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Toxicity of cypermethrin on growth, pigments, and superoxide dismutase of *Scenedesmus obliquus*

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Abstract

Cypermethrin is a synthetic pyrethroid that is particularly toxic to crustaceans. It is therefore applied as a chemotherapeutant in farms for the treatment of pests. The effective concentrations of cypermethrin on the inhibition of *Scenedesmus obliquus* growth at 96 h (96 h EC50) were determined to be 50, 100, 150, 200, and 250 mg/L. Algal growth, pigment fractions, and the activity of superoxide dismutase (SOD) in the algal cells were measured in the exponential phase after exposure to cypermethrin. The results show that higher concentration of cypermethrin is inhibitory for growth and other metabolic activities and the 96 h EC50 of cypermethrin to *S. obliquus* is 112 ± 9 mg/L; the potential application of SOD activity in *S. obliquus* as a sensitive biomarker for cypermethrin exposure is also discussed.

Keywords: Ecotoxicology; *Scenedesmus obliquus*; Cypermethrin; Super oxide dismutase

1. Introduction

A variety of organic toxic agents including herbicides, insecticides, surfactants, and other organic compounds have been detected in freshwater systems. The insecticides are reported by Stratton (1987) to affect both target and nontarget organisms when discharged into a water body, thus destroying the structure of the ecosystem. Cypermethrin, one of the widely used insecticides nowadays, is very efficient in agricultural and sanitary pest control. After use, cypermethrin is released directly into the environment, enters the water body by runoff, and affects the aquatic ecosystem. A decrease in algae density and species as primary producers in food webs affects the aquatic ecosystem directly by reducing their biodiversity and primary products. Therefore, algae are frequently used in various bioassays. *Scenedesmus obliquus* was chosen in this study because, as reported by Huang et al. (1994), it is easy to cultivate and its response is highly reproducible. Toxic compounds may affect microