Ass. Univ. Bull. Environ. Res. Vol. 6 No. 1, March 2003

AUCES

INTERACTIVE EFFECT OF THIAMIN (VITAMIN B_1) AND COPPER ON GROWTH, TOTAL PIGMENTS, PHOTOSYNTHESIS AND SOME RELATED METABOLIC ACTIVITIES OF *CHLORELLA VULGARIS BEIJER* CULTURES

Sayed A. Desouky

Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Assiut Branch)

ABSTRACT :

This study followed the interactive effect of thiamin (vitamin B_1) and $CuSO_4$ on growth criteria, total pigments, photosynthesis, respiration, carbohydrates, proteins and free amino acids of *Chlorella vulgaris* Beiger cultured for 7 days.

When *Chlorella vulgaris* cultures was treatment with only $CuSO_4$, the values of growth parameters, total carbohydrate contents, total proteins and free amino acids were significantly raised up to the treatment level of 4 µM of $CuSO_4$. Under relatively higher levels (8 and 10 µM) of $CuSO_4$, the values of these parameters were significantly lowered. On the other side, respiration rate, the contents of soluble carbohydrates and soluble proteins were significantly raised with the rising of $CuSO_4$ concentrations up to the level of 10 µM $CuSO_4$.

On the other hand, the interactive effect of $CuSO_4$ and thiamin (200 ppm) on growth parameters, total pigments, photosynthetic rate, and some related metabolic activities of *Chlorella* exerted a significant increase under the various levels (2, 4, 6, 8 and 10 µM) of $CuSO_4$. On the contrary, this interactive effect of 200 ppm thiamin and $CuSO_4$ significantly reduced the respiration rate of *Chlorella,* subjected to various levels (2, 4, 6, 8 and 10 µM) of $CuSO_4$.

INTRODUCTION:

Excess copper in soil leads to toxic effects on plant metabolism such as growth inhibition, oxidative stress and membrane damage, alteration of enzyme activity, inhibition of photosynthesis etc. (Lidon *et al.,* 1993, Ciscato et al, 1997). On the other side, copper is an essential micronutrient, being a component of enzymes involved in several important metabolic and physiological processes. The function of copper as a plant nutrient is based mostly on the participation of enzymatically bound copper in redox reactions (Marschner, 1998). Besides other functions, copper is also involved in the photosynthetic electron transport, being a component of plastocyanine, and plays an important role in the regulation of

PSII-mediated electron transport (Barón *et al.,* 1995).Commonly observed toxic effect on photosynthetic apparatus include decrease of photochemical activities, damage to the structure and composition of the thylakoid membrane and alterations at the pigment level (Ouzounidou, 1996 and Ciscato *et al.,* 1997). On the other hand, excess copper seems to have an inhibitory effect on the activity of both photosystems (Droppa and Horwath, 1990). Similarly, the results of several studies on the photosynthetic apparatus in excess Cu-treated plants reported that Cu have a complex toxic action at the level of primary reactions of photosynthesis. In this context, Lidon *et al.,* (1993) found that the decrease of photochemical activities (mainly by PSII) was shown to be accompanied by an alternation of the inner structure and composition of the thylakoid membrane. However other works, reported that the decrease in those pigment is accompanied by an inhibiting aminolevulinic dehydratase and / or stimulates chlorophyll per oxidation by inducing production of hydroxyl radicals (Martinon *et al.,* 1982 and Fernando and Henriques 1991). On the contrary, Lidon and Henriques (1991) found that a small increase in chlorophylls and carotenoids in *Spinacea oleracea* when exposed to higher levels of Cu.

