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Effect of Pendimethalin on Growth and Photosynthetic Activity of *Protosiphon botryoides* in Different Nutrient States

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The effect of pendimethalin on the green alga *Protosiphon botryoides* was investigated. Results indicate that specific growth rate, cell number, chlorophyll a level, and dry weight yield significantly decrease with increasing pendimethalin concentrations, while protein and carbohydrate contents increase significantly. On the other hand, photosynthetic activity decreases whereas dark respiration increases with high pendimethalin concentrations. High doses of pendimethalin exhibited no clear trend with 77 K fluorescence (F_v/F_m). Increasing nitrate and phosphate levels led to a decrease in cell number, chlorophyll a, and dry weight as compared with the control at high doses of pendimethalin. The results obtained revealed that N : P < 1 increases the inhibitory effect of high doses of the herbicide. © 2001 Academic Press

Key Words: **algae; nutrients; respiration; herbicides; pendimethalin; *Protosiphon botryoides*; toxicity; growth; photosynthesis.**

INTRODUCTION

Many investigators who have studied herbicide toxicity to soil algae have found that higher doses are toxic to growth and metabolism of algal cells (Vaishampayan *et al*., 1978; Kobbia and El-Sharouny, 1983; Pandey, 1985; Caux *et al*., 1996). The effects of drepamon, ordram, rifit, and avirosan herbicides on growth and nitrogen fixation of *Nostoc*, *Anabaena*, and *Calothrix* isolated from an Egyptian rice field revealed that growth of *Calothrix* was inhibited by all the herbicides used; *Anabaena* was the most tolerant and *Nostoc* was moderately affected. However, complete recovery from such effects has occurred (Higazy and Fayez, 1989).

There are some reports related to the toxic effects of herbicides and pesticides in the presence of various levels of nutrients in culture medium (Tubea *et al*., 1981; Singh *et al*., 1983; Pandey *et al*., 1984; Mishra and Pandey, 1989; Mohapatra and Mohanty, 1992). The aim of the present study was to investigate the toxicity of pendimethalin to growth and photosynthesis of the green alga *Protosiphon botryoides* and the effect of nitrate and phosphate nutrients on herbicide toxicity.

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MATERIALS AND METHODS

Protosiphon botryoides (Kutezing) Kelbs, a simple siphonous chlorophyte alga, was isolated from a paddy field (Egypt, 1996). Isolation and purification were carried out using a dilution and plating technique. Cultures were grown in Bold's basal medium (BBM) (Nichols, 1973) and incubated in a controlled growth chamber at $24 \pm 2°C$ and $70\ \mu mol\ m^{-2}\ s^{-1}$ photosynthetically active radiation provided by cool white fluorescent lamps set on 16 : 8 light : dark photoregimen for 16 days. All cultures were shaken twice daily to prevent cells from clumping. Pendimethalin (stomp), a dinitroaniline herbicide with poor water solubility and a purity of 95%, was used as a rice field herbicide in this study. Aliquots of the stock in acetone were added to each culture to obtain final concentrations of 0, 0.25, 0.5, and $1.0\ mg\ L^{-1}$. The carrier solvent was completely evaporated to dryness, the culture medium was dispensed and equilibrated, and the flasks were left for 1 day to obtain aqueous solutions (Megharaj *et al*., 1986). All cultures (three per treatment) received the same inoculum ($200\ \mu g\ L^{-1}$ chlorophyll a) and were incubated under the prescribed growth conditions. The first experiment considered the effect of pendimethalin concentrations on *Protosiphon* in standard BBM and algal growth, photosynthetic activity, metabolism, and recovery after herbicide removal. In the next two experiments, BBM was modified to contain different levels of nitrate (0.6, 3.0, or 15 mM) and phosphate (0.3, 1.7, or 8.5 mM), to study the effect of nutrient availability on the toxicity of pendimethalin to *P. botryoides*.

Chlorophyll a content was determined at 2-day intervals up to the end of experiment fluorometrically with a Turner 111 fluorometer. The specific growth rate ($\mu\ day^{-1}$) was determined for individual cultures by linear regression through chlorophyll a data. Algal cells were counted at

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2-day intervals using hemocytometer counting chamber, and the harvested cells were dried at 105°C for 8 h to obtain dry weight according to APHA (1992). Protein was determined by the Bradford method following the extraction of Jones *et al.* (1989). Total carbohydrates were extracted according to Myklestad and Haug (1972), then determined as glucose following Dubois *et al.* (1956).

