recycling

Table (11): Effect of different concentrations of cobalt nitrate (µM) on dry. wt (mg/l) and lipid contents (mg lipid /l and % of lipid yield) of *Chlorella vulgaris*.

Time (Day)	1 µM			1.68 µM (control)			2 µM			2.5 µM		
	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %
0	122.50	14.47	11.82	122.50	14.47	11.82	122.50	14.47	11.82	122.50	14.47	11.82
2	156.80	18.53	11.82	159.25	18.82	11.82	161.70	19.11	11.82	151.90	17.95	11.82
4	166.60	14.10	8.4.65	173.90	22.94	13.19	176.40	22.65	12.84	171.50	22.65	13.21
6	176.40	22.65	12.84	183.70	31.60	17.20	191.10	39.74	20.79	181.30	31.20	17.21
8	181.30	31.20	17.21	200.00	40.48	20.24	210.70	48.29	22.92	200.90	39.74	19.78
10	181.30	39.74	21.92	198.45	48.91	24.65	200.90	56.84	28.29	191.10	48.29	25.27
12	176.40	48.30	27.38	193.55	57.56	29.74	191.10	65.38	34.21	186.20	56.84	30.53

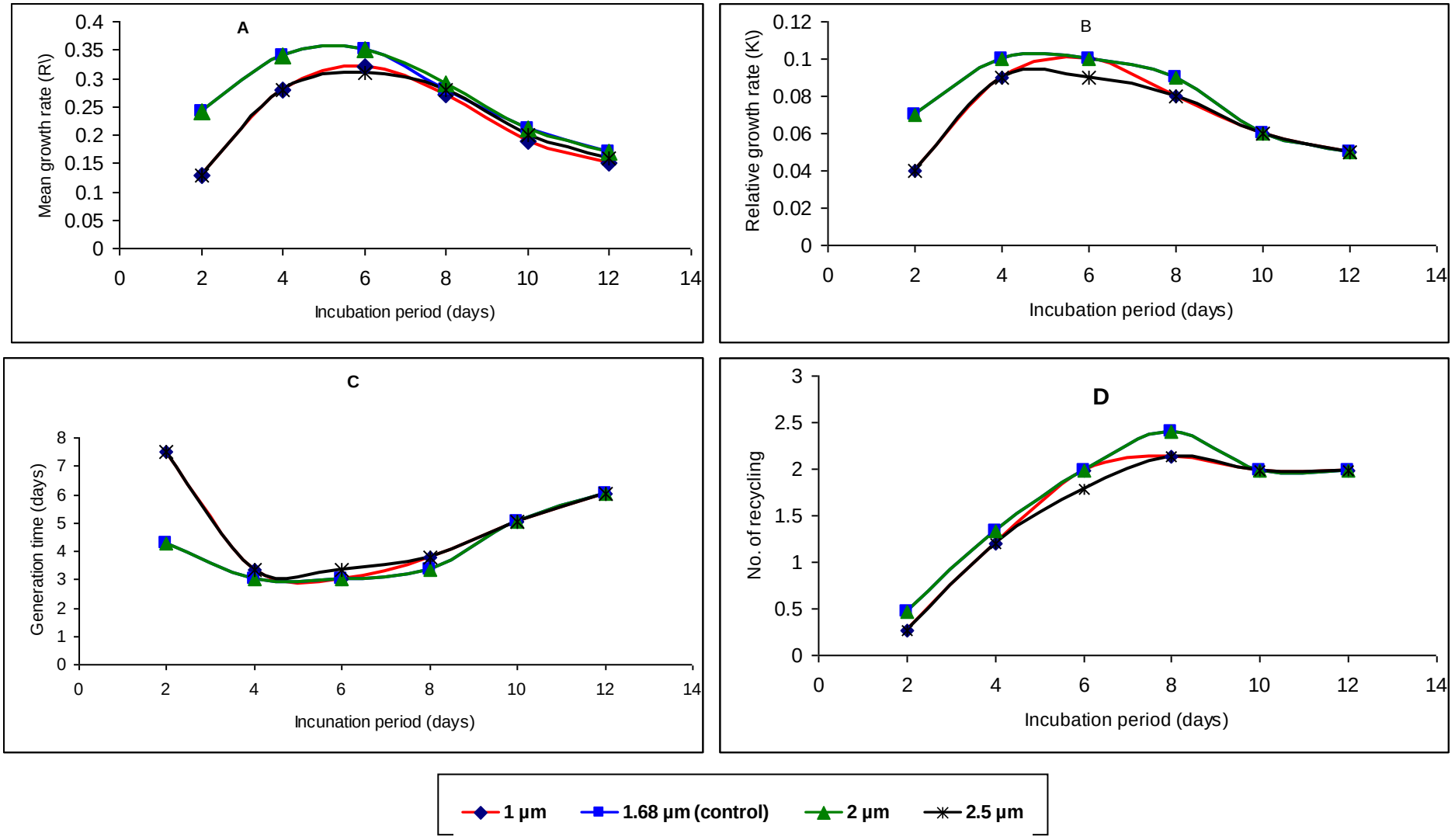

Fig.16. Effect of different concentrations of cobalt nitrate (µM) on growth parameters of *Chlorella vulgaris* measured as: **A**-Mean growth rate (R\) **B**-Relative growth rate (K\) **C**-Generation time (G) **D**-Number of recycling.

