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THE EFFECT OF CARBETAMIDE HERBICIDE ON PIGMENT CONTENTS, CARBOHYDRATE AND NITROGEN LEVELS AND $^{14}CO_2$-PHOTOASSIMILATION IN *CHLORELLA VULGARIS*

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Abstract

Tratment of *Chlorella vulgaris* with Carbetamide (-1- (ethylcarbamyol) ethyl phenyl carbamate) induced changes in pigment content, 5 ppm showed considerable increase in pigment content, when 25 and 50 ppm induced bleaching after 5 and 7 days, respectively. Thereafter, a remarkable re-greening of these cultures was observed. Carbetamide treatment reduced carbohydrate components associated with an increase in nitrogen component at high concentration of the herbicide.

Carbetamide induced changes in the pattern of $^{14}CO_2$-photoassimilation. A significant increase in the ratio of soluble/insoluble products was obtained, this being indicative that the rate of transformation of soluble to insoluble photosynthates tended to decrease as a result of herbicide treatment. Percentage of labelling in methionine, arginine, aspartic, glutamic, glycine and serine decreased with increasing concentration of carbetamide in the culture medium. Low labelling of phenylalanine detected in the control culture was considerably increased at higher carbetamide concentrations. This may indicate stimulation of the transamination reactions in *C. vulgaris*.

Introduction

Increasing attention is being given towards the possible effects of herbicides on algae since many of the common herbicides inhibit photosynthesis (Ciszewska, 1973; Dodge, 1975; Tchan *et al.*, 1975). Although the effect of some herbicides on growth and photosynthetic activity as measured by O_2-evolution has been studied (Davis *et al.*, 1976; Singh *et al.*, 1978; Richardson *et al.*, 1979; Swader & Howe, 1979; Blythe *et al.*, 1979), the effect of these toxic substances on algal metabolism has received little attention. In the present study the pattern of change in pigment contents, carbohydrate and nitrogen levels as well as in $^{14}CO_2$-photoassimilation in one of the most dominant green alga in Egypt (*Chlorella vulgaris*) grown in the presence of a herbicide, carbetamide is reported.

Material and Methods

Chlorella vulgaris (Beij. var. *vulgaris* Fott.) was isolated from water sample collected from the Damietta branch of the river Nile. Bacteria – free cultures were obtained by using the technique described by Venkatarman (1969). The technique of mass culture

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(Lorenzen, 1964) and the medium used for cultivation was the fresh water algal medium recommended by Khul (1962). The culture conditions and growth criteria of Ahmed & Osman (1973) and Osman *et al.*, (1984) were adopted. Growth rate was determined by measuring the optical density at 540 nm (Ziellinski & Price, 1978), dry weight by the method used by Ahmed & Osman (1973) and photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids were determined by the method described by Metzner *et al.*, (1965).

Determination of carbohydrates: A known volume of algal suspension was dried at 80°C to a constant weight and then ground to a fine powder. Sugars were extracted according to the method described by Younis (1963). The direct reducing value (DRV) which was considered to be equivalent to reducing sugars was estimated by the modified Nelson's method (see Naguib, 1964). Total reducing value (TRV) was estimated by determination of the optical density after hydrolysis by an adequate amount of invertase. Sucrose content was calculated from the difference between TRV and DRV. Polysaccharides were determined in the dry residue left after thorough alcohol extraction of soluble carbohydrates (Younis *et al.*, 1969).

Determination of nitrogen: The different nitrogenous compounds were extracted by the method described by Yemm & Willis (1956). Total-N was determined in the extracts by the conventional micro-Kjeldahl method (Pirie, 1955). Aliquots of the extracts were used for estimation of amino-N (Muting & Kaiser, 1963). The data presented are the mean values of at least triplicate samples.

Photosynthetic activity determinations and pattern of ^{14}C-incorporation: The photosynthetic activities of the cultures as well as the pattern of $^{14}CO_2$-incorporation into the different metabolic intermediates were followed as described by Bassham & Calvin (1963). At the end of the fixation time (3 min.), the algal suspension was quickly mixed with boiling methanol which kills the cells and extracts the relevant compounds. The extract was then subjected to radioactive determination. To follow the pattern of ^{14}C-incorporation into the soluble metabolic intermediates autoradiography technique was applied and the data were expressed as % labelling.

