Ecotoxicology and Environmental Safety **49,** 235–239 (2001)
Environmental Research, Section B
doi:10.1006/eesa.2001.2056, available online at http://www.idealibrary.com on IDEAL®

Differential Effects of Thiobencarb Toxicity on Growth and Photosynthesis of *Anabaena variabilis* with Changes in Phosphate Level

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Received June 5, 2000

A thiobencarb dose of 3 mg L^{-1} reduced the protein content of *Anabaena variabilis*, whereas it elevated the carbohydrate content. Measurements of *Anabaena* growth, photosynthetic activity, and respiration rate revealed that the high dose of phosphate (0.53 mM) exerted no additional toxic effect to thiobencarb toxicity. Recovery of *Anabaena* cells from the inhibitory effect of thiobencarb occurred immediately after its reculture in herbicide free-medium. Maximum uptake of thiobencarb was associated with high biomass yield. © 2001 Academic Press

Key Words: **cyanobacteria; *Anabaena*; herbicide; thiobencarb; phosphate; photosynthesis; growth; recovery.**

INTRODUCTION

Algal growth is affected by many environmental factors, such as the nutrient levels. Ryan *et al.* (1972) found that increased levels of dissolved nutrients can change the aquatic ecosystem by stimulating algal growth. Many studies concerning the toxic effects of pesticides in the presence of various levels of nutrients in culture media have been conducted (Tubea *et al.*, 1981; Kashyap and Pandey, 1982; Harrison *et al.*, 1990; Mohapatra and Mohanty, 1992).

Corbett *et al.* (1984) and Tomlin (1994), reported that thiobencarb herbicide inhibited protein synthesis, which in turn can exert many secondary effects on growth. Wide variations in sensitivity to thiobencarb have been reported in Chlorophyta and Cyanophyta, with inhibitory concentrations ranging from about 0.01 to 4 mg L^{-1} (Mishra and Pandey, 1989; Bhunia *et al.*, 1991; Kolte and Goyal, 1992; Kasai and Hatakeyama, 1993; Mansour *et al.*, 1994; Sabater and Carrasco, 1996).

Alleviation of growth inhibition and reduction of heterocyst formation in the blue green alga *Nostoc linckia* by glucose under herbicide treatment clearly revealed that thiobencarb has an inhibitory effect on photosynthetic CO_2 assimilation (Singh *et al.*, 1983). Carbon sources including glucose, acetate, and some amino acids (glutamine, arginine, serine, and tryptophan) resulted in protection of *N. linckia* against thiobencarb toxicity (Mishra and Pandey, 1989). The aim of the present investigation was to try to reduce the toxicity of thiobencarb herbicide to *Anabaena variabilis* via the addition of different levels of phosphorus.

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MATERIALS AND METHODS

Anabaena variabilis Kutezing, a filamentous cyanobacter, was isolated from a paddy field in Qalubia, Egypt (1996). Isolation and purification were conducted by the moist plate method. *A. variabilis* were grown in 100 mL of sterile BG-11 medium free of a combined nitrogen source (Stainer *et al.*, 1971) and incubated in a controlled growth chamber at $28 \pm 2°C$ and 50 μ $Em^{-2}s^{-1}$ photosynthetically active radiation (PAR) provided by cool white fluorescent lamps set on a 16/8 light/dark photoregimen for 10 days. Thiobencarb (Saturn 95%) was used in this study. Concentrated herbicide stock was prepared by appropriate dilution in acetone, which is then completely evaporated to dryness. Based on the results of preliminary experiments, aliquots of the prepared stock herbicide were added to each culture to obtain final concentrations of 0.0, 1.0, 2.0, and 3.0 mg L^{-1}. BG-11 medium was modified to contain different levels of phosphate (0.06, 0.18, and 0.53 mM) to study the effect of phosphate availability on the toxicity of thiobencarb. All culture flasks (three per treatment) received the same inoculum (200 μg L^{-1} chlorophyll *a*) and were incubated under the prescribed growth conditions. Growth recovery was followed after resuspension in BG-11 medium of *Anabaena* cells previously exposed to thiobencarb and reincubated under the prescribed growth conditions.