In this respect, some works were conducted to try to alleviate the inhibitory effects of some heavy metals and salinity stress by using some exogenously added (Zidan, 1991, Arrigoni *et al.,* 1992; Desouky,1995 and Desouky, 2001).The counteractive effects of thiamin might linked with the physiological role of this vitamin. Such vitamins can be used as coenzyme performed generally the major physiological functions in the plant cells (Kefeli, 1981). Mohamed *et al.,* (1988) reported that thiamin increased the protein contents of *Chlorella vulgaris*, and the highest protein contents were produced in the presence of the concentration of 100 ppm thiamin. Similarly, Makled (1995) working with two green algae; namely *Chlorella vulgaris* and *Akistrodesmus falcatus,* reported that the addition of thiamin, nicotinic acid or pyridoxine exerted a considerable increase in growth rate, the synthesis of total pigments, photosynthetic oxygen evolution, carbohydrate contents and protein contents. On the other side, some other investigators found that the growth criteria, total pigments, photosynthetic oxygen evolution, carbohydrate contents and protein contents and free amino acids were markedly raised, when exposed to salinity stress and treatment with any of the vitamins applied (200 ppm of ascorbic acid, thiamin and pyridoxine) (Zidan ,1991, Arrigoni *et al.*, 1992 and Desouky, 1995). Similarly, Desouky, (2001) found that the growth parameters (cell number and dry weight) total pigments of *Chlorella vulgaris* cultures were significantly stimulated, when the algal cultures were subjected to various concentrations (20, 40, 60 80 and 100 ppm) of $ZnCl_2$ and treated with any of the vitamins applied (200 ppm of thiamin, pyridoxine and riboflavin).

As far as the works available, rarely studies were intentionally conducted using thiamin aiming to alleviate the toxic effects of higher concentrations of copper on the algae cells. Therefore, this work was conducted to try to alleviate the inhibitory effects of excess copper after addition of thiamin.

MATERIALS AND METHODS:

Tested alga:

***Chlorella vulgaris* Beijer was collected from the River Nile and used as a test organism. Beijerinck's nutritive culture the test algal was**

used as a medium for enrichment and growth of the tested algal (Stein, 1966).

Treatments:

Chlorella vulgaris **Beijer cultures were subjected to various concentrations (2,4,6,8 and 10 µM) of $CuSO_4$ without and with 200 ppm of thiamin for 7 days. The control cultures (00) Cu^{2+} and (00) thiamin was left without any treatments.**

. . . . . .

Analytical methods:

1-Determination of cell number: Haemocytometer, (0.1mm depth) having improved Naubuer ruling was used. One drop of the algal suspension was pipetted on the slide, covered and left two minutes for algal setting. The mean counts of four replicates were taken into consideration and the results were measured as cells ml^{-1} alga1 suspension.

2-Determination of dry weight: A definite volume (50-ml) of algal suspension was filtered through weighed glass fiber filter. The cells after being precipitated on the filter were washed twice with distilled water and dried over night in an oven at 105 ^{o}C. The data were measured as µg ml^{-1} algal suspension.

3-Determination of photosynthetic pigments: The pigment fractions chlorophyll a, chlorophyll b and carotenoids were calculated by using the equations mentioned by Metzner *et al.*, (1965). The results were expressed as (µg $m1^{-1}$ algal suspension).

4-Determination of oxygen evolution and oxygen uptake: Oxygen evolution (photosynthetic rate) and oxygen uptakes (dark respiration) were measured by using oxygen electrode (ORION Model 97-08) according to the method adopted by Lessler *et al.*, (1956). The results were measured as µ mole O_2 ml^{-1} algal suspension hr^{-1}.

5-Determination of carbohydrates: By using the anthrone-sulphoric acid reagent according to the method Badour (1959). The data were measured as µg mg^{-1} dry weight.

6-Determination of proteins: By using folin reagent according to the method adopted by Lowry *et al.*,(1951). The data were expressed as µg mg^{-1} dry weight.

7-Determination of free amino acids: By using the method of Moore and Stein (1948). The results were measured as µg mg^{-1} dry weight.

8-Statistical Analysis: Four replicates were used in this study and the data were statistically analyzed to calculate the Least Significant Difference (L.S.D) according to Snedecor and Cochran (1972).

RESULTS:

The results present in this study elucidated the important role of thiamin on counteraction the toxic effects of $CuSO_4$ on growth, photosynthetic pigments, photosynthetic oxygen evolution and some related metabolic activities of *Chlorells vulgaris* after the culture being treated for 7 day.

