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EFFECT OF MOLYBDENUM, VITAMIN (C) AND VITAMIN (B_1) ON THE GROWTH CRITERIA, PHOTOSYNTHETIC PIGMENTS AND METABOLIC ACTIVITIES OF STRESSED-*SCENEDESMUS OBLIQUUS* CULTURES

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ABSTRACT:

The growth criteria (cell count and dry weight) and total photosynthetic pigments, total carbohydrates, total proteins, free amino acids and proline of stressed- *Scenedesmus obliquus* cultures were significantly increased up to the level 3 ppm of $MoCl_2$ of that the control cultures, there above the values all these parameters were significantly decreased.

When treated stressed-*Scenedesmus obliquus* cultures with 200 ppm of either ascorbic acid or thiamine were both supplemented of them separately, the growth criteria, total photosynthetic pigments, total proteins, free amino acids and proline of stressed-*Scenedesmus obliquus* cultures were significantly increased, as comparison with that the control cultures. Conversely, the total carbohydrate contents significantly decreased, when the algal cultures subjected to various levels (1.5, 3 and 4.5 ppm) of $MoCl_2$ and treated with 200 of either ascorbic acid or thiamine.

INTRODUCTION:

Molybdenum (Mo^{+2}) was established to be essential in higher plants and algae by Arnon and Stout (1939). Molybdenum is known as a constituent of enzymes such as nitrate reductase, which reduces nitrate to nitrite, and the enzyme nitrogenase, which reduces molecular nitrogen (N) to ammonia in all N-fixing organisms. Further details can found elsewhere (Hewitt and Smith 1975, Epstein and Bloom, 2005 and Malavolta, 2006).

Mo^{+2} have a catalytic effect on N_2 fixation by blue-green algae; along with iron, Mo^{+2} is a constituent of the nitrogenase enzyme complex (Vega, *et al.* 1971 and Bothe, 1982). Regarding the essentiality of molybdenum for algae, it has been shown that molybdenum-deficient cell of the green algae *Scenedesmus obliquus* fail to assimilate nitrate nitrogen (Arnon, *et al.* 1961 and Bothe 1982).

Thus, the role of molybdenum as an essential trace element for algae and higher plants in the processes of nitrate reduction has been firmly established (Beevers and Hageman

1969 and Vega *et al.*, 1971). The requirement of molybdenum by the green algae *Scenedesmus obliquus* found to be extremely low (Arnon *et al.* 1961). The optimal concentrations being about (75 to 200 µg L^{-1}) of Mo^{+2} (Wolfe 1954).In this context, Young and Langille (1958) found the range of 0.23 to 1.36 mg/kg for eleven species of marine seaweeds. Molybdenum has been shown to be a limited factor in nature for the growth of algae (Godman 1965 and 1966). Subsequently, it has been reported that Mo^{+2} stimulate photosynthesis (Allen, 1972 and Godman, 1972).

Vitamins act as trigger in the biochemical reactions in the plant cells. Also, vitamins are among the organic nutritional factors required for continued growth and metabolic activities of living organisms. These compounds scarcely tried to counter some the adverse effects of salinity and heavy metals stress Desouky, (1995, 2003 and 2011). In this context, some authors working with the normally cultured (non-treatment) algal groups found that the additional of various vitamins was necessary for continued growth of these algae (Berland, *et al.*, 1978, Swift, 1980 and Desouky, 2003).

Aim of this study illustrated the main important role of some exogenously organic additives such as ascorbic acid and thiamin, which counteracted the toxicity of the adverse effects of molybdenum chloride on growth criteria, photosynthetic pigments, the carbohydrates contents, total protein contents, free amino acid and proline contents of stressed-*Scenedesmus obliquus* cultures.

MATERIALS AND METHODS:

Tested alga:

Scenedesmus obliquus collected from the river Nile and used as a test organism. Beijerinck's nutritive culture was used as a medium for enrichment and growth of the tested alga, (Stein, 1966).

Treatments:

Scenedesmus obliquus cultures subjected to 00 (control) and 200 ppm of either ascorbic acid (vitamin C) or thiamine (vitamin B_1) in the absence or presence of different levels (00, 1.5, 3, and 4.5 ppm) of molybdenum chloride for 7 days.