Chlorophyll *a* (Chl *a*) content was determined fluorometrically with a Turner 111 fluorometer; the specific growth rate (μ day^{-1}) was determined for individual cultures by linear regression through Chl *a* data. Turbidity and dry weight were measured (APHA, 1992). Proteins were determined by the Bradford method (Jones *et al.*, 1989). Total carbohydrates were extracted according to the method of

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TABLE 1
Growth and Photosynthetic Parameters of *Anabaena variabilis* on Day 10 of Thiobencarb Treatment and Recovery Period[a]

Thioben carb ($mg\,L^{-1}$)	$\mu^{Chl\,b}$ (day^{-1})	Turbidity A_{750}	Chl *a* ($mg\,L^{-1}$)	Dry wt ($mg\,L^{-1}$)	Chl *a* ($mg\,g^{-1}$ dry wt)	Protein ($mg\,g^{-1}$ dry wt)	Carbohydrate ($mg\,g^{-1}$ dry wt)	F_v/F_m	Gross P_m ($mol\,O_2\,g^{-1}$ chl h^{-1})	R_d ($mol\,O_2\,g^{-1}$ chl h^{-1})
Treatment										
Control	0.17 ± 0.02	0.63 ± 0.02	2.30 ± 0.22	247 ± 2	9.3 ± 0.8	102 ± 9	79 ± 11	0.48 ± 0.01	307 ± 53	-40 ± 8
1.0	0.12 ± 0.02**	0.39 ± 0.02***	1.36 ± 0.17***	243 ± 6	5.6 ± 0.6***	25 ± 7***	80 ± 3	0.48 ± 0.02	296 ± 84	-48 ± 21
2.0	0.13 ± 0.02**	0.21 ± 0.01***	1.43 ± 0.05***	149 ± 6***	9.6 ± 0.3	52 ± 28**	137 ± 18***	0.45 ± 0.04	439 ± 77*	-35 ± 21
3.0	0.12 ± 0.02**	0.21 ± 0.01***	1.53 ± 0.15***	161 ± 5***	9.5 ± 0.7	50 ± 12**	130 ± 15**	0.39 ± 0.04*	405 ± 16	-26 ± 17
One-way ANOVA	**	***	***	***	***	**	***	*	NS	NS
Recovery										
Control	0.19 ± 0.04	0.92 ± 0.10	1.87 ± 0.14	360 ± 32	5.2 ± 0.2	145 ± 22	102 ± 36	0.41 ± 0.02	440 ± 49	-44 ± 6
1.0	0.23 ± 0.01	0.90 ± 0.04	1.75 ± 0.17	357 ± 34	4.9 ± 0.2	122 ± 24	83 ± 9	0.40 ± 0.02	453 ± 28	-38 ± 12
2.0	0.17 ± 0.01	1.02 ± 0.04	2.09 ± 0.18	388 ± 17	5.4 ± 0.2	176 ± 25	133 ± 18	0.44 ± 0.02	603 ± 271	-80 ± 47
3.0	0.17 ± 0.02	0.98 ± 0.05	1.95 ± 0.06	372 ± 21	5.3 ± 0.4	155 ± 41	156 ± 14*	0.43 ± 0.02	621 ± 139	-51 ± 14
One-way ANOVA	*	NS	NS	NS	NS	NS	*	NS	NS	NS

[a]Means $\pm$ SD ($n = 3$). Results of one-way ANOVA and Dunnett's one-sided comparison of treatments with controls indicate $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. NS, not significant.

[b]Specific growth rate over Days 2–6.

Myklestad and Haug (1972) and then determined (Dubois *et al*., 1956).

Gross photosynthesis (P_m) and dark respiration (R_d), were determined as O_2 exchange at $24 \pm 2°C$ in a Hansatech DW3 water-jacketed, 10-mL polarographic electrode chamber, and a customized computer-controlled fluorometer was used for measurement of fluorescence at 77 K (Henley *et al*., 1991). Uptake of herbicide by algae was determined by measuring its residue in culture medium at the end of the incubation period. Thiobencarb residue was extracted via solid-phase technique, and 10-μL samples were injected into the high-performance liquid chromatograph (Redondo *et al*., 1994).

Results were tested (SYSTAT 7.0) by either one-way analysis of variance (ANOVA), followed by one-sided Dunnett post hoc comparisons with the corresponding controls (thiobencarb-free) with each herbicide treatment. ANOVA effects and treatment differences were considered significant when $P < 0.05$.