Treating *Chlorells vulgaris* cultures with only $CuSO_4$, the growth criteria (cell number and dry weight), total pigments, photosynthetic oxygen evolution rate, total carbohydrate contents, total protein contents and free amino acid were significantly elevated up to the level of 4µM $CuSO_4$ Under relatively higher concentrations (6, 8 and 10 µM) of $CuSO_4$, the values of these parameters were significantly decreased (Tables 1-a, 2-a, 3-a & 4 -a). On the other side, respiration rate, the contents of soluble carbohydrates and soluble protein contents were significantly raised with rising of treatment levels of $CuSO_4$. The maximum value of respiration was obtained at the level of 10 µM $CuSO_4$, used only (Table 2-a). The maximum values of soluble carbohydrates and soluble

proteins were recorded at the level of 6 µM $CuSO_4$ as compared with those of the control (Tables 3-a. and 4-a).

On the other hand, the interactive effect of 200 ppm thiamin and $CuSO_4$ on cell number, dry weight, photosynthetic pigments, photosynthetic oxygen evolution, respiration, carbohydrate contents, protein contents and free amino aids of *Chlorella vulgaris* cultured for 7 days. A significant increased in the values of cell number, dry weight, photosynthetic pigments, photosynthetic oxygen evolution, the contents of total carbohydrate, total protein contents and free amino acids of *Chlorella vulgaris* cultured were obtained under the various treatment levels of $CuSO_4$ and 200 ppm of thiamin, when compared with those of the control (Tables 1-b and 2-b, 3-b & 4-b). On the other side, the respiration rate of *Chlorella* was significantly decreased, under the various concentrations of $CuSO_4$ and treatment with 200 ppm thiamin, when compared with that of the control (Table 2-b). As it can seen from the above results the toxic of effect $CuSO_4$ on plant metabolism such as growth, photosynthetic pigments photosynthetic rate and some related metabolic activities was partially alleviated on *Chlorells vulgaris* cells.

Table (1-a): Effects of copper sulfate on cell number (cell ml^{-1} algal suspension) , dry weight (µg ml^{-1} algal suspension) and total pigments (µg ml^{-1} algal suspension) of *Chlorella vulgaris* Beiger cultured for 7 days.

Treatment $CuSO_4$ (µM) : Thiamin (ppm)	Cell number	% control	Dry weight	% control	Chloro. a	Chloro. b	Carotenoids	Total Pigments	% control
00 : 00	500 $x10^4$	100.00	485	100.00	3.20	1.75	2.16	7.11	100.00
2 : 00	520$x10^4$**	104.00	52.0**	107.21	3.50*	2.10**	3.18**	8.78**	123.48
4 : 00	540$x10^4$**	105.00	580**	119.58	3.80**	2.70**	4.20**	10.70**	150.49
6 : 00	480$x0^4$**	96.00	430**	88.65	2.50**	1.23**	2.01**	5.76**	80.73
8 : 00	420$x10^4$**	84	400**	82.47	2.12**	1.08**	1.50**	4.70**	66.10
10 : 00	400$x10^4$**	80	380**	78.35	1.80**	0.95**	1.21**	3.96**	55.69
*L .S.D at 5%	6.205		12.400		0.201	0.080	0.046	0.490	
**L .S. D at 1%	12.250		25.300		0.401	0.016	0.088	0.690	

Table (1-b): The interactive effect of copper sulfate and 200 ppm of thiamin on cell number (cell ml^{-1} algal suspension), dry weight (µg ml^{-1} algal suspension) and total pigments (µg ml^{-1} algal suspension) of *Chlorella vulgaris* Beiger cultured for 7days.