Results and Discussion

A steady growth of the control alga throughout the experimental period was observed (Fig. 1). There was no appreciable change by the addition of 5 or 10 ppm carbetamide, but at 25 and 50 ppm a considerable reduction was observed. These results confirm our observation on *Scendesmus* (Osman *et al.*, 1984) and is in conformity with those obtained by Sika & Pramer (1968) who found that fluometuron at 10 ppm (w/v), reduced the growth of *Euglena* by 52%. Dry weights of the control and of the carbetamide treatments followed a more or less similar pattern to that of growth (Fig. 2).

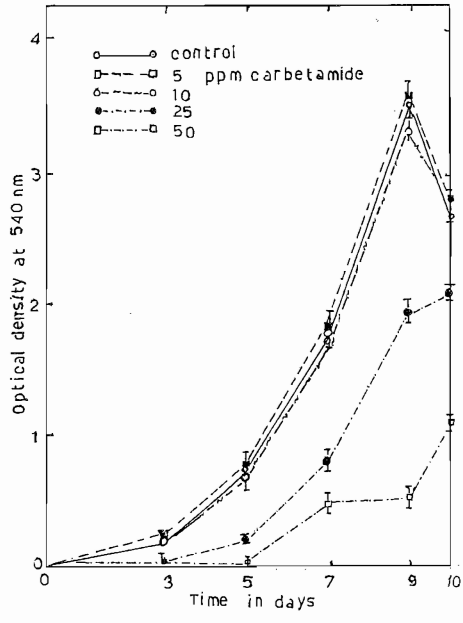

Fig. 1. Effect of different concentrations of carbetamide on the growth of *Chlorella vulgaris*. The mean values listed are given as optical density at 540 nm.

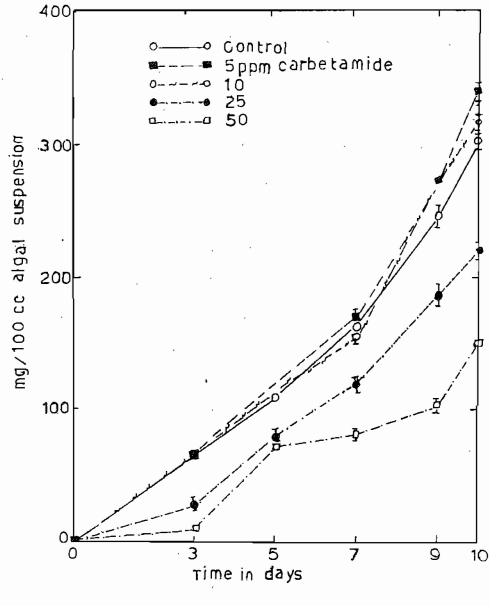

Fig. 2. Effect of different concentrations of carbetamide on the dry weight of *Chlorella vulgaris*. The mean values listed are expressed as mg/100 ml of culture.

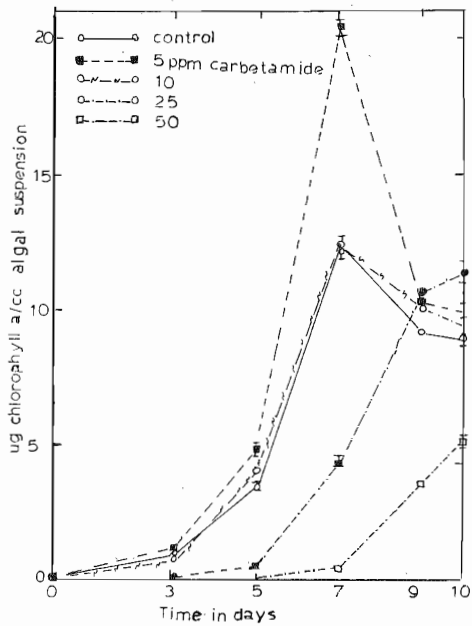

Fig. 3. Effect of different concentrations of the carbetamide on chlorophyll a content of the cultures of *Chlorella vulgaris*. The mean values listed are given as µg chlorophyll a/ml algal suspension.