ANALYTICAL METHODS:

1-Determination of cell count:

One drop of the algal suspension was pipette on the Haemocytometer slide (0.1mm depth), covered and left two minutes for algal setting. The mean counts of four replicates were taken into consideration and the results measured as cells ml^{-1} algal suspension.

2-Determination of dry weight:

A definite volume (100 mls.) of alga 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°C. The data were expressed as µg 100 ml^{-1} algal suspension.

3-Determination of total photosynthetic pigments:

The pigment fractions (µg/m algal suspension) chlorophyll a, chlorophyll b and carotenoids calculated using the equations mentioned by Metzner *et al.*, (1965).

4-Determination of Carbohydrate contents:

Using of anthrone-sulphoric acid reagent according to the method by Badour (1959). The data measured as µg/mg dry weight.

5-Determination of Protein Contents:

Using folin phenol reagent according the method adapted by Lowry *et al.*, (1951). The data measured as µg/mg dry weight.

6-Determination of Proline:

It was determined according to Bates *et al.*, (1973) methods. The results of proline contents are calculated (µg/mg dry weight).

7-Determination of Free amino acids:

Free amino acids extracted from fresh water algal suspension and calorimetrically determined using the method of Moore and Stein (1948). The free amino acid contents are calculated as µg/mg dry weight.

8-Statistical Analysis:

Four replicates used in this study and the data were statistically analyzed to calculate the Least Significant Difference (L.S.D) according to Snedecor and Cochray (1980).

RESULTS:

The date present in this investigation showed the effect of exogenously natural organic additives and toxicity of some heavy metals on growth parameters (cell count and dry weight), total photosynthetic pigments, total carbohydrate, total protein, and free amino acid and proline contents of *Scenedesmus obliquus* cultures for 7 days.

In this study, the growth criteria (cell count and dry weight) and total photosynthetic pigments of *Scenedesmus* cultures significantly increased up to level 3 ppm of $MoCl_2$ only. However, under higher level (4.5 ppm) of $MoCl_2$, all these parameters were significantly decreased, as compared with that of the control cultures. The maximum values of growth parameters (cell count and dry weight) and total photosynthetic pigments of *Scenedesmus* cultures were 107%, 127% and 136 % of that of the control cultures, when the algal cultures subjected to 3 ppm of $MoCl_2$, respectively. Whereas, the minimum values of growth parameters (cell number and dry weight) and total photosynthetic pigments were 52%, 67% and 52% of that of the control cultures, when algal cultures subjected to 4.5 ppm $MoCl_2$, respectively (Fig. 1-a).

The maximum values of the cell number, dry weight and total photosynthetic pigments were 195%, 149% and 171% of that the control cultures, when the algal cultures subjected to 3 ppm $MoCl_2$ and treated with 200 ppm ascorbic acid respectively. The minimum values of the cell count, dry weight and total photosynthetic

pigments were 107%, 103% and 113% of that the control cultures, when the algal cultures subjected to 4.5 ppm $MoCl_2$ and treated with 200 ascorbic acid, respectively (Fig. 1-b). Also, the maximum values of cell count, dry weight and total photosynthetic pigments were 167%, 141% and 155% of that the control cultures, when the algal cultures subjected to 3 ppm $MoCl_2$ and treated with 200 ppm thiamine, respectively. Therefore, the minimum values of the cell count, dry weight and total photosynthetic pigments were 112%, 108% and 122% of that the control cultures, when the algal cultures subjected to 4.5 ppm $MoCl_2$ and treated with 200 thiamine, respectively (Fig. 1-c).