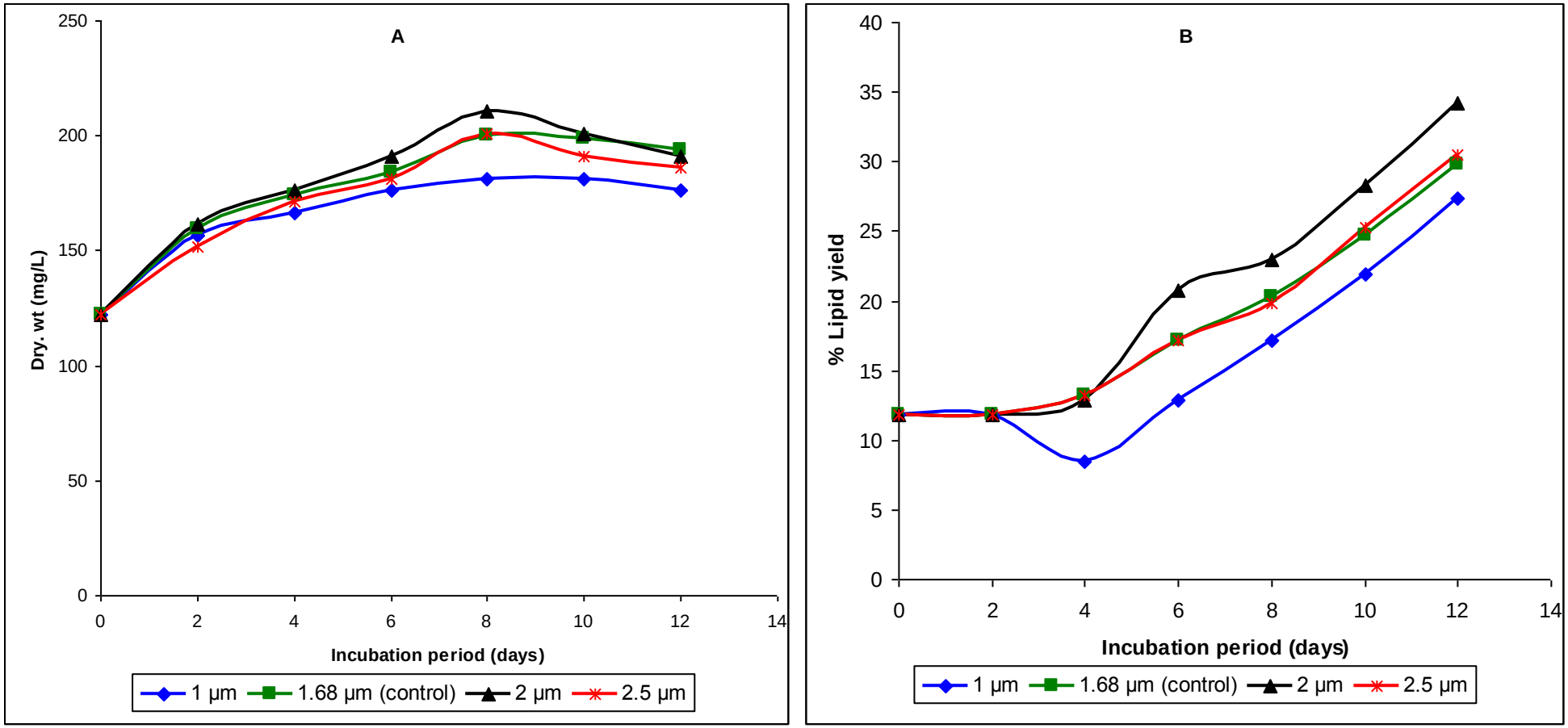

Fig.17. Effect different concentrations of cobalt nitrate (µM) on *Chlorella vulgaris*:

A- Dry wt. (mg/l). **B-** % of Lipid yield.

Experiment (6): Effect of different concentrations of hydrogen peroxide (mM) on the growth and lipid content of *Chlorella vulgaris*

The mean growth rates and cell productivity of *Chlorella vulgaris* grown at different concentrations of H_2O_2 (mM) were shown in Tab. (12) and Fig. (18). It was observed that the growth of *Chlorella vulgaris* was subsequently decreased as it treated with different concentrations of H_2O_2 up to 4mM. While treating the alga with 6mM H_2O_2 causes a great suppression in the growth and the alga enter the death phase after 2 days of incubation period, so this concentration is consider the lethal one. Also, after 6 days of treating *Chlorella vulgaris* with 2mM and 4mM the mean growth rate inhibited by 25.8% and 45.2% respectively as compared with the corresponding control. The maximum growth was recorded in normal control conditions where the mean growth rate was 0.31 d^{-1} with generation time 3.27 d and during this period the alga recycled itself 1.8 cycle/ day.