Chlorophyll a and b levels of the control and 10 ppm treated cultures increased upto 7 days and then declined (Fig. 3 and 4). The cultures treated with 5 ppm carbetamide increased their chlorophyll contents, suggesting a stimulation of chlorophyll synthesis. In the 25 and 50 ppm treated cultures the chlorophyll levels decreased during the early growth accompanied by bleaching of the cells. Thereafter chlorophyll content increased inducing re-greening of the cultures. Carotenoids (Fig. 5) showed more or less similar behaviour to that of chlorophylls. Chlorophyll bleaching at high concentrations of carbetamide may be attributed to inhibition of the reactions leading to the pigment biosynthesis as suggested by Kunert & Böeger (1978) for *Scenedesmus acutus* and *Chlorella kessleri* treated with the herbicide EMD-IT 5914. The re-greening phenomena of the culture treated with the higher concentrations of herbicide after 5 or 7 days may indicate that carbetamide has a reversible toxic action on the photosynthetic apparatus or the reactions leading to its formation as reported by Böger & Schule (1976) for the herbicides diuron, atrasin and mitribuzine added to cultures of *Bumelleriopsis* sp.

The carbohydrate fractions (reducing sugars, sucrose, polysaccharides and total carbohydrates) of 10 days-old treated cultures, showed a progressive reduction as the herbicide concentration increased (Fig. 6). These reductions could be accounted for the respiratory loss exceeding photosynthetic production. The reduction in the labelling percent-

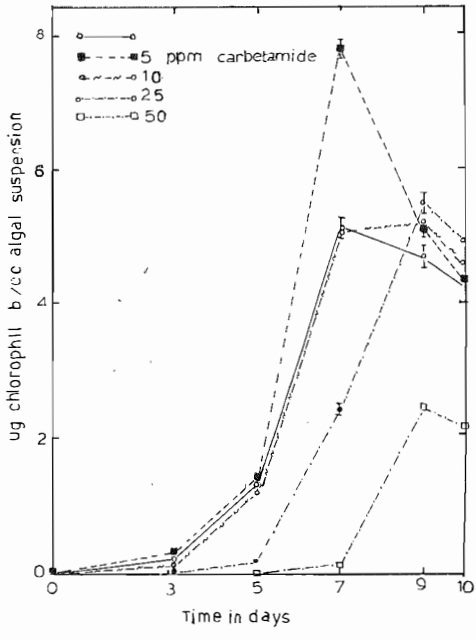

Fig. 4. Effect of different concentrations of carbetamide on chlorophyll b content of *Chlolrela vulgaris* cultures. The mean values listed are given as µg chlorophyll b/ml algal suspension.

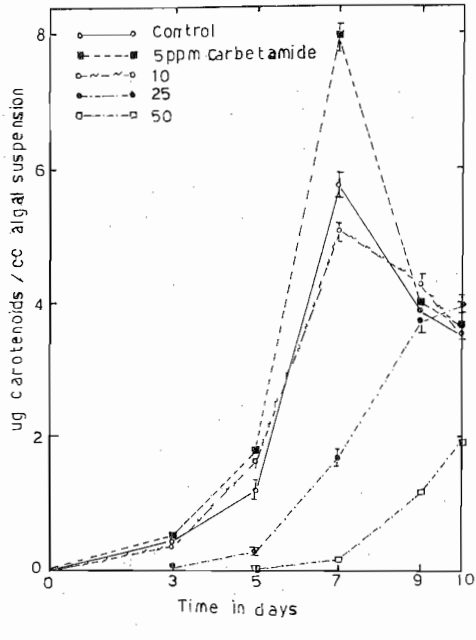

Fig. 5. Effect of different concentrations of carbetamide on caroteniods contents of *Chlorella vulgaris*. The mean values listed are given as µg carotenoides/ml algal suspension.

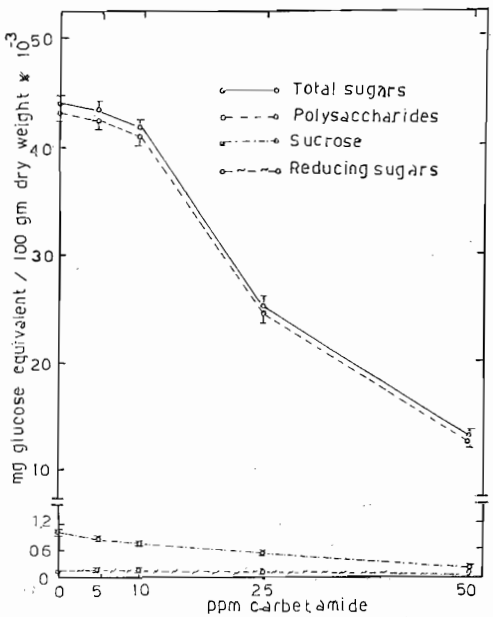

Fig 6. Carbohydrate content of *Chlorella vulgaris* treated with carbetamide. The mean values listed are expressed as mg glucose/100gm dry weight *Chlorella*.