On the other hand, the maximum value of soluble carbohydrates content was 103 % of that of the control cultures when subjected to 3 ppm $MoCl_2$ only. Also, the maximum values of insoluble and total carbohydrates content amounted to 119% and 113% of that of the control cultures when algal cultures subjected to 1.5 ppm $MoCl_2$. While, the minimum values of soluble, insoluble and total carbohydrates content when treated with $MoCl_2$ only amounted to 66%, 82% and 79% of that of the control cultures when algal cultures subjected to 4.5 ppm $MoCl_2$, respectively (Table 1-a)

In this respect, the maximum values of soluble carbohydrates content amounted to 67%, when algal cultures subjected to1.5 ppm $MoCl_2$ and treated with 200 ppm ascorbic acid, respectively. The maximum values of insoluble and total carbohydrates content were 89% and 82 % of that the control cultures, when the algal cultures subjected to 3 ppm $MoCl_2$ and treated with 200 ppm ascorbic acid, respectively (Table 1-b). On the other side, the maximum values of soluble carbohydrates content amounted to 61% of that the control cultures, when the *Scenedesmus* cultures subjected to 1.5 $MoCl_2$ and treated with 200 ppm thiamin. Also, the maximum values of insoluble and total carbohydrate were 85% and 78% of that the control cultures, when the algal cultures subjected to 3 ppm $MoCl_2$ and treatment with 200 ppm thiamine, respectively (Table 1-c).

Similarly, the maximum value of soluble protein contents when treated with $MoCl_2$ only amounted to 96%, of that of the control cultures when algal cultures subjected to 1.5 ppm $MoCl_2$, and the maximum values of insoluble and total protein contents when treated with $MoCl_2$ only amounted to 111% and 102% of that of the control cultures when algal cultures subjected to 3 ppm $MoCl_2$, respectively. Whereas, the minimum values of soluble, insoluble and total protein contents when treated with $MoCl_2$ only amounted to 46 %, 62% and 59% of that of the control cultures when algal cultures subjected to 4.5 ppm $MoCl_2$, respectively (Table 2-a).

Similarly, the maximum values of soluble, insoluble and total protein contents amounted to 167%, 189% and 185% of that the control cultures, when the algal cultures subjected to 3 ppm $MoCl_2$ and treated to with 200 ppm ascorbic acid, respectively (Table 2-b). Whereas, the maximum values of soluble, insoluble and total protein contents amounted to 160%, 175% and 172% of that control cultures, when algal cultures subjected to 3 ppm $MoCl_2$, respectively (Table 2-c).

Table 1 : Carbohydrate contents (µg/mg dry weight), of stressed *Scenedesmus obliquus* cultures subjected to various combinations of $MoCl_2$ and 200 ppm of either ascorbic acid or thiamin for 7 days

Treatments / $MoCl_2$ (ppm)	Water-soluble Carbohydrates	% Control	Water-insoluble Carbohydrates	% Control	Total Carbohydrates	% Control
			(a)			
00 : 00	38.00	100.00	173.00	100.00	211.00	100.00
1.5 :00	32.00**	84.20	207.25**	119.80	239.25**	113.40
3: 00	39.38**	103.60	125.37 **	72.50	164.75**	78.08
4.5:00	25.42**	66.80	141.84**	82.00	167.25**	79.27
L.S.D. at 1%	0.012		6.123		11.193	
L.S.D. at 5%	0.018		9.015		15.075	
			(b)			
$MoCl_2$ (ppm) & 200 (ppm) Ascorbic acid) :						
00:200	70.28	185.00	254.84**	142.10	316.12**	149.82
1.5:200	25.64**	67.40	132.64**	77.25	159.25**	75.50
3:200	19.85**	52.40	154.00**	89.02	173.85**	82.40
4.5:200	14.00**	36.80	118.50**	6850	132.50**	62.80
L.S.D. at 1%	6.125		8.002		15.124	
L.S.D. at 5%	8.142		11.045		18.125	
			(c)			
$MoCl_2$ (ppm) & 200 (ppm) Thiamine:						
00:200	66.70**	175.53	231.24**	123.66	297.94**	141.20
1.5:200	23.50**	61.80	122.07**	70.60	145.57**	67.00
3:200	18.34**	48.60	147.33**	85.00	165.67**	78.52
4.5:200	12.38**	32.50	113.50**	65.61	125.88**	59.66
L.S.D. at 1%	10.002		10.001		14.001	
L.S.D. at 5%	12.003		12.004		16.002	

Table 2 : Protein contents (µg mg^{-1} dry weight) of *Scenedesmus obliquus* cultures subjected to various combinations of $MoCl_2$ and 200 ppm of either ascorbic acid or thiamine for 7 days