Gross photosynthesis (P_m) and dark respiration (R_d), were determined as O_2 exchange at 24 ± 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).

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

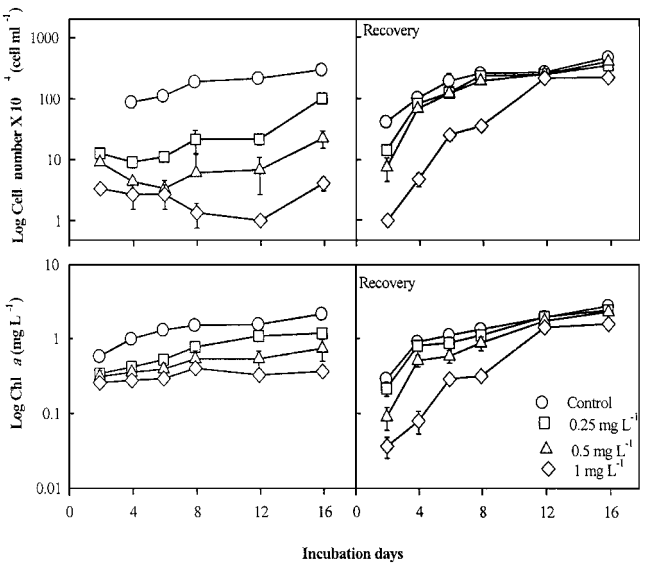

FIG. 1. Effect of pendimethalin on cell number and chlorophyll a level of *Protosiphon botryoides* and recovery. Means ± SD ($n = 3$).

RESULTS

Growth and physiological measurements of *P. botryoides* incubated for 16 days in standard BBM containing different pendimethalin concentrations, using one-way ANOVA, revealed a significant effect of pendimethalin on *P. botryoides* for all variables except gross photosynthesis (P_m^{Chl}) and dark respiration (R_d^{Chl}) with respect to chlorophyll a. Specific growth rate (μg Days 2–8), cell number, chlorophyll a, and dry weight yields significantly decreased with increasing pendimethalin concentration as presented in Table 1 and the time/dose-dependent growth curves in Fig. 1. Chlorophyll a (mg g^{-1} dry wt) increased at 0.25 mg L^{-1} pendimethalin, but decreased at higher concentration, a trend that was clear at Days 12 to 16 (Fig 1). Protein and carbohydrate contents significantly increased with increase in pendimethalin concentration. The ratio of variable fluorescence to maximum fluorescence (F_v/F_m) was slightly but significantly reduced at all concentrations of pendimethalin. Gross P_m^{Chl} significantly decreased at the highest pendimethalin concentration (1.0 mg^{-1}), whereas R_d^{Chl} was unaffected by pendimethalin.

P. botryoides did not fully recover within 8 days of subculture in pendimethalin-free medium as provided in

TABLE 1
Growth and Photosynthetic Activity of *P. botryoides* on Day 16 of Pendimethalin Treatment and Recovery Periods[a]

Pendimethalin (mg L^{-1})	μ^{Chl} day^{-1} [b]	Cell count (×10^{-4} mL^{-1})	Chl a (mg L^{-1})	Dry wt (mg L^{-1})	mg/g dry wt			F_v/F_m	mol O_2 g^{-1} Chl h^{-1}	
					Chl a	Protein	Carbohydrate		Gross P_m	R_d
Treatment										
Control	0.16±0.03	288±25	2.10±0.11	240±7	8.8±0.3	111±8	136±8	0.72±0.01	188±8	−54±16
0.25	0.13±0.01	100±10***	1.18±0.14***	115±15***	10.3±0.4*	195±24*	167±21	0.65±0.03**	169±28	−33±8
0.5	0.08±0.03**	22±7***	0.73±0.24***	84±21***	8.6±0.9	264±68**	178±38	0.68±0.01*	178±25	−63±53
1.0	0.07±0.01**	4±1***	0.37±0.05***	56±7***	6.5±0.6**	382±42***	206±22	0.65±0.01**	115±49*	−78±88
One-way ANOVA	**	***	***	***	***	***	*	**	NS	NS
Recovery										
Control	0.24±0.02	453±28	2.71±0.26	417±15	6.5±0.4	77±3	161±8	0.79±0.00	169±17	−31±4
0.25	0.25±0.04	335±50**	2.38±0.19	357±15*	6.7±0.8	90±2	168±34	0.81±0.02	305±39***	−40±16
0.5	0.35±0.02**	388±33	2.26±0.22*	327±29**	6.9±0.2	93±12	158±19	0.76±0.00*	288±15***	−39±9
1.0	0.40±0.05***	217±29***	1.57±0.11***	290±36***	5.5±1.1	97±18	139±43	0.74±0.02**	296±2***	−53±20
One-way ANOVA	***	***	***	**	NS	NS	NS	***	***	NS