Treatment $CuSO_4$ (µM) : Thiamin (ppm)	Cell number	% control	Dry weight	% control	Chloro. a	Chloro. b	Carotenoids	Total Pigments	% control
00: 00	500 $x10^4$	100.00	485	100.00	3.20	1.75	2.16	7.11	100
00 : 200	540 $x10^4$**	108.00	515**	106.18	3.82*	2.50	2.18**	8.50*	119.54
2 : 200	580$x10^4$**	116.00	565**	116.49	4.20**	2.68	3.55**	10.43**	146.69
4 : 200	650$x10^4$**	130.00	640**	131.95	5.53**	3.10	3.90**	12.53**	176.23
6 : 200	550$x10^4$**	110.00	520**	107.21	3.50**	2.44	3.12**	9.06**	127.42
8 : 200	530$x10^4$**	106.00	505**	104.12	3.25*	2.33	3.95**	8.53*	199.97
10 : 200	510$x10^4$	102.00	500*	103.09	3.01**	2.01	2.22	7.24*	101.82
*L .S.D at 5%	10.212		9.22		0.045	0.012	0.995	1.220	
**L .S. D at 1%	20.236		18.250		0.095	0.030	1.520	2.001	

Table (2-a): Effects of copper sulfate on photosynthesis (Oxygen evolution) and respiration (Oxygen uptake) (µ mole O_2 ml^{-1} algal suspension $hr.^{-1)}$ of *Chlorella vulgaris* Beiger cultured for 7days.

Treatment $CuSO_4$ (µM) : Thiamin (ppm)	Oxygen evolution	% Control	Oxygen uptake	% Control
00 : 00	1.250	100.00	0.858	100.00
2 : 00	1.385**	110.80	0.636**	74.12
4 : 00	2.120**	169.60	0.838**	97.73
6 : 00	1.025**	82.60	1.230**	143.34
8 : 00	0.925**	74.00	1.305**	152.09
10 : 00	0.857**	68.56	1.425**	166.08
*L .S.D at 5%	0.002		0.001	
**L .S. D at 1%	0.004		0.003	

Table (2-b):The interactive effects of copper and 200 ppm of thiamin on photosynthesis and respiration (µ mole O_2 ml^{-1} algal suspension $hr.^{-1)}$ of *Chlorella vulgaris* Beiger cultured for 7days.

Treatment $CuSO_4$ (µM) : Thiamin (ppm)	Oxygen evolution	% Control	Oxygen uptake	% Control
00 : 00	1.250	100.00	0.858	100.00
00 : 200	1.420**	113.60	0.555	94.87
2 : 200	1.560**	124.80	0.540**	92.30
4 : 200	2.780**	222.40	0.490**	57.10
6 : 200	1.520**	121.60	0.554**	64.56
8 : 200	0.450**	116.00	0.570**	66.43
10 : 200	0.310**	104.80	0.600**	76.92
*L .S.D at 5%	0.010		0.001	
**L .S. D at 1%	0.040		0.004	

Table (3-a): Effect of copper sulfate on soluble, Insoluble and total carbohydrates (µg mg^{-1} dry weight) algal suspension) of *Chlorella vulgaris* Beiger cultured for 7days.

Treatment $CuSO_4$ (µM): Thiamin (ppm)	Soluble Carbohydrates	% Control	Insoluble Carbohydrates	% Control	Total Carbohydrates	% Control
00 : 00	40.20	100.00	168.12	100.00	208.32	100.00
2 : 00	53.22**	132.38	180.72	107.99	233.97**	112.29
4 : 00	60.44**	150.34	218.55	129.99	278.99**	133.92
6 : 00	70.50**	175.37	160.80	95.64	231.30**	11.03
8 : 00	39.10	97.26	150.70	89.63	153.53**	73.70
10 : 00	30.20**	75.12	120.83	71.87	151.03	72.49
*L .S.D at 5%	4.180		6.174		7.336	
**L .S. D at 1%	6.330		9.350		11.110	

Table (3-b): The interactive effects of copper and 200 ppm of thiamin on soluble, Insoluble and total carbohydrates (µg mg^{-1} dry weight) of *Chlorella vulgaris* Beiger cultured for 7days.