Treatments / $MoCl_2$ (ppm)	Water-soluble Proteins	% Control	Water-insoluble Proteins	% Control	Total Proteins	% Control
			(a)			
00:00	33.00	100.00	142.00	100.00	175.00	100.00
1.5:00	31.90 **	96.70	144.37 **	101.70	176.27**	100.73
3:00	21.45 **	65.00	158.50 **	111.62	179.95**	102.83
4.5:00	15.20 **	46.06	88.00 **	62.00	103.20**	59.00
L.S.D. at 1%	0.008		1.002		0.003	
L.S.D. at 5%	0.010		1.020		0.008	
			(b)			
$MoCl_2$ (ppm) & 200 (ppm) Ascorbic acid:						
00:00	53.05**	160.76	230.00**	162.00	283.05**	161.74
1.5:200	48.75**	147.73	232.54 **	163.80	281.29 **	160.74
3:200	55.28**	167.52	269.00 **	189.44	324.28 **	185.30
4.5:200	39.80**	120.61	167.65**	118.10	207.45**	118.54
L.S.D. at 1%	9.003		10.025		14.006	
L.S.D. at 5%	12.045		12.014		18.005	
			(C)			
$MoCl_2$ (ppm) & 200 (ppm) Thiamine:						
00:200	50.453 **	152.82	215.00**	151.40	265.43**	151.70
1.5:200	48.00 **	145.50	230.50 **	154.93	268.50**	153.43
3:200	52.84 **	160.12	249.48 **	175.70	3.2.32 **	172.75
4.5:200	43.70**	132.42	176.42**	124.24	220.12 **	125.80
L.S.D. at 1%	7.023		4.012		12.087	
L.S.D. at 5%	10.033		6.025		17.089	

* Significant difference ** High Significant difference..... as compared absolute control