The impact of different concentrations of H_2O_2 on the lipid contents of *Chlorella vulgaris* was shown in Tab. (13) and Fig. (19). It was revealed that the biosynthesis and accumulation of lipid was subsequently increased with increasing concentrations of H_2O_2 up to 4mM. It was calculated from the data that after 12 days of treating the alga with 2mM and 4mM the lipid yield was increased by 3.2% and 53.6% respectively more than the corresponding control. The maximum yield of lipid was recorded 45.86% after 12 days old cultures treated with 4 mM H_2O_2.

Table (12): Effect of different concentrations of hydrogen peroxide (mM) on the growth parameters of *Chlorella vulgaris*.

Time (days)	(0.00 mM) Control					2mM					4mM					6mM				
	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N	OD (665)	R\	K\	G	N
0	0.05± 0.008	-	-	-	-	0.05± 0.00	-	-	-	-	0.05± 0.004	-	-	-	-	0.05± 0.004	-	-	-	-
2	0.07 ± 0.00	0.24	0.07	4.12	0.49	0.07± 0.004	0.19	0.06	2.30	0.87	0.06± 0.004	0.13	0.04	7.72	0.26	0.04± 0.004	-	-	-	-
4	0.11± 0.008	0.28	0.09	3.54	1.13	0.09± 0.008	0.21	0.06	4.72	0.85	0.08± 0.001	0.15	0.04	6.84	0.59	0.00	0.00	0.00	0.00	0.00
6	0.18± 0.008	0.31	0.09	3.27	1.8	0.13± 0.004	0.23	0.07	4.36	1.38	0.10± 0.007	0.17	0.05	6.02	0.99	0.00	0.00	0.00	0.00	0.00
8	0.23 ± 0.00	0.28	0.08	3.67	2.18	0.16± 0.002	0.21	0.06	4.77	1.68	0.12± 0.000	0.16	0.05	6.27	1.28	0.00	0.00	0.00	0.00	0.00
10	0.20± 0.004	0.20	0.06	5.10	1.96	0.18± 0.004	0.18	0.06	5.41	1.85	0.10± 0.000	0.09	0.03	10.75	0.93	0.00	0.00	0.00	0.00	0.00
12	0.19 ± 0.00	0.16	0.05	6.27	1.91	0.17± 0.004	0.15	0.05	6.64	1.81	0.09± 0.000	0.07	0.02	14.14	0.85	0.00	0.00	0.00	0.00	0.00

R\ = Mean growth rate
K\= Relative growth rate
G= Generation time
N= Number of recycling

Table (13): Effect of different concentrations of hydrogen peroxide (mM) on dry. wt (mg/l) and lipid contents (mg lipid /l and % of lipid yield) of *Chlorella vulgaris*.

Time (Day)	(Control)			2mM			4mM			6mM		
	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %	Dry. wt (mg/l)	Lipid content (mg lipid/l)	Lipid yield %
0	122.50	14.47	11.82	120.54	14.47	11.82	122.50	14.47	11.82	122.50	14.47	11.82
2	159.25	18.82	11.82	135.24	15.98	11.82	128.87	15.23	11.82	112.00	9.52	8.50
4	173.90	22.94	13.19	153.37	22.94	14.96	135.20	22.94	16.97	86.00	0.00	6.00
6	183.70	31.60	17.20	166.00	31.60	19.04	145.00	31.60	21.79	50.00	0.00	0.00
8	200.00	40.48	20.24	177.80	43.35	24.38	151.90	57.57	37.90	0.00	0.00	0.00
10	198.45	48.91	24.65	189.14	57.57	30.44	147.00	66.15	45.05	0.00	0.00	0.00
12	193.55	57.57	29.74	187.67	57.57	30.68	145.00	66.23	45.68	0.00	0.00	0.00