Treatment $CuSO_4$ (µM): Thiamin (ppm)	Soluble Carbohydrates	% Control	Insoluble Carbohydrates	% Control	Total Carbohydrates	% Control
00 : 00	40.20	100.00	168.12	100.00	208.32	100.00
00 : 200	44.60**	110.94	177.60**	105.63	222.63**	106.66
2 : 200	49.60**	123.38	200.90**	119.49	250.50**	120.24
4 : 200	50.30**	125.12	250.40**	148.41	300.70**	144.34
6 : 200	52.60**	130.84	220.30**	131.03	272.90**	131.00
8 : 200	45.30**	122.68	210.40**	125.14	255.70**	112.74
10 : 200	42.20*	104.97	190.60**	113.37	232.80**	111.75
*L .S.D at 5%	1.800		4.122		8.787	
**L .S. D at 1%	3.330		6.150		13.306	

Table (4-a): Effects of copper sulfate on soluble, Insoluble, total proteins and free amino acids (µg mg^{-1} dry weight) of *Chlorella vulgaris* Beiger cultured for 7days.

Treatment $CuSO_4$ (µM) : Thiamin (ppm)	Soluble Proteins	% Control	Insoluble Proteins	% Control	Total Proteins	% Control	Free Amino acids	% Control
00 : 00	38.15	100.00	240.60	100.00	278.75	100.00	18.02	100.00
2 : 00	52.92**	138.71	266.52**	110.77	319.44**	114.59	22.12**	122.75
4 : 00	62.17**	162.96	305.21**	126.85	367.38**	131.79	28.12**	156.04
6 : 00	77.98**	204.40	250.72**	104.20	328.70**	117.91	17.81**	98.83
8 : 00	33.82**	88.65	180.90**	75.18	214.72**	77.02	16.40**	91.00
10 : 00	30.90**	80.99	166.84**	69.34	197.74**	70.93	12.30**	68.25
*L .S.D at 5%	2.280		5.490		10.552		0.090	
**L .S. D at 1%	5.995		8.320		20.233		0.180	

Table (4 –b): The interactive effect of copper sulfate and 200 ppm of thiamin on soluble , Insoluble , total proteins and free amino acids (µg mg^{-1} dry weight) of *Chlorella vulgaris* Beiger cultured for 7 days.

Treatment $CuSO_4$ (µM) : Thiamin (ppm)	Soluble Proteins	% Control	Insoluble Proteins	% Control	Total Proteins	% Control	Free Amino acids	% Control
00 : 00	38.15	100.00	240.60	100.00	278.75	100.00	18.02	100.00
00 : 200	45.30	118.74	260.99	108.47	306.290	109.87	22.80	126.52
2 : 200	55.20	144.69	34.90	143.34	400.10	143.53	28.16	156.27
4 : 200	67.23	176.22	390.90	162.46	458.13	164.35	44.30	246.83
6 : 200	60.40	158.32	330.50	137.36	390.90	140.23	40.60	225.30
8 : 200	52.30	137.09	310.44	129.02	362.74	130.13	35.40	196.44
10 : 200	44.19	115.83	299.30	124.39	343.49	123.22	25.60	142.06
*L .S.D at 5%	3.750		6.250		12.258		1.010	
**L .S. D at 1%	5.522		12.260		4.568		3.020	

DISCUSSION:

This study elucidated the interactive effects of copper and thiamin on growth, photosynthetic pigments, photosynthesis and some related metabolic activities of *Chlorella vnlgaris* cultured for 7 days. These parameters were significantly elevated up to the level of only 4 µM of $CuSO_4$. Under relatively higher levels of $CuSO_4$, the values of these parameters were significantly lowered. On the other side,the respiration rate, soluble carbohydrates and soluble proteins were raised whatever the levels of $CuSO_4$. In accordance with this, Sandmann and Böger (1980) found that the influence of copper ions (Cu^{2+}) on *Scendesmus* exhibits many inhibiting effects according to copper concentration in the medium. The same authors reported that the growth parameters and chlorophyll contents of *Scendesmus* cells were decreased when treated with higher concentrations of Cu. This is also in accordance with the results for higher plants, algae and cyanobacteria (Shioi *et al*., 1978 a,b; Sandmann and Böger, 1980 and Ouzonoidou *et al.,* 1996). In accordance with this, some other investigators found that the excess copper may result in membrane damage, suppression of enzyme activity, cell death and inhibition of both the light reaction of photosynthesis and root growth (Kennedy and Gonsalves, 1987 and Walker and Web, 1981; Lanaras *et al.*, 1993 and Doncheva *et al.*, 1996). In this context, several workers reported that photosynthesis is greatly affected by copper availability (Fernando and Henriques 1991). However, there have been some conflicting results about the effects of copper toxicity on the photosynthetic apparatus. In accordance with this, that the decrease in the pigment levels the results this could be due to inhibition aminolevulinic dehydratase and/or stimulating chlorophyll peroxidation by inducing ridicals (Martinon *et al.,* 1981, Fernando and Henriques 1991 and Nathalie *et al.* 2001). On the contrary, Baszynski, *et al*., (1982), reported a small increase in chlorophylls and carotenoids in *Spinaces oleracea* when exposed to high concentrations treatment of copper.