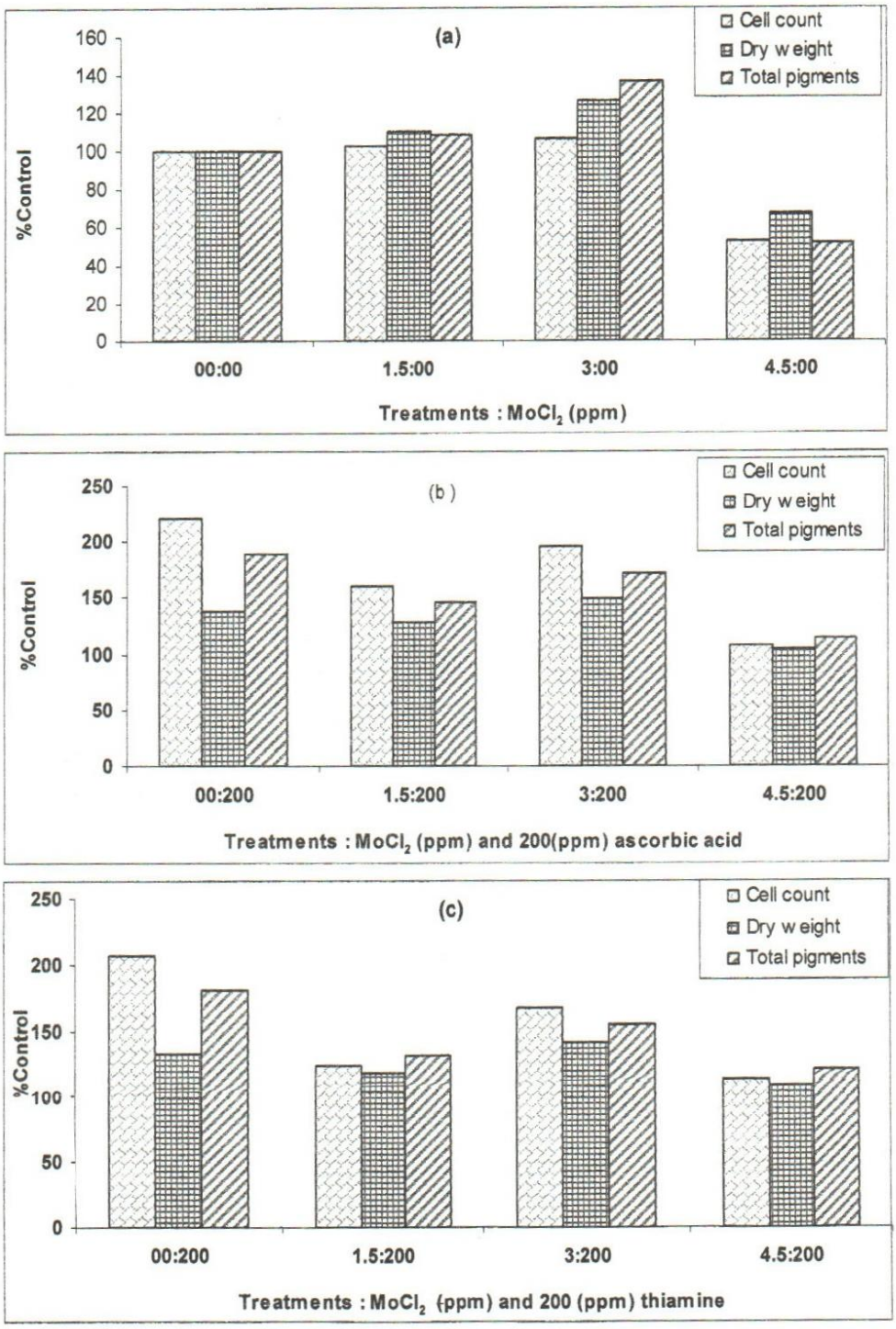

Figure 1 : Cell count (cell ml^{-1} algal suspension), dry weight (µg/ml algal suspension) and total photosynthetic pigments (µg ml^{-1} algal suspension) of stressed -*Scenedesmus obliquus* cultures subjected to various combinations of $MoCl_2$ and 200 ppm of either ascorbic acid or thiamin for 7 days