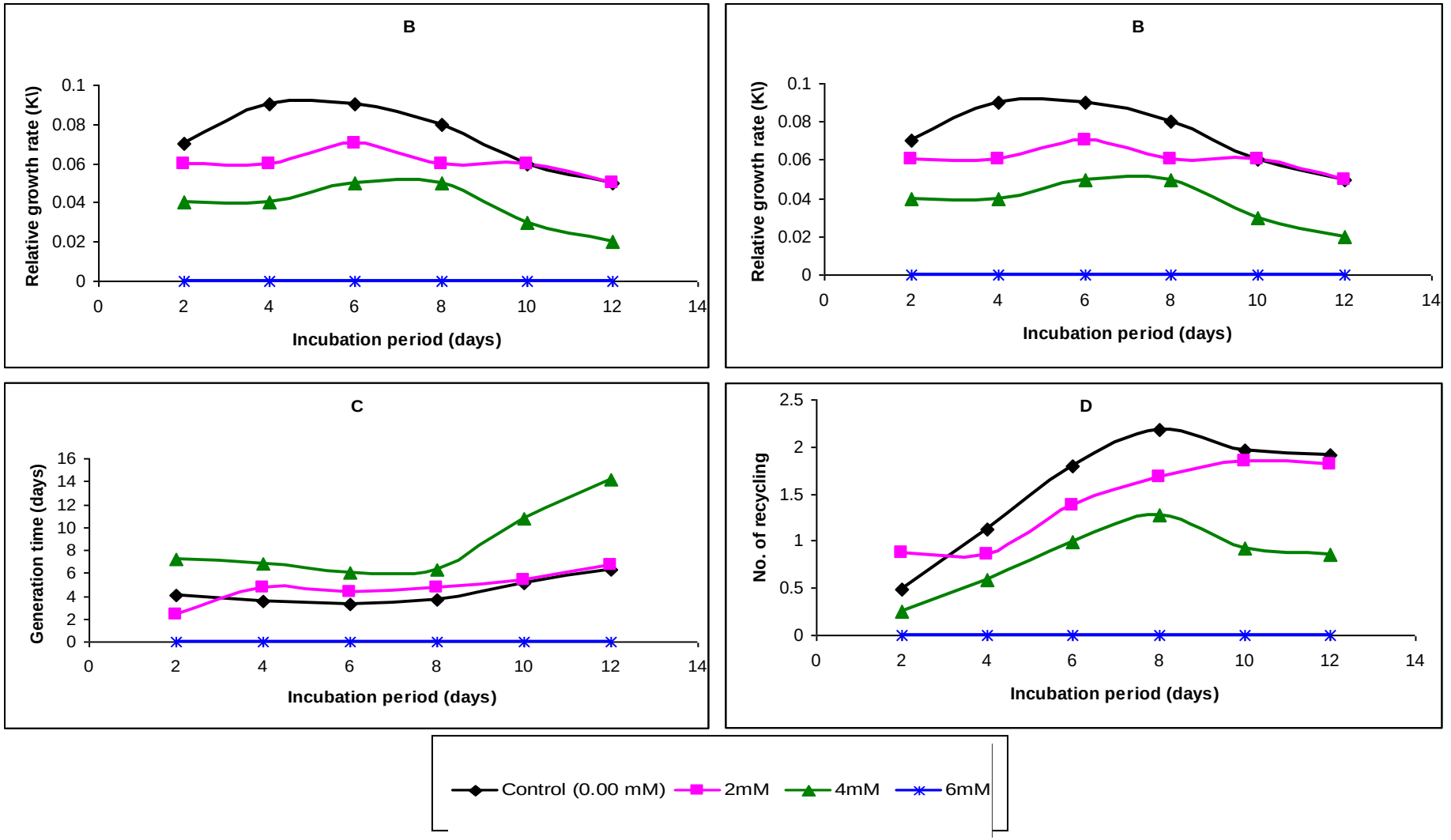

Fig.18. Effect of different concentrations of H_2O_2 (mM) on growth parameters of *Chlorella vulgaris* measured as: **A**-Mean growth rate (R\`) **B**-Relative growth rate (K\`) **C**-Generation time (G) **D**-Number of recycling.