RESULTS

One-way ANOVA of growth and physiological measurements of *A. variabilis* incubated for 10 days in standard BG-11 medium containing different thiobencarb concentrations revealed a significant negative effect of thiobencarb on *A. variabilis*. These effects were reflected for all growth and physiological variables, except gross photosynthesis (P_m^{Chl}) and dark respiration (R_d^{Chl}) which were normalized to chlorophyll *a* (Table 1). Specific growth rate (μ, Days 2–6) significantly decreased (Table 1), Turbidity, Chl *a* (Fig. 1), and dry weight yields significantly decreased with increasing thiobencarb concentration. Chl *a* ($mg\,g^{-1}$ dry wt) decreased significantly at $1\,mg\,L^{-1}$ thiobencarb. Dry weight and, normalized protein decreased significantly in the presence of thiobencarb, whereas carbohydrate content significantly increased at 2 and $3\,mg\,L^{-1}$ thiobencarb. The ratio of variable fluorescence to maximal fluorescence (F_v/F_m), which is an indicator of the photochemical efficiency of photosystem II, decreased slightly but significantly at $3\,mg\,L^{-1}$ thiobencarb. Gross P_m^{Chl} increased significantly at $2\,mg\,L^{-1}$ compared with control, whereas R_d^{Chl} was not significantly affected by thiobencarb.

In a recovery experiment on previously thiobencarb-treated *Anabaena* cells in thiobencarb-free medium, none of the growth or physiological parameters was significantly different from those of control. However, the carbohydrate content of cells recovered from $3\,mg\,L^{-1}$ thiobencarb treatments was somewhat higher (Table 1).

The entire contents of *Anabaena* cultures and control media (without algal cells) were harvested after 10 days to follow the partitioning of thiobencarb residue. Residual thiobencarb concentrations in control medium and culture media increased progressively with increasing initial thiobencarb addition. However the residual thiobencarb concentrations in the cells were negligible (Table 2). About one-third of the thiobencarb was detected after 10 days in sterile control medium, but 16% remained in algal culture medium and 2% in the cells. This decrease may be due to degradation, volatilization, or adherence to the culture flasks. Generally, maximum uptake was attained in the presence of a low thiobencarb concentration where growth measurements for *Anabaena* were at their highest values.

Studying the effect of phosphate concentration on the sensitivity of *A. variabilis* to thiobencarb using two-way ANOVA revealed that the phosphate–thiobencarb interaction

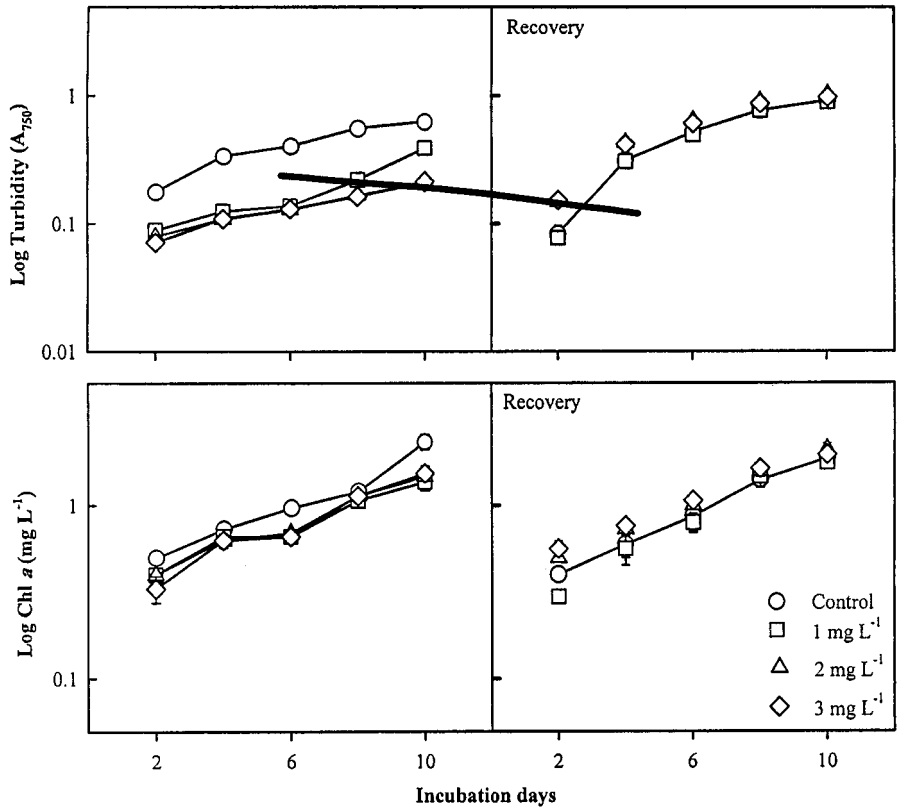

FIG. 1. Effect of thiobencarb on turbidity and chlorophyll *a* content of *Anabaena variabilis* and recovery. Means ± SD ($n = 3$).