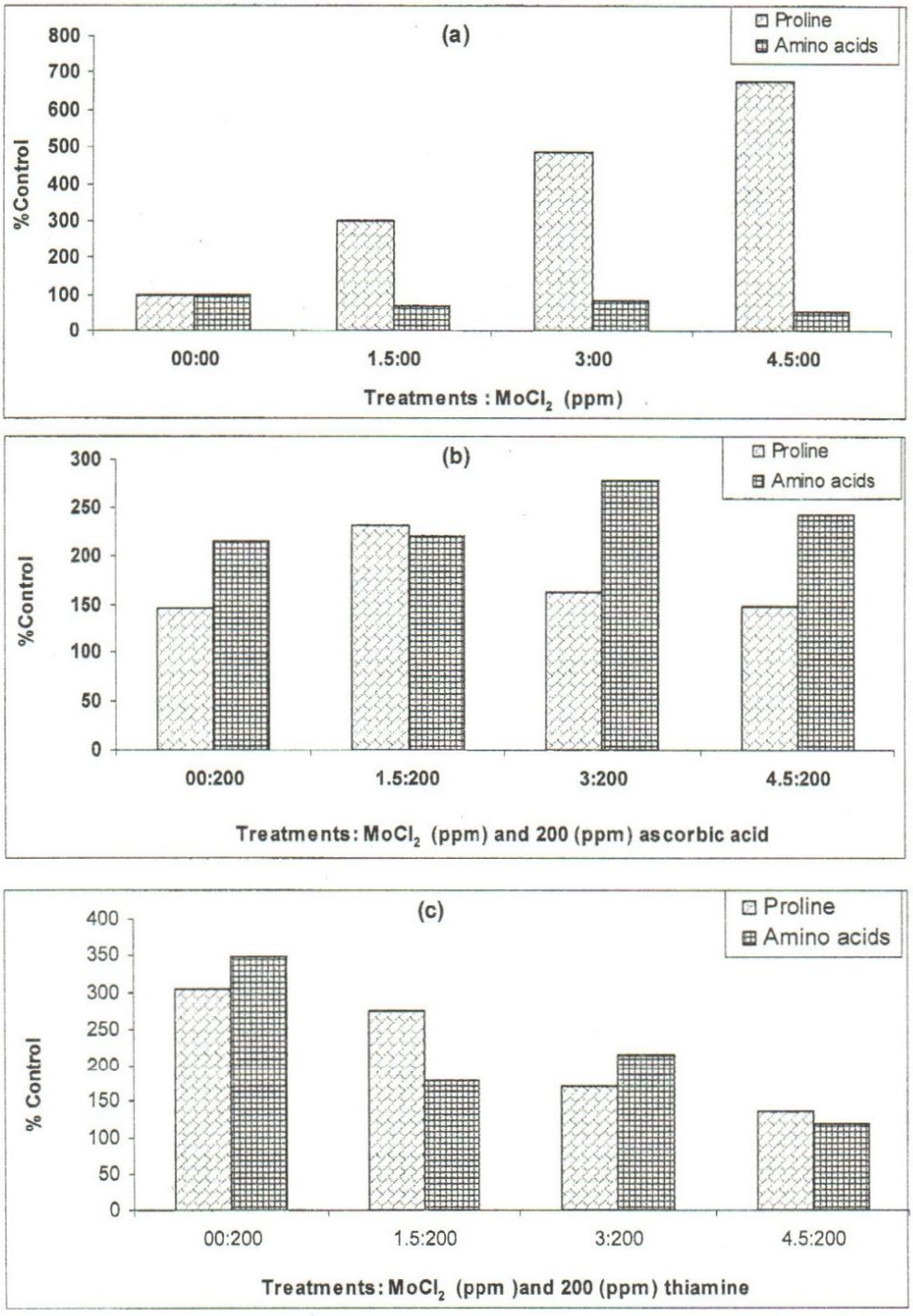

Figure 2 : Proline contents and free amino acids (µg mg^{-1} dry weight) of stressed *Scenedesmus obliquus* cultures subjected to various combinations of $MoCl_2$ and 200 ppm of either ascorbic acid or thiamin for 7 days

Whereas, the maximum value of proline content was 675% of that of the control cultures, when algal cultures subjected to 4.5 ppm $MoCl_2$ only (Fig. 2-a).

Whereas, the maximum values of proline contents reached to 231% and 274% of that of the control cultures, when algal cultures subjected to 4.5 ppm $MoCl_2$ and treated with 200 ppm of either ascorbic acid or thiamine, respectively. Also, the minimum values of proline contents were 148% and 136% of that the control cultures, when the algal cultures subjected to 1.5 ppm $MoCl_2$ and treated with 200 ppm of either ascorbic acid or thiamine (Fig. 2-b and c).

The minimum value of free amino acids content of *Scenedesmus* reached to 52% of that of the control cultures, when algal cultures subjected to the level of 4.5 ppm $MoCl_2$ only. Whereas, the maximum value of free amino acids content was 82%, when algal cultures subjected to 3 ppm $MoCl_2$ (Fig. 2-a).

The maximum value of free amino acids content amounted to 277% and 216% of that of the control cultures, when algal cultures subjected to 3 ppm $MoCl_2$ and treated with 200 ppm of either ascorbic acid or thiamine, respectively (Fig. 2- b and c).

DISCUSSION:

This study elucidated the effect of some natural organic additives namely; (ascorbic acid (vitamin C) and thiamine (vitamin B_1) and heavy metal $MoCl_2$ on the growth criteria (cell count and dry weight), total photosynthetic pigments, total carbohydrates, total proteins, other free amino acids and proline of *stressed-Scenedesmus obliquus* cultures for 7 days.

$MoCl_2$ plays an important role in the growth and metabolic activities in living organisms. Thus, when the algal cultures were subjected to lower doses of Mo^{+2} the growth parameters were significantly increased. However, under relatively higher doses of $MoCl_2$ the growth parameters were significantly decreased. In this context, these results are in agreement with some other authors who reported that the molybdenum element is necessary for the growth and metabolic activities of the algal groups (Arnon and Stout 1939, and Arnon, *et al.* 1961, Godman 1965, Godman 1966 and Bothe 1982).