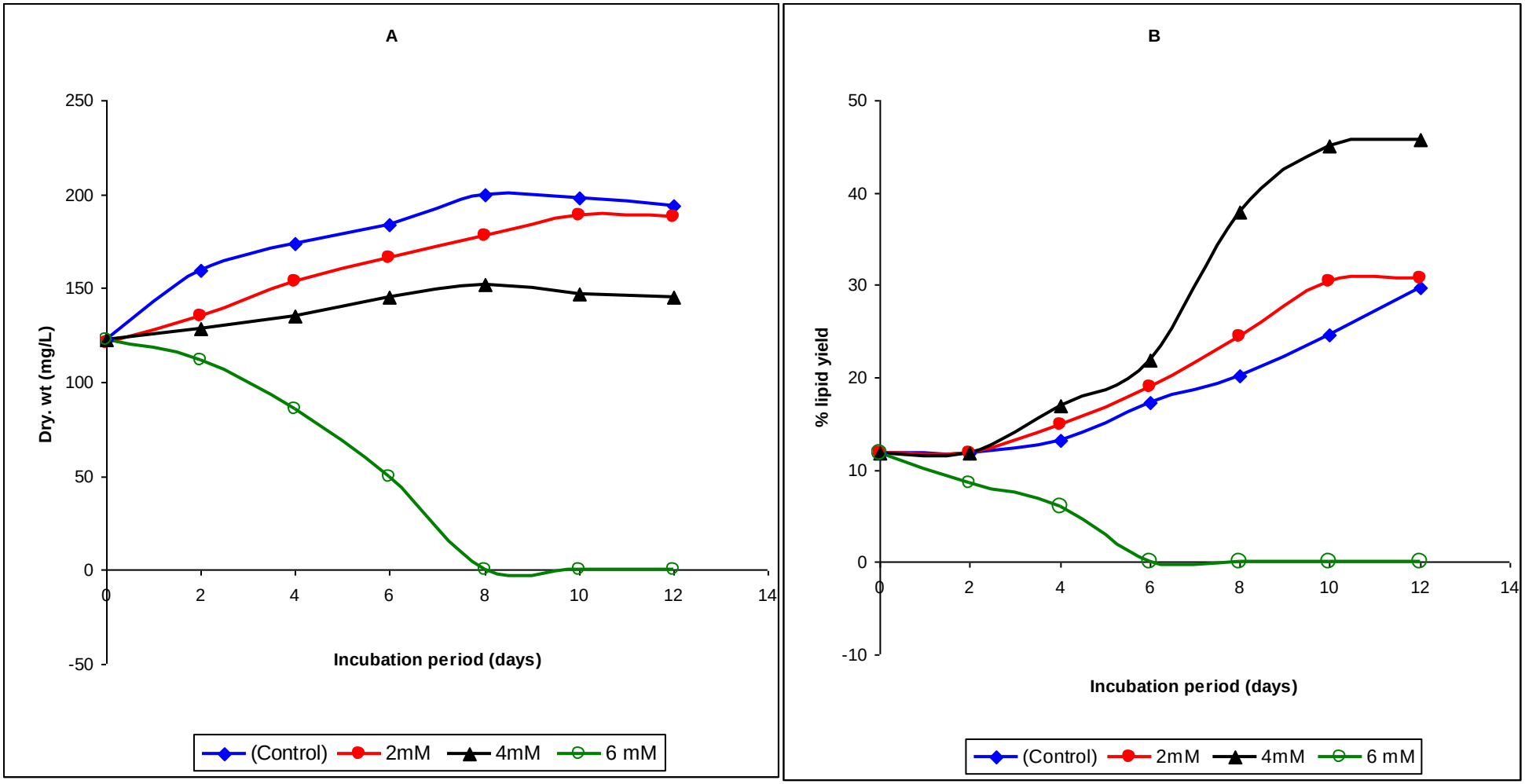

Fig.19. Effect of different concentrations of H_2O_2 (mM) on *Chlorella vulgaris*:

A- Dry wt. (mg/l). **B-** % of Lipid yield.

Experiment (7): Photosynthetic pigment contents of *Chlorella vulgaris*

The effect of the previous stress factors (specially the concentrations that gives the highest lipid content) on *Chlorella vulgaris* as shown in Tab. (14) and Fig. (20) revealed that all treatments resulted in an obvious decreases in photosynthetic pigments of (Chlorophyll a, Chlorophyll b, carotenoids and total pigments) compared with the corresponding control. On the other hand the other hand results indicated that the only increases in photosynthetic pigments were recorded when the alga treated with 35.25 µM ferrous sulphate.

Data revealed that, the highest stimulatory effect on the photosynthetic pigments was recorded in cultures subjected to 35.25 µM ferrous sulphate, where the pigments contents recorded (Ch. a=149.6, Ch. b=90.8 and Car. =38 µg/g dry. wt) while it recorded (Ch. a =122.40, Ch. b =70.40 and Car. =37.34 µg/g dry. wt) for untreated control. The highest inhibitory effect on the photosynthetic pigments was recorded in cultures subjected to 4mM H_2O_2, where the pigments contents recorded (Ch. a =79.60, Ch. b =32.08 and Car. =22.80 µg/g dry. wt).

Table (14): Photosynthetic pigment contents of *Chlorella vulgaris* treated with the best concentrations of different nutrients for lipid production.

Treatment	Selected concentration	Pigment contents (µg/g dry. wt)			
		Ch. a	Ch. b	Car.	Total pigments
Control	**-**	122.40 ±0.55	70.40 ±2.3	37.34±0.58	230.14±1.8
Sodium nitrate	**0.10 mM**	83.22 ±2.6	38.41 ±1.2	32.60±1.3	154.23±4.4
Sodium chloride	**0.45 mM**	94.84 ±4.0	49.60 ±2.3	25.20±0.57	169.64±5.0
Ferrous sulphate	**35.25 µM**	149.60 ±1.8	90.80 ±0.69	38.00±0.57	278.40±2.5
Manganese chloride	**12.00 µM**	112.70 ±0.85	68.35 ±0.20	36.95±0.59	218.00±1.15
Cobalt nitrate	**2.00 µM**	105.80±0.52	65.00 ±0.28	30.37±0.50	201.17±0.78
Hydrogen peroxide	**4.00 mM**	79.60±0.62	32.08 ±0.90	22.80±0.48	134.48±0.52

Values expressed as ± SD * Each value is a mean of 3 replicates.