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

[b] μ = specific growth rate over Days 2–8.

Table 1 and Fig. 1. Only chlorophyll a (mg g^{-1} dry wt), protein, carbohydrate, and R_d^{Chl} were not significantly different from control. However, μ and gross P_m^{Chl} were actually higher than control values, where the other variables were still slightly lower.

With varying nitrate concentration at normal BBM and one phosphate concentration (1.7 mM), two-way ANOVA revealed a significant effect of pendimethalin on all measured variables and a significant effect of nitrate concentration and nitrate with pendimethalin interaction on cell number, chlorophyll a, and dry weight yields, and gross P_m^{Chl} (Table 2). Within each nitrate level, specific growth rate (μ, Days 2–8), cell number, chlorophyll a, and dry weight yields significantly decreased with increasing pendimethalin concentration. Within each pendimethalin level, cell number, chlorophyll a, and dry weight yield values tended to decrease at 15 mM nitrate as compared with the control nitrate level (3 mM). At 0.25 mg L^{-1} pendimethalin, biomass yield also decreased at 0.6 mM nitrate relative to normal BBM (3 mM nitrate). Gross P_m^{Chl} and dark respiration rate significantly increased at 1 mg L^{-1} pendimethalin as compared with control.

Different phosphate concentrations at normal BBM medium with one nitrate concentration (3 mM) were conducted. Two-way ANOVA revealed that cell number, chlorophyll a, and dry weight yields and F_v/F_m were significantly affected by phosphate, while all variables except chlorophyll a (mg g^{-1} dry wt) were significantly affected by pendimethalin. Phosphate–pendimethalin interaction significantly affected biomass yield (Table 3). Comparing pendimethalin treatments within phosphate levels, specific growth rate (μ, Days 2–8), cell number, chlorophyll a, and dry weight yields decreased continuously with increasing pendimethalin concentration. High phosphate level (8.5 mM) reduced cell number, chlorophyll a, and dry weight yields, confirming the detrimental effect of $N:P < 1$ on *P. botryoides*. Biomass yields were inversely related to both phosphate and pendimethalin concentrations. Chlorophyll a (mg g^{-1} dry wt) and F_v/F_m were unaffected by pendimethalin interaction with phosphate. Gross P_m^{Chl} significantly increased at 1 mg L^{-1} pendimethalin at all phosphate levels, while R_d^{Chl} significantly increased only at 8.5 mM phosphate.

DISCUSSION

Pendimethalin mode of action is as an inhibitor for cell division and cell elongation (Hess and Bayer, 1974; Tomlin, 1994).

The present results indicated that 1.0 mg L^{-1} pendimethalin decreased specific growth rate and significantly reduced biomass yields of *P. botryoides*, while protein and carbohydrate content were increased. Pendimethalin reduced photosystem II efficiency (F_v/F_m). Gross photosynthetic capacity of *P. botryoides* decreased at high concentrations of pendimethalin. These data are in agreement with Kolte and Goyal (1992), who reported that pendimethalin herbicide affected growth of the blue-green algae

TABLE 2
Growth and Photosynthetic Activity of *P. botryoides* on Day 16 of Nitrate and Pendimethalin Treatment Period[a]