Exogenously added 200 ppm thiamin (vitamins B_1) alleviate the inhibitory effects of some heavy metals and salinity stress (Zidan, 1991; Arrigoni *et al.,* 1992 ; Desouky,1995 and Desouky, 2001). Such vitamins can be used as coenzyme performed generally the major physiological functions in the plant cells (Kefeli, 1981). In this context, Mohamed *et al*., (1988) reported that thiamin increased the protein contents of *Chlorella vulgaris*, and the highest protein content was produced in the presence of the concentration of 100 ppm thiamin. Similarly, Makled (1995) working with two green algae; namely *Chlorella vulgaris* and *Akistrodesmus falcatus,* reported that the addition of thiamin, nicotinic acid or pyridoxine exerted a considerable increase in growth rate, the synthesis of total pigments, photosynthetic oxygen evolution, carbohydrate contents and protein contents, whatever the concentration of thiamin applied. On the other side, some other investigators working with higher plants and algae found that growth criteria, total pigments, photosynthetic oxygen evolution, carbohydrate contents and protein contents and free amino acids were markedly raised, when exposed to various salinity levels and treated with any of the vitamins applied (200 ppm of ascorbic acid, thiamin and pyridoxine) (Zidan ,1991; Arrigoni *et al.,* 1992 and Desouky, 1995). Similarly, Desouky, (2001) found that the growth parameters (cell number and dry weight) total pigments of *Chlorella vulgaris* cultures were significantly stimulated, when the algal cultured were subjected to various concentrations (20, 40, 60 80 and 100 ppm) of $ZnCl_2$ and treated with any of the vitamins applied (200 ppm of thiamin, pyridoxine and riboflavin). Finally, the excess of copper concentration is considered as a toxic effect on growth and some related metabolic activities of *Chlorella vulgaris* cells. On the other side, the exougenously added thiamin has partially alleviated the toxic effect of higher concentrations of $CuSO_4$ on *Chlorella vulgaris* cultures.

REFERENCES:

1-Arrigoni, O., De-Gara, L.,Tommasi, F. and Liso, R.(1992): Changes in the ascrobate system during seed development in *Vicia faba* L.Plant Physiol.59 : 235-238.

2-Badour, S. S. A. (1959): Analytisch-chemisch untersuchung des kaliummangles bei *Chlorella* im vergleich mit anderen Mangelzustän den, Ph. Dissertation. Goettingen

3-Baròn, M., Arellano, J. B. and Lpez Gorge (1995): Copper and photosystem II: A controversial relationship. Physiol. Plant. 94: 174-180.

4-Baszynski, T. M. Kròl, Z. Krupa, M. Ruazkowska, U.Wojcieska and D.Wolinska (1982): Photosynthetic apparatus of spinach exposed to excess copper. Z. Pflanzenphysiol. 108 : 385-395

5-Ciscato, L. Y., Roland, V., Van Loven, K. Clijsters, H. and Navari-Izoo, F. (1997): Effects of in vivo copper treatment on photosynthetic apparatus of *Triticum durum* cultivars with different stress sensitivity. Physiol. Plant. 100: 901-908.