In this investigation it, was generally found that the concentration of the applied vitamins [200 ppm ascorbic acid (vitamin C and thiamine (vitamin (B_1)] could stimulated the growth parameters (cell count and dry weight) and total photosynthetic pigments of *stressed-Scenedesmus obliquus*, when subjected to various doses (1.5, 3 and 4.5 ppm) of $MoCl_2$. At the higher concentration (4.5 ppm) of this heavy metale also, the growth parameters and total photosynthetic pigments were significantly increased, when the algal cultures treated with 200 ppm of either ascorbic acid or thiamine.

Ascorbic acid was also recorded to affect the chlorophyll contents (Choudhury *et al.*, 1993) through promoting the capacity of chlorophyll by stabilizing and protecting these molecules from being oxidized. This stimulation of growth of

both organisms mentioned above in response to treatments with thiamine (vitamin B_1) could be attributed to the significant role played by thiamine (vitamin B_1) in cellular metabolism as coenzymes in the oxidative decarboxylation of pyruvate or of α-ketoglutarate (Harper, 1991; Makled, 1995). In this context, Desouky (2001 and 2011) reported that that the growth parameter (cell number and dry weight), total photosynthetic pigments and some metabolic activities of *Chlorella vulgaris* cultures significantly increased, when the algal cultures subjected to 200 ppm thiamine.

The results this study showed the total carbohydrate contents of *Senedesmus obliquus* cultures were markedly increased, when the algal cultures subjected to lower levels of, Mo^{+2}. While under higher doses of this element the carbohydrate contents were markedly decreased. However, the soluble carbohydrate contents were increased with the increased of treatment elements in the medium cultures. Under higher concentration of this element (Mo^{+2}), the total carbohydrates decreased, when compared with that the control cultures. These results present in this study are in agreement with Afkar *et al.*, (2010) who reported that the carbohydrate contents of the *Chlorella vulgaris* alga declined in manner dependent on the metal concentration exist in the medium, but the inhibitory effect of the three tested metals (Co^{+2}, Cu^{+2} and Zn^{+2}) were not pronounced as on protein content. In this context, Fathi *et al.* (2005) reported that the higher doses of heavy metals severely attenuate chlorophyll synthesis coupled with severe drop in protein resulting in increased carbohydrates. Torres *et al.* (1998) demonstrated that algae *Cylindrothica fusiformis* produce carbohydrate as a defense mechanism against copper toxicity in stationary phase when cells are exposed to lower dose of (5 ppm) of Cu^{+2}. In this respect, Desouky, (2004) recorded that the total carbohydrate contents of *Chlorella vulgaris* cultures were significantly decreased when, the algal cultures were subjected to various concentrations of $Cd\ Cl_2$.

Generally, the accumulation of free amino acids in response to metals concentrations may lead to the assumption that suppressed protein biosynthesis encouraged free amino acids accumulation, or may be due to some counteracting chelating mechanism against heavy metals toxicity (El-Sheekh *et al.*, 2003;Osman *et al.*, 2004 and Fathi *et al.*, 2005).

Also, the results in this study showed the proline contents of stressed- *Senedesmus obliquus* cultures were significantly increased, when the algal cultures subjected to lower level of Mo^{+2}. While under higher level of $MoCl_2$ the proline accumulation was significantly increased (Bassi and Sharma, 1993).

The results of this investigation show that the free amino acids of *stressed-Senedesmus obliquus* cultures were markedly increased, when the algal cultures subjected to lower levels of Mo^{+2}. While under higher doses of all these elements the free amino acid contents were markedly decreased. These results in this investigation in accordance with, Afkar *et al.*, (2010) reported that the total free amino acids

of *Chlorella vulgaris* gradually increased with increasing metals concentration. The most pronounced stimulation was detected at the culture supplemented with 10^{-7} M copper in comparison to the other tested metals. The same authors reported that, the increasing copper concentration more than 10^{-7} M, the total amount of free amino acids partially reduced. On the other hand, cobalt and zinc also stimulated the biosynthesis of the total free amino acids, but the stimulatory effect is less than that obtained with copper. In this context, Omar (2002) showed that the free amino acids of *Scenedesmus obliquues* and *Scenedesmus quadricauda* was gradually increased total amino acid contents under lower levels of Zn^{+2}, however, decreased it at high concentrations.