NO_3 (mM)	Pendimethalin (mg L^{-1})	μ^{Chl} day^{-1} [b]	Cell count ($\times 10^4$ mL $^{-1}$)	mg L^{-1}		Chl a (mg g^{-1} dry wt)	F_v/F_m	mol O_2 g^{-1} Chl h^{-1}	
				Chl a	Dry wt			Gross P_m	R^d
0.6	Control	0.17±0.02	252±37	1.50±0.22	291±6	5.2±0.7	0.73±0.06	218±7	−27±4
	0.25	0.08±0.02***	90±5***	0.97±0.06***	138±34***	7.3±1.7*	0.77±0.02	221±35	−48±30
	0.5	0.10±0.01**	185±28*	1.17±0.07*	180±12***	6.5±0.5	0.77±0.01	113±24	−31±16
	1.0	0.09±0.01***	24±14***	0.46±0.06***	63±8***	7.4±0.7*	0.75±0.02	750±178***	−68±26
3.0	Control	0.17±0.06	183±21	1.88±0.19	341±22	5.5±0.3	0.69±0.07	175±22	−24±6
	0.25	0.12±0.01	273±87	1.71±0.41	199±32***	8.6±1.9*	0.78±0.00*	211±7	−34±6
	0.5	0.13±0.06	225±67	1.01±0.09**	177±21***	5.8±1.0	0.75±0.01	124±11	−33±3
	1.0	0.07±0.03*	18±6**	0.48±0.09***	70±10***	6.8±0.4	0.74±0.00	591±168***	−71±27**
15	Control	0.14±0.01	160±65	1.42±0.15	216±56	6.9±1.8	0.76±0.08	165±40	−22±12
	0.25	0.07±0.01**	65±26*	0.76±0.09***	107±10**	7.1±0.6	0.80±0.01	157±17	−13±8
	0.5	0.10±0.00	63±24*	0.60±0.02***	96±12**	6.3±0.6	0.78±0.02	192±30	−53±17*
	1.0	0.11±0.03	13±0**	0.42±0.02***	69±8***	6.2±0.4	0.77±0.00	294±39**	−36±13
Two-way ANOVA									
Nitrate		NS	***	***	***	NS	NS	**	NS
Pendimethalin		***	***	***	***	**	*	***	**
NO_3 ± Pendimethalin		NS	***	***	**	NS	NS	***	NS

[a] Means±SD ($n = 3$). Results of two-way ANOVA and Dunnett's one-sided comparison of treatments to controls at the same nitrate level indicate * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS, not significant.

[b] μ = specific growth rate over Days 2–8.

TABLE 3
Growth and Photosynthetic Activity of *P. botryoides* on Day 16 of Phosphate and Pendimethalin Treatment Period

PO_4 (mM)	Pendimethalin (mg L^{-1})	μ^{Chl} day^{-1} [b]	Cell count ($\times 10^4$ mL^{-1})	mg L^{-1}		Chl a (mg g^{-1} dry wt)	F_v/F_m	mol O_2 g^{-1} Chl h^{-1}	
				Chl a	Dry wt			Gross P_m	R^d
0.3	Control	0.13±0.02	260±26	2.92±0.29	404±34	7.3±1.0	0.79±0.01	51±10	−19±7
	0.25	0.13±0.05	72±19***	1.24±0.44***	159±78***	8.2±1.1	0.80±0.01	182±45**	−46±21
	0.5	0.06±0.03*	37±6***	0.74±0.26***	105±55***	7.8±3.0	0.73±0.05*	204±74**	−71±39
	1.0	0.05±0.02*	23±9***	0.54±0.17***	63±18***	8.8±1.8	0.77±0.02	219±34**	−86±56
1.7	Control	0.12±0.03	332±10	2.20±0.25	287±94	8.4±3.4	0.77±0.01	100±17	−27±8
	0.25	0.08±0.03	92±34***	1.11±0.14***	155±38*	7.4±1.7	0.79±0.01	170±27	−27±2
	0.5	0.08±0.03	38±5***	0.83±0.06***	94±5**	8.9±0.5	0.74±0.02	145±7*	−35±11
	1.0	0.08±0.02	25±6***	0.50±0.04***	56±19***	9.6±3.1	0.75±0.03	183±32**	−86±67
8.5	Control	0.13±0.03	137±24	1.32±0.15	171±11	7.8±1.4	0.79±0.00	131±9	−20±15
	0.25	0.08±0.02**	62±26***	0.95±0.05**	97±3***	9.8±0.5	0.80±0.01	179±33	−69±25*
	0.5	0.06±0.01***	33±6***	0.72±0.06***	73±2***	9.8±1.0*	0.78±0.01	148±8	−31±10
	1.0	0.08±0.00**	14±7***	0.41±0.10***	53±8***	7.7±1.1	0.78±0.01	220±51**	−75±27*
Two-way ANOVA									
Phosphate		NS	***	***	***	NS	*	NS	NS
Pendimethalin		***	***	***	***	NS	***	***	**
PO_4^+ pendimethalin		NS	***	***	**	NS	NS	NS	NS

[a] Means±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] μ = specific growth rate over Days 2–8.