6-Desouky, A. S. (2001): Effect of some externally added vitamins on growth criteria, photosynthesis and some related activities of $ZnCl_2$ stressed *Chlorella vulgaris* Beijger cultures. Ass. Univ. Bull. Envion. 2: 1-10 .

7-Desouky, S.A. (1995): Effect of some organic additives on salinized *Chlorella vulgaris*. Ph.D. Thesis, Fac. Sci., Assiut Univ., Assiut, Egypt. pp :1-199.

8-Doncheva, S., N. Kolov and Ogneva, V. (1996): Effect of copper excess on the morphology of the mucleus in maize root meristem cells. Phsiol. Plant. 96: 118-122.

9-Droppa, M. and Horwath, G. (1990): The role of copper in photosynthesis – CRC Plant Sci. 9: 111-123.

10-Fernando, C., and Henriques, F. S. (1991): Limiting step on photosynthesis of Rice plants treated with varying copper levels. J. Plant Physiol 38: 115-118.

11-Kefeli, V.I. (1981): Vitamin and some other representatives of non hormonal plant growth regulators. Priki Biokhim.Microbiol.17: 5-15.

12-Kennedy, C. D. and Gonsalves, F. A. N. (1987): The action of divalent Zinc, cadmium, mercury, copper and lead on the trans-root potential and H^+ efflux of exceed roots. J. Exp. Bot. 38: 800-817.

13-Lanaras, T. Moustakas, M., Symeonidis, L., Dian. Antoglou, S. and Korataglis, S. (1993): Plant metal content, growth responses and some photosynthetic measurements on field cultivated wheat growing on are bodies enriched in Cu. Physiol. Plant 88: 307-318.

14-Lessler, M. A.; Malloy, E. and Schwab, C. M. (1956): Measurement of oxidative activity in biological systems by automated oxygen electrode. Fed. Proc. 24: 336-345.

15-Lidon, F. C. and F. S. Henriques (1991): Effects cupper on the ascorbate, diamine and O-diphenol oxidases activities of rice leaves. Phyton (Intern J. Exp. Bot.) 52: 97-104.

16-Lidon, F. C., Ramalho, J. C. and Henriques, F. S. (1993): Copper inhibition in rice photosynthesis. J. Plant Physiol. 142: 12-17.

17-Lowry, O.H.,Rosebroiugh, N.J., Farr, A.L., and Randall, R.J. (1951): Protein measurement with folin phenol reagent. J. Biol. Chem.193:291-299

18-Makled, M.M.(1995): Physiological studies on some species of unicellular green algae with special reference to protein production. M.Sc. Thesis, Fasc.Sci., Menoufia Univ., Egypt, pp: 1- 156.

19-Marschner, H- (1998): Mineral Nutrition plants. pp. 333-347.- Academic Press. London. ISBN O – 12-473543-6.

20-Martinon, E., M.J. Dalling, and P.H. Matile (1982): Catabolism of chlorophyll: demonst-ration of chloroplast localized peroxidative and oxidative activities. Z. Pflanzenphysiol. 107: 289-279.

21-Metzener,H.,Rau,H.und Senger, H.(1965): untersuchungen Zur Synchronisierbarkeit einzelener Pigment-Mangel Mutanten Von *Chlorella.*Planta 65 : 186-194.

22-Mohamed, Y.A., Osman. M.E.H. and El-Sheekh. (1988): Factors affecting growth and protein contents of *Chlorella vulgaris.* Bull.Fac.Sci., Assiut Univ., Egypt, 17 : 103-114.

23-Moore, S. and Stein, W. (1948): Photo-mertric ninhydrine method for use in the Chromatography of amino acids. From the laboratories of Rockefeler institute for Medical Research, New York.

24-Nathalie, A. L. M. Van Hoof, Panl L. M. Koevotes, Henk Schat, Jos, A. C. Verkleij and Wil Fried H.O. Ernst (2001): Enhanced ATP-dependent copper efflux across the root cell plasma membrane in copper tolerant *Silene Valgaris.* Physiol. Plant. 113: 225-232.