age of organic acid in response to the carbetamide treatment (Table 2) may support this interpretation. This reduction may be due to the stimulation of respiration leading to high rates of consumption of such acids in the tricarboxylic acid cycle. In accordance with this conclusion, Richardson *et al.*, (1979) found that some herbicides (metribuzin, fluometuron and glyphosate) reduced the photosynthetic activity of *Euglena* cells (measured as O_2-evolution), whereas the rate of respiration was elevated by up to 20% by glyphosate. Moreover, Davis *et al.*, (1976) have found that prometryn at 5 x 10^{-5}M or diuron at 5 x 10^{-7} increased the rate of respiration of *Chlorella pyrenoidosa* by 70 and 44% respectively after 80 minutes of treatment.

The pattern of changes in different nitrogen fractions of 10 days-old cultures in response to herbicide treatment is given in Fig. 7. Except for treatment with 5 ppm carbetamide, which induced a slight decrease in protein-and total-N, there apeared to be a progressive increase in different nitrogen fractions (amino-, protein-, total soluble-, and total-N) as the concentration of carbetamide increased. Comparing Fig. 6 and 7, the reduction in different carbohydrate levels was associated with a considerable increase in the different nitrogen fractions. These changes can be correlated with the balance between the metabolic activities (respiration and photosynthesis) and growth.

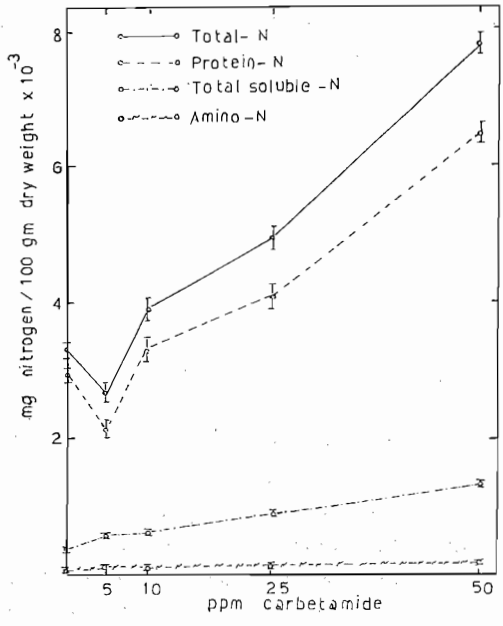

Fig. 7. Nitrogen contents of *Chlorella vulgaris* treated with carbetamide. Values are expressed as mg N/ 100gm dry weight *Chlorella*.

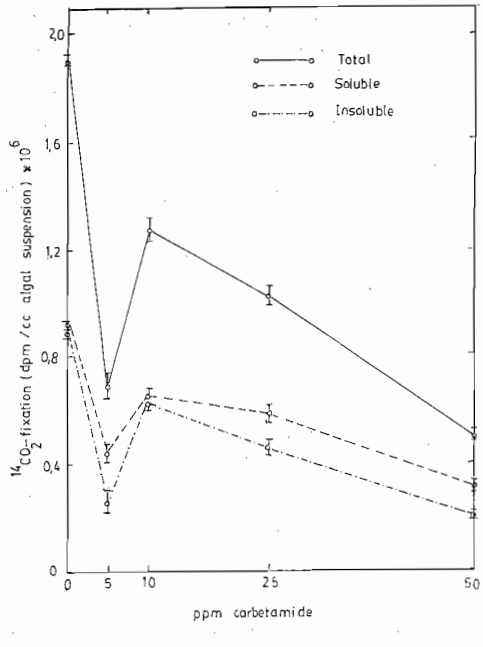

Fig. 8. Effects of different concentrations of carbetamide media at photosynthetic activity at 25°C after 10 days of herbicide application.

Table 1. Effect of different concentrations of carbetamide on the ratio of soluble/insoluble photosynthates in *Chlorella vulgaris*.