On the other hand , the results in this study showed the protein contents of *Senedesmus obliquus* cultures were markedly increased, when the algal cultures subjected to lower levels of Mo^{+2} .While under higher doses of this element the protein contents were markedly decreased. However, the soluble protein contents were increased with the increased of treatment elements in the medium cultures. However, under higher concentrations of Mo^{+2} the total protein contents were decreased, when compared with that the control cultures. This results in this investigation in accordance with, Afkhar *et al.*, (2010) who recorded that the total protein contents of the green alga *Chlorella vulgaris* gradually decreased in a manner dependent on the metal concentration in the medium. The same authors reported that all the three heavy metals (Co^{+2}, Cu^{+2} and Zn^{+2}) affected negatively on the total protein contents at higher doses. On the other hand, the supplementation of copper and zinc by concentration 10^{-9} M increases the total protein content as compared to the control. However, no marked change in total protein contents occurred in cells of *Chlorella vulgaris* ,which exposed to cobalt. It could be suggested that accumulation of protein at low heavy metal concentrations may be one of the ways through which the algae can abolish their toxic effects, or to increase respiration leading to the utilization of carbohydrate in favor of protein accumulation (Osman *et al.*, 2004).Whereas, the suppression of protein accumulation may be attributed to shortage of carbon skeleton results from low photosynthetic rate.

Thus, Maggio *et al.*, (2002) reported that the depicted an inverse relationship between biomass (dry weight) and proline accumulation in the test algae under stressed conditions. The same authors found that proline might be produced at the expense of material(s) required for the development of the algae. This might involve reduction in cell division or delay of exponential growth due to proline accumulation.

The concentration of these noxious effect of heavy metal on plant growth and metabolism represents a main goal for many works in the field of plant physiology (Osman *et al.*, 2004, Fathi *et al.*, 2005 ,Afkar *et al.*, 2010 and Desouky, 2011). Hence the plant activities need always to be investigated under heavy metals

treatments using the various techniques and treatments.

The present investigation was conducted to study the effect of some exogenously added vitamins (vitamin C and vitamin B_1) on stressed- *Scenedesmus obliquus* cultures. It was aimed to tested whether these vitamins can counteract the adverse effects of heavy metals on the growth and some metabolic activities of *Scenedesmus*. It should pointed out that responses of stressed algal cultures with heavy metals to vitamins treatments was poorly investigated.

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تأثير الموليبدنم وفيتامين ج وفيتامين ب١ على معدلات النمو والأصباغ النباتية وبعض الأنشطة الفسيولوجية لمزارع طحلب " السينيدسمس أوبليكس" المقساة

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اهتمت هذه الدراسة بإظهار تأثير الموليبدنم وفيتامين (ج) وفيتامين (ب١) على معدلات النمو (عدد الخلايا والوزن الجاف)، الأصباغ النباتية، محتوى المواد الكربوهيدراتية، محتوى المواد البروتينية، الأحماض الأمينية والبرولين على مزارع طحلب " السينيدسمس أوبليكس" المقساة لمدة ٧ أيام.

ويمكن تلخيص النتائج التي أمكن الحصول عليها من هذه الدراسة كما يلي :

١- عند وضع طحلب مزارع طحلب " السينيدسمس أوبليكس المقساة بكلوريد الموليبدنيم منفرداً. ويلاحظ أن معدلات النمو متمثلاً في (عدد الخلايا والوزن الجاف) والأصباغ النباتية للطحلب المقساة تزداد زيادة معنوية.

٢- تناقص قيم تلك معدلات النمو للطحلب والأصباغ النباتية تتناقصاً معنوياً عند المستوى العالى ٤,٥ جزء من المليون مع مزارع " السينيدسمس أوبليكس" المقساة.

٣- عند معالجة مزارع الطحلب بتركيز ٢٠٠ جزء من المليون من أي من فيتامين (ج) وفيتامين (ب١) إلى مزارع الطحلب، ويلاحظ أن معدلات نمو الأصباغ النباتية ومحتوى المواد البروتنية الكلية والأحماض الأمينية والبرولين للطحلب تزداد زيادة معنوية.

٤- محتوى المواد الكربوهيدراتية يتناقص تناقصاً معنوياً، وذلك عند المعالجة بأي من الفيتامينين.