25-Ouzounidou, G. (1996): The use of photoacoustic spectroscopy in assessing leaf photosynthesis under copper stress: Correlation of energy storage to photos-ythestem II Fluorescence parameters and redox change of P 700. Plant Sci. 113: 229-237.

26-Sandmann, G. and P. Böger (1980): copper mediated lipid peroxidations processes in photosynthetic membranes. Plant Physiol. 66: 797-800.

27-Shioi., Y., H. Tamai and T. Sasa (1978a): Effects of copper on photosynthetic electron system in Spinach chloroplasts. Plant cell Physiol. 19: 203-209.

28-Shioi, Y., H. Tamai and T. Sasa (1978 b): Inhibition of photosystem II in green algal *Ankistrodesmus flactus* by copper. Physiol. Plant 434-438.

29-Snedecor,G.A and Cochran,W.G. (1972): Statistical Methods,11th Ed.,The Iowa State Univ.Press, Ames, Iowa, U.S.A, PP :172-334.

30-Stein,J. R. (1966): Growth and mating of *Gonium pectoral* (Volvolcales) in defined media. J. Phycol. 2: 23 – 28.

31-Walker, C. D. and Webl, J. (1981): Copper in plants. Forms and behaviors: In Soils Plants (J. F. Loneragon, A. D. Robson and R. D. Graham, eds), pp 182-212. Academic Press, London. ISBN 0-12-455520-9.

32-Zidan, M.A. (1991): Alleviation of salinity stress on growth and related parameters in wheat sprayed with thiamin, nicotinic acid and pyridoxine. Arab. gulf. J. Scient. Res. 9 : 103-107.

التأثيرات المتداخلة لكل من عنصر النحاس والثيامين (فيتامين ب١) على النمو والأصباغ الكلية والبناء الضوئى والتنفس وبعض الأنشطة الأيضية لمزارع طحلب " كلوريللا فولجاريس " بيجر

سيد عباس دسوقى عبد الحليم

قسم النبات والميكروبيولوجى – جامعة الأزهر – فرع أسيوط

يهدف هذا البحث إلي إظهار التأثيرات المتداخلة لكل من عنصر النحاس والثيامين (٢٠٠ جزء / المليون) في إيقاف التأثير العكسي الضار والسام الناتج من معالجة مزارع طحلب " *كلوريللافولجاريس* بيجر" بتركيزات مختلفة (٢، ٤، ٦، ٨، ١٠ ميكرو مول) من كبريتات النحاس علي محددات النمو والأصباغ الكلية ومعدل البناء الضوئي والتنفس والمواد الكربوهيدراتية الكلية والبروتينيات الكلية والأحماض الأمينية.

وقد أظهرت النتائج ما يأتي :

١- زيادة محددات النمو(عدد الخلايا والوزن الجاف) والأصباغ الكلية ومعدل البناء الضوئي (الأوكسجين المتصاعد) والمواد الكربوهيدراتية الكلية والبروتينيات الكلية والأحماض الأمينية زيادة معنوية كبيرة تفوق مثيلاتها في المزرعة الضابطة عند المستويات المنخفضة حتي مستوى ٤ ميكرو مول من كبريتات النحاس. أما عند المستويات العالية من كبريتات النحاس حتى مستوى ١٠ ميكرو مول فإنها تؤدي إلي تثبيط تلك العوامل. كما يلاحظ زيادة معدلات كل معدل التنفس والمواد الكربوهيدراتية الذائبة والمواد البروتينية الذائبة عند المستويات العالية من كبريتات النحاس .

٢- أما عند معالجة مزارع طحلب *"كلوريللا فولجاريس"* بيجر المجهدة بتركيزات مختلفة من كبريتات النحاس بواسطة تركيز ٢٠٠ جزء/مليون من الثيامين فإنه يلاحظ أيضاً زيادة محددات النمو(عدد الخلايا والوزن الجاف) والأصباغ الكلية ومعدل البناء الضوئي (الأوكسجين المتصاعد) والبروتينيات الكلية، الأحماض الأمينية زيادة معنوية كبيرة تفوق مثيلاتها في المزرعة الضابطة.