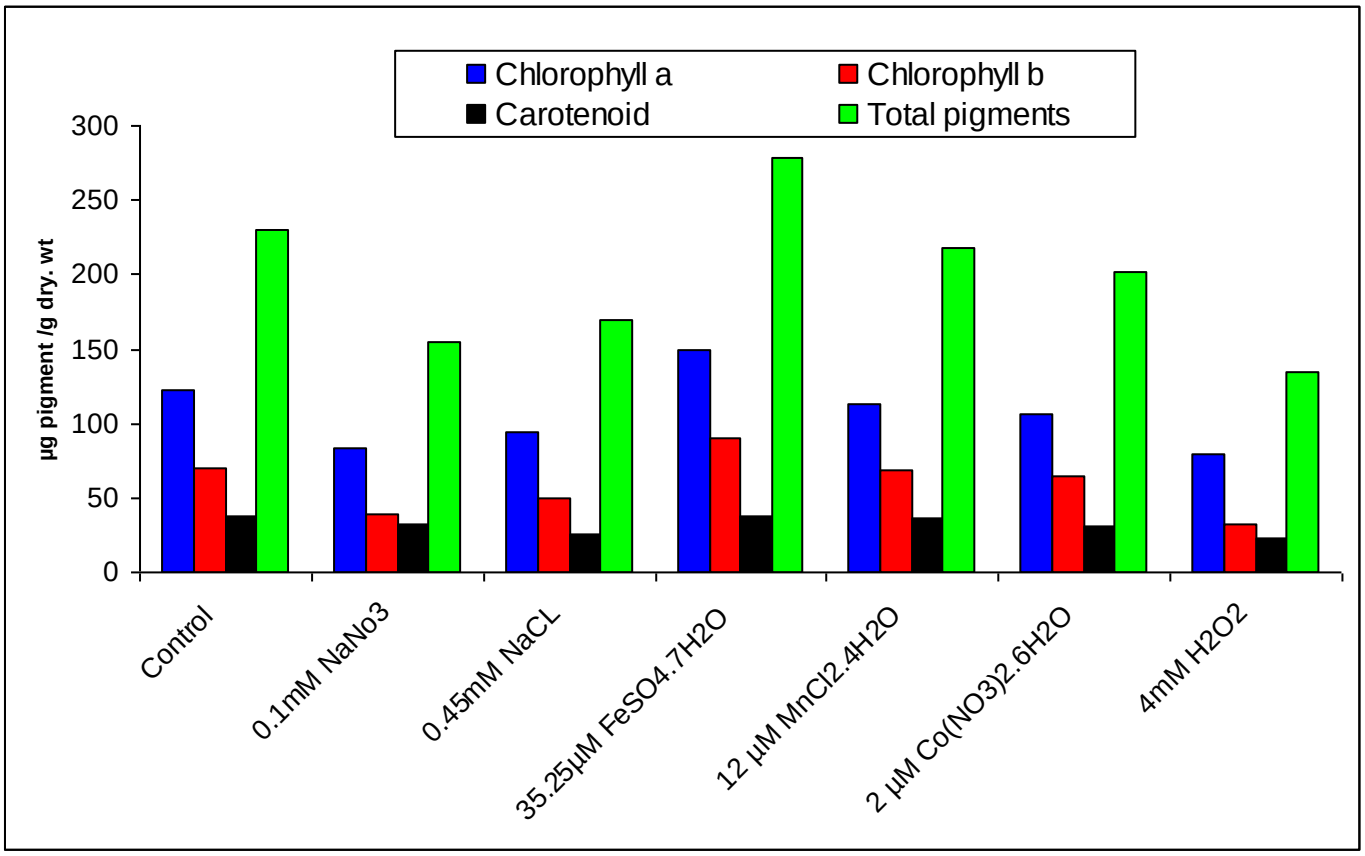

Fig.20. Photosynthetic pigment contents (expressed as µg/g dry. wt) of *Chlorella vulgaris* treated with best concentrations of different nutrients for lipid production.

Experiment (8): Major biochemical components of the dry biomass of *Chlorella vulgaris*

The major biochemical components of the dry biomass of *Chlorella vulgaris* treated with the best concentrations of different nutrients for lipid production were shown in Tab. (15) and Fig. (21). Results revealed that, under the maximum stress factors (as previously mentioned from experiment 1 to 7) there were great variations in the major biochemical contents of *Chlorella vulgaris*. The resulted data indicated that under the previous stress factors the biosynthesis and accumulation of lipids in *Chlorella vulgaris* varies inversely with the accumulation of carbohydrates and protein contents. The resulted data also, manifested that among the used stress factors 0.10 mM $NaNO_3$ considered a feasible tool for the over production of lipid where, it maximally recorded 52.3% with increasing of 83.5% from the corresponding control. On the other hand the maximum accumulation of carbohydrates and proteins with observed in un treated cultures of *Chlorella vulgaris*

The data also, showed that the lipid content determined gravimetrically don't differ so much from the previous amount of lipid determined spectrofluorometrically using Nile red stain.

Table (15): Major biochemical components of the dry biomass of *Chlorella vulgaris* treated with the best concentrations of different nutrients for lipid production

Treatment	Selected concentration	Lipid %	Carbohydrates %	Protein %
Control	-	28.50	14.34	25.68
Sodium nitrate	**0.10 mM**	52.30	9.79	14.28
Sodium chloride	**0.45 mM**	44.00	10.33	19.28
Ferrous sulphate	**35.25 µM**	32.70	11.97	24.48
Manganese chloride	**12.00 µM**	31.00	12.88	23.28
Cobalt nitrate	**2.00 µM**	32.50	11.61	22.48
Hydrogen peroxide	**4.00 mM**	46.20	9.61	20.88