significantly affected *Anabaena*-specific growth rate (μ, Days 2–6), dry weight yields, Chl *a* (mg g^{-1} dry wt), and gross P_m^{Chl}; thus, Dunntt's test was performed to compare the means of thiobencarb treatments within different phosphate levels with the mean of the control. Specific growth rate (μ) was not consistently related to thiobencarb concentration, but Chl *a* and dry weight yields significantly decreased with increasing thiobencarb concentration (Table 3). F_v/F_m was not significantly different with respect to the control at all thiobencarb treatments. Gross P_m^{Chl} and R_d^{Chl} were nonsignificantly affected by thiobencarb, while they were significantly affected by phosphate level. Gross P_m^{Chl} values tended to decrease at 0.06 mM phosphate compared with the highest phosphate levels. This means that the low phosphate level increased the inhibitory effect of the herbicide, while the highest phosphate level decreased thiobencarb toxicity.

TABLE 2
Residual Thiobencarb Concentration in *Anabaena variabilis* Culture and Uninoculated Control Media[a]

	Initial thiobencarb		
	1 mg L^{-1}	2 mg L^{-1}	3 mg L^{-1}
	Residual thiobencarb (mg L^{-1})		
Control medium	0.346 ± 0.033	0.792 ± 0.115	1.073 ± 0.249
Culture medium	0.004 ± 0.002	0.29 ± 0.023	0.484 ± 0.028
Cells	0.021 ± 0.001	0.005 ± 0.005	0.001 ± 0.000

[a]Means ± SD ($n = 3$). In all thiobencarb treatments, culture medium and cells contained significantly ($P < 0.01$) less thiobencarb residue than control medium without algae.

DISCUSSION

The present results indicate that 3 mg L^{-1} thiobencarb decreases the specific growth rate, which significantly reduces the biomass yield of *A. variabilis*. Consistent with these observation, Sabater and Carrasco (1996) reported that thiobencarb inhibited growth rates of *Chlorella saccharophila* and *Scenedesmus acutus*. Thiobencarb reduced the cellular protein content and photosystem II efficiency (F_v/F_m) of *A. variabilis*. This reduction indicates that thiobencarb herbicide exerted a direct effect on protein metabolism in the test organism, a trend that agrees with that reported by Tomlin (1994). Orus *et al.* (1990) reported decreased content of total protein and increased content of

TABLE 3
Growth and Photosynthetic Parameters of *A. variabilis* on Day 10 of Phosphate and Thiobencarb Treatment Period[a]

PO_4 (mM)	Thiobencarb ($mg\,L^{-1}$)	$\mu^{Chl\,b}$ (day^{-1})	Turbidity A_{750}	Chl *a* ($mg\,L^{-1}$)	Dry wt ($mg\,L^{-1}$)	Chl *a* ($mg\,g^{-1}$ dry wt)	F_v/F_m	Gross P_m ($mol\,O_2\,g^{-1}$ chl h^{-1})	R_d ($mol\,O_2\,g^{-1}$ chl h^{-1})
0.06	Control	0.21 ± 0.01	0.95 ± 0.02	1.91 ± 0.13	420 ± 30	4.6 ± 0.2	0.42 ± 0.02	373 ± 38	-18 ± 5
	1.0	0.18 ± 0.01	0.33 ± 0.02***	1.26 ± 0.05***	193 ± 8***	6.5 ± 0.0*	0.46 ± 0.03	434 ± 37	-22 ± 28
	2.0	0.22 ± 0.06	0.26 ± 0.00***	1.22 ± 0.14***	200 ± 35***	6.3 ± 1.6	0.40 ± 0.03	318 ± 55*	-36 ± 3
	3.0	0.18 ± 0.05	0.26 ± 0.02***	1.26 ± 0.09***	177 ± 13***	7.1 ± 0.7**	0.38 ± 0.01	316 ± 60*	-34 ± 4
0.18	Control	0.17 ± 0.02	0.63 ± 0.02	2.30 ± 0.22	247 ± 2	9.3 ± 0.8	0.48 ± 0.01	307 ± 53	-40 ± 8
	1.0	0.12 ± 0.02**	0.39 ± 0.02***	1.36 ± 0.17***	243 ± 6	5.6 ± 0.6***	0.48 ± 0.02	296 ± 84	-48 ± 21
	2.0	0.13 ± 0.02**	0.21 ± 0.01***	1.43 ± 0.05***	149 ± 6***	9.6 ± 0.2	0.45 ± 0.04	439 ± 77*	-35 ± 21
	3.0	0.12 ± 0.02**	0.21 ± 0.01***	1.53 ± 0.15***	161 ± 5***	9.5 ± 0.7	0.39 ± 0.04*	405 ± 16	-26 ± 17
0.53	Control	0.13 ± 0.03	0.87 ± 0.03	1.85 ± 0.19	396 ± 17	4.7 ± 0.6	0.39 ± 0.01	694 ± 48	-44 ± 24
	1.0	0.17 ± 0.03	0.33 ± 0.01***	1.23 ± 0.08***	224 ± 4***	5.5 ± 0.3	0.42 ± 0.01	473 ± 87*	-77 ± 19
	2.0	0.17 ± 0.05	0.27 ± 0.03***	1.25 ± 0.18***	212 ± 4***	5.9 ± 0.7	0.38 ± 0.02	503 ± 120	-70 ± 24
	3.0	0.23 ± 0.01*	0.27 ± 0.01***	1.26 ± 0.08***	213 ± 21***	6.0 ± 0.9	0.37 ± 0.06	486 ± 125*	-80 ± 19
Two-way ANOVA									
Phosphate		***	***	***	***	***	*	***	***
Thiobencarb		NS	***	***	***	***	***	NS	NS
PO_4 + thiobencarb		*	***	NS	**	***	NS	**	NS