Calothrix, *Nostoc*, and *Tolypothrix* at normal field dose (1.0 ppm), whereas *Anabaena* remained unaffected at the supernormal field dose. However, pendimethalin has been found to be completely harmless to diazotrophic cyanobacteria when it is applied at the recommended field dose (Goyal, 1986, 1989). Shabana and Abou-Waly (1995) stated that the drop in carbohydrate content of *Nostoc muscorum*, which matched the suppression of chlorophyll a synthesis at higher concentrations of triazine herbicides, is attributed to a respective inhibition of algal photosynthesis.

Complete recovery of *Protosiphon* was delayed after its reincubation in pendimethalin-free medium, which can be attributed to the poor water solubility of pendimethalin and its adsorption to glass vessels and algal cells. In this connection, Fliedner (1997) reported that 77% of pendimethalin adsorbed to algal cells after 48 h of treatment and only 12% was recovered from the water phase. Since the main exposure route for aquatic organisms is via the water phase, the adsorbed pendimethalin adsorbed to the algal cells worked as a renewable herbicide source, maintains the herbicide at a high concentration for a long time and may explain the delayed algal recovery in this study.

The effects of toxic substances as (herbicides) vary greatly depending on the different environmental factors, one of which is the composition of the growth nutritional medium. As a general rule, it is easier for any organism to resist toxic effects if all other living conditions are optimal.

Effects of different levels of nutrients (nitrate and phosphate) on herbicide toxicity to the algae studied revealed that pendimethalin toxicity to *P. botryoides* was increased at higher levels of nitrate as compared with low levels. These data are contrary to those reported by Tubea *et al.* (1981), who found that the toxicity of herbicides to *N. muscorum* was reduced when the alga was provided with higher doses of phosphate or nitrate. On the other hand, gross photosynthesis and dark respiration decreased when high nitrate levels interacted with high pendimethalin concentration, indicating that there is a correlation between biomass yield of *Protosiphon* and its photosynthetic activity, which confirms the findings El-Sheekh *et al.* (1994) and contradicts those of Stratton (1984), who reported that no correlation appears to exist between photosynthetic activity and growth patterns.

It is possible that the high nitrate level (fivefold) used in the pendimethalin experiment was toxic to *P. botryoides*. From these observations, perhaps much higher (toxic) or lower (limiting) nutrient concentration stress would increase the effect of the herbicides. It is well established that nitrogen and phosphorus sources accelerate the growth rate, due to their powerful effects as nutrients for growth. In the presence of herbicide, higher nitrogen and phosphorus concentrations probably exerted additional stress on the test organisms, thus increasing the herbicide's toxic effect. This strengthens the findings that suggested that regulation of the toxic effects of different pesticides and other toxic chemicals is influenced by nutrient sources (Eladel *et al.*, 1999; Mohapatra and Mohanty, 1992; Megharaj *et al.*, 1989; Somasundaram *et al.*, 1987). Photosystem II efficiency

(F_v/F_m) remained unchanged because of the strong interaction of nitrate with pendimethalin, whereas herbicide treatment without nutrient interaction reduced photosystem II efficiency, indicating the strong effect of nitrate on herbicide toxicity. Results of nitrate and phosphate experiments for pendimethalin herbicide indicated that $N:P < 1$ inhibited *P. botryoides* growth and increased the inhibitory effect of the studied herbicide.

CONCLUSION

In the presence of pendimethalin herbicide, higher nitrogen (15 mM) and phosphorus (8.5 mM) concentrations exerted additional stress on *P. botryoides*, thus increasing pendimethalin's toxic effect. It must be emphasized that the effects of environmental factors such as nutrients on the stability and potency of herbicides pose a problem in the evaluation and correlation of the results.

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