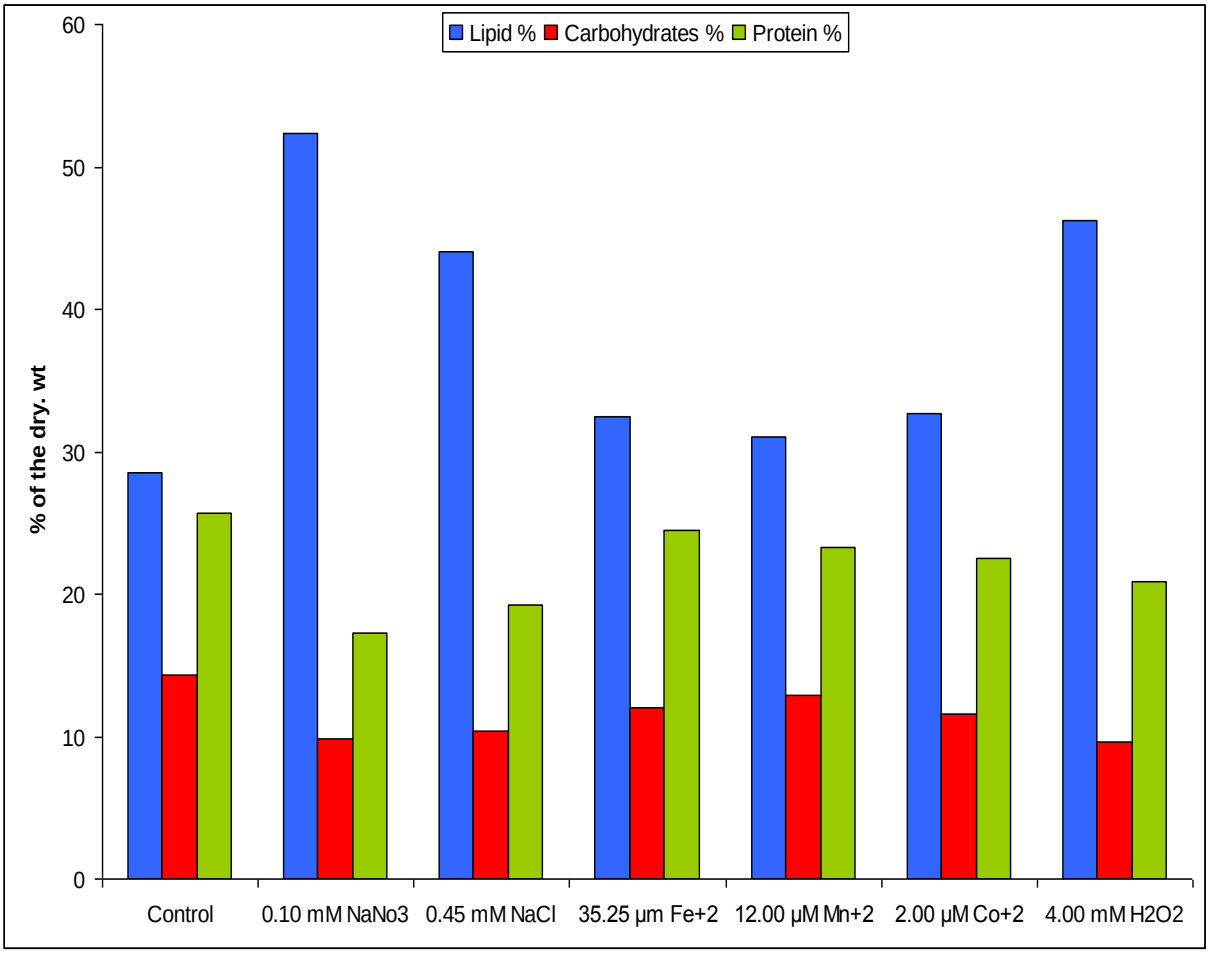

Fig.21. Major Biochemical components of the dry biomass of *Chlorella vulgaris* comprises (lipids %, Carbohydrates % and protein %) treated with the best concentrations of different nutrients for lipid production.

Experiment (9): Gas Liqiud Chromatography analysis (GLC) of fatty acid methyl esters (%) of *Chlorella vulgaris*

Gas chromatographic analysis of fatty acid methyl esters % of *Chlorella vulgaris* treated with the best concentrations of different nutrients for lipid production were shown in Tab. (16). Thirteen fatty acids were identified from Capric (C12:0) to Lignoceric fatty acids (C24:0). It was found that treating *Chlorella vulgaris* with 0.1mM of $NaNO_3$ and 0.45mM of NaCl resulted in an obvious increase in total saturated fatty acids (SFA) with percent 14% and 3.3% respectively from their corresponding control. On the other hand, treating the alga with 35.25 µM $FeSO_4$, 12 µM $MnCl_2$, 2 µM $Co(NO_3)_2$ and 4 mM H_2O_2 resulted in an obvious decreases in total SFA with percentages 3.3%, 13.7%, 21.8% and 9.9% respectively from their corresponding control. So, the resulted decreases in total SFA may be shifted towards the biosynthesis of unsaturated fatty acids (USFA) and vice versa. The total SFA percentages ranged from 40.54-59.07 % and the maximum yield of saturated fatty acid was found in the lipid extract of cultures treated with 0.1 mM of $NaNO_3$. Data also showed that the major constituents of the lipid fraction of *Chlorella vulgaris* were found to be C14, C16, C18, C18:1, C18:2 and C18:3 fatty acids. Also, it was found that all of the treatments in the present work resulted in formation of C24 fatty acid which wasn't recorded in the lipid profile of untreated alga.

Treating the alga with 0.1 mM of $NaNO_3$ resulted in an obvious increase in the relative percentages of saturated fatty acid (SFA) more than its corresponding control. The increase in SFA is due to increases in the content of stearic acid (C18). On the other hand, there was a decrease in unsaturated fatty acid (USFA) contents from the corresponding control (especially in palmitolec (C16:1), linoleic (C18:2) and α-linoleic (C18:3)).