[a]Means $\pm$ SD ($n = 3$). Results of Two-way ANOVA and Dunnett's one-sided comparison of treatments to controls at the same phosphate level indicate $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. NS, not significant.
[b]Specific growth rate over Days 2–6.

carbohydrate in *Anabaena* cells as a result of trichlorofon treatment. This unbalanced cell composition could be due to disturbances in nitrogen metabolism and photosynthetic activity. It is generally known that when protein synthesis is suppressed by various factors, algal cells depending, on genotype, are transformed to synthesize either carbohydrates or lipids (Averamova and Rossler, 1975). Bhunia *et al.* (1991) stated that reduction of protein content in *N. muscorum* after thiobencarb treatment could be due to an increase in protease enzyme activity.

All measures of growth yield and physiology recovered for *A. variabilis* within 10 days of transfer to thiobencarb-free medium were more or less similar to those observed for *Chlorella pyrenoidosa* (Yoo, 1979). The decomposition of thiobencarb in the medium, apparently accelerated by the algae, could limit its effect to fairly short periods. This is in consistent with their lifetime in the field of 2–3 weeks in aerobic soils (Tomlin, 1994).

The residual thiobencarb concentrations in blank set and inoculated culture media increased progressively with increasing initial thiobencarb concentration. Variations between detected residual thiobencarb and initial concentrations may be attributed to its decomposition by algal cells, which use it as an energy source, or its degradation to less toxic intermediates, which is in accordance with the results obtained by Mostafa (1995). Maximum uptake was associated with high values of biomass and growth rate, particularly at low thiobencarb concentration. This may be attributed to the fact that an increase in algal biomass is linked to an increase in surface area of algal cells and hence the rate of uptake, a trend that is in agreement with the results of some other authors (Shehata *et al.*, 1984; El-Dib *et al.*, 1989, 1990).

It was observed that the toxicity of thiobencarb to *Anabaena* increased gradually with an increase in thiobencarb dose at different phosphate levels. There were no differences in growth of *A. variabilis* (control set) when treated with different levels of phosphate. These findings are in agreement with results obtained by Tubea *et al.* (1981).

It is well established that phosphorus accelerates the growth rate because of its powerful effects as a nutrient for growth. However, higher phosphorus concentrations probably exerted additional stress on the test organisms, thus increasing the toxic effect of herbicides (Eladel *et al.*, 1999; Mohapatra and Mohanty, 1992). This strengthens the findings that suggested that regulation of the toxic effects of different pesticides and other toxic chemicals is influenced by nutrient sources (Somasundaram *et al.*, 1987; Megharaj *et al.*, 1989).

CONCLUSION

Thiobencarb toxicity to *Anbaena variabilis* growth and photosynthetic activity was alleviated by the relatively

higher concentrations of phosphate. It is clear that *Anabaena* has the ability to take up thiobencarb herbicide from growth media.

ACKNOWLEDGMENT

The authors appreciate the helpful cooperation, kind hospitality, and provision of research facilities for this study by Dr. William J. Henley, Botany Department, Oklahoma State University.

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