Ratio	ppm carbetamide 0.0	5	10	25	50
Soluble/insoluble	1.1	1.7 v.h.s	1.0 n.s	1.3 s	1.5 v.h.s

v.h.s very highly significant (at 0.1% level)
s significant (at 5% level)
n.s non significant

A progressive reduction in the labelling percentage of soluble, insoluble and total photosynthates was observed as the concentration of carbetamide was increased (Fig. 8). Treatment with 5 ppm gave an anomalous low figure. These results give a strong evidence that carbetamide inhibited the photosynthetic activities in *C. vulgaris*. This conclusion is in agreement with the data obtained by some workers who reported reductions in photosynthetic oxygen evolution and growth rate of algae in response to the herbicide treatment (Davis *et al.*, 1976; Singh *et al.*, 1978; Richardson *et al.*, 1979; Swader & Howe, 1979; Blythe *et al.*, 1979).

Data presented in Table 1 show significant increase in the values of soluble/insoluble ratio in response to the carbetamide treatment, except for treatment with 10 ppm, which induced a slight reduction. This means that the rate of transformation of soluble to insoluble photosynthates tended to decrease in the treated *C. vulgaris* cultures. Lipoid and methionine represent the highest labelling percentages of the control culture (Table 2). Both fractions showed significant decreases in response to the addition of carbetamide. The reduction in the lipoid fraction could be accounted for by an inhibitory effect of the herbicide on the reactions leading to lipid biosynthesis. Ashton, *et al.*, (1977) also observed an inhibition of 26% in lipid synthesis in bean cells, in response to chloropropham tratment.

The labellings of free amino acids viz., arginine, aspartic, glutamic, glycine and serine show reductions in response to the different treatments with carbetamide. On the other hand, the low labelling percentage of phenylalanin and proline in the control sample was considerably increased by addition of carbetamide to the culture medium. Aspargine was hardly detectable in the control culture, but carbetamide appeared to induce incorporation of ^{14}C into this amide.

The reduction of labelling in some amino acids in response to carbetamide treatment, was associated with an increase in others. This result suggests a stimulatory action of car-

Table 2. Percentage labellings of photosynthetic intermediates of carbetamide treated and untreated cultures of *Chlorella vulgaris* after 10 days incubation.

Treatment	Carbetamide (ppm)									
	Control		5		10		25		50	
Intermediate	% labelling	± S.D	% labelling	± S.D	% labelling	± S.D	% labelling	± S.D	% lebelling	± S.D
Phsphorylated sugars	0.4	0.02	0.9	0.07	0.5	0.04	0.3	0.02	0.7	0.06
Arginine	14.6	1.1	19.7	1.47	14.4	0.88	13.3	1.19	4.4	0.21
Aspartic acid	1.3	0.07	0.7	0.05	0.6	0.03	0.2	0.02	0.5	0.04
Asparagine	—	—	0.9	0.06	1.0	0.06	0.2	0.02	0.4	0.05
Glutamic acid	2.5	0.23	2.2	0.02	0.2	0.03	0.5	0.04	1.0	0.09
Glutamine	4.6	0.55	8.7	0.66	6.7	0.53	17.00	1.45	11.3	1.2
Glycine	0.9	0.06	0.7	0.06	0.3	0.02	0.3	0.02	0.2	0.02
Methionine	25.9	2.8	22.4	1.52	16.1	1.25	15.3	1.7	10.8	0.93
Phenylalanine	4.3	0.38	24.0	2.06	19.2	1.7	20.8	2.48	30.5	2.28
Proline	1.5	0.12	4.4	0.35	16.3	1.8	10.4	0.48	10.5	0.7
Serine	1.0	0.08	1.1	0.06	0.9	0.07	0.3	0.02	0.4	0.04
Citric acid	2.3	0.15	0.4	0.05	0.4	0.04	0.2	0.02	0.2	0.02
Fumaric acid	0.2	0.02	0.4	0.04	0.3	0.02	0.2	0.02	0.2	0.02
X_5	—	—	0.3	0.02	0.2	0.02	0.3	0.02	0.4	0.03
X_6	—	—	0.4	0.04	0.8	0.06	—	—	—	—
X_8	—	—	—	—	—	—	—	—	0.9	0.06
Lipoid fraction	39.7	3.4	12.5	1.1	22.5	1.8	20.7	1.9	27.3	2.18

X_5, X_6 and X_8 represent unidentified intermediates.

betamide on the transamination reaction and/or inhibition of some amino acid biosynthesis. In agreement with our results, Ekanage *et al.*, (1979) reported that phenylalanine was absent in the glyphosate treated leaves of *Panicum repens*, whereas tyrosine content increased in such treated leaves.

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