Also it was found that C10, C12, C16:1, C17, C20 and C22 fatty acids weren't recorded in the resulted lipid profile at 0.1 mM of $NaNO_3$ compared with the control lipid profile.

Not only 0.1 mM of $NaNO_3$ but also**,** treating the alga with 0.45mM NaCl resulted in an obvious increase in SFA contents more than their corresponding control. This increase is due to the biosynthesis of Lignoceric acid (C24:0= 24% of total content) which wasn't recorded in the corresponding control. On the other hand, there were a slightly decreases in USFA (especially in C16:1 and C18:2) from the corresponding control.

On the other hand, treating *Chlorella vulgaris* with 35.25µM $FeSO_4$ resulted in a slightly decreases in the total contents of SFA with a slightly increases in total USFA as compared with control. The previous decreases in SFA (especially in the contents of C14:0 and C16:0) may be shifted in the synthesis of long chain fatty acid (C24:0=12.72% of total content) which wasn't recorded in untreated culture

In the same manner treating the alga with 12µM $MnCl_2$ causes an obvious decrease in SFA with noticeable increases in USFA as compared with corresponding control. The reduction in SFA from the control profile is due to the reduction in the contents of C10:0, C14:0, C16:0, C18:0 fatty acids and absence of C12:0 fatty acid. Where as the increases in USFA is due to increases the content of C18:1 and C18:2 fatty acids .The resulted profile also shows the formation of Lignoceric acid (C24:0=10.34%) which wasn't detected in the un treated lipid profile.

Also, it was found that treating *Chlorella vulgaris* with 2µM $Co(NO_3)_2$ causes an obvious decrease in SFA fatty acids and increases in USFA as compared with the corresponding control. The formed profile

indicates that the decrease in SFA from the control profile is due to decrease in contents of C14:0, C16:0, C17:0, C18:0, C20:0, C22:0 fatty acids and absence of C10:0 and C12 fatty acids. While the increases in USFA is due to increases in the contents of C18:1, C18:2, C18:3 fatty acid as compared with the control profile. The results of methyl ester profile also shows the formation of Lignoceric acid (C24:0=26.04%) which wasn't detected in the untreated lipid profile.

Also, the lipid profile obtained from treating the alga with 4mM H_2O_2 resulted in an obvious decrease in total content of SFA with an increase in the total USFA as compared with the untreated lipid profile. The formed profile indicated that the decreases in SFA from the control profile are due to decreases in contents of C14:0, C16:0, C17:0, C18:0, C20:0 fatty acids and absence of C12:0 and C22 fatty acids. While the increases in USFA is due to increases in the contents of C18:1, C18:2, C18:3 fatty acid as compared with the control profile. The resulted profile also shows the formation of Lignoceric acid (C24:0=23.14%) which wasn't detected in the untreated lipid profile.

Finally, the results of methyl ester profiles for all treatments suggested that 0.1mM of $NaNO_3$ and 0.45mM of NaCl considered suitable conditions for a lipid production with highly SFA in *Chlorella vulgaris*.

Table (16): Gas liqiud Chromatography analysis (GLC) of fatty acid methyl esters (%) of *Chlorella vulgaris* treated with best concentrations of different nutrients for lipid production

Fatty acids	*C-Number of fatty acids*	Control	0.1mM $NaNO_3$	0.45mM NaCl	35.25µM $FeSO_4$	12 µM $MnCl_2$	2 µM $Co(NO_3)_2$	4mM H_2O_2
Capric	**C10:0**	1.38	-	2.5	2.36	0.64	-	2.58
Lauric	**C12:0**	4.67	-	2.13	2.51	-	-	-
Myristic	**C14:0**	14.57	1.45	7.02	6.46	2.86	0.99	4.50
Palmitic	**C16:0**	19.83	11.17	12.93	14.24	14.89	8.09	10.50
Palmitoleic	**C16:1**	7.56	-	3.7	4.47	0.18	-	1.59
Margaric	**C17:0**	2.79	-	0.42	2.75	2.89	1.04	1.36
Stearic	**C18:0**	5.54	44.11	2.33	5.52	1.43	3.33	4.13
Oleic	**C18:1**	10.69	38.50	11.48	14.66	23.26	17.44	16.25
Linoleic	**C18:2**	17.64	1.64	16.49	17.50	27.07	19.95	19.31
α-Linoleic	**C18:3**	12.24	0.77	14.27	13.22	4.70	22.07	16.15
Arachidic	**C20:0**	1.57	-	0.66	2.27	9.03	0.39	0.48
Behenic	**C22:0**	1.49	-	1.57	1.30	2.67	0.66	-
Lignoceric	**C24:0**	-	2.34	24.00	12.72	10.34	26.04	23.14
Saturated fatty acid (SFA)		**51.84**	**59.07**	**53.56**	**50.13**	**44.75**	**40.54**	**46.69**
Unsaturated fatty acid (USFA)		**48.13**	**40.90**	**45.94**	**49.85**	**55.03**	**59.46**	**53.30**
Ratio of SFA / USFA		**1.08**	**1.44**	**1.17**	**1.01**	**0.81**	**0.68**	**0.88**
Total fatty acid		**99.97**	**99.97**	**99.50**	**99.98**	**99.78**	**100**	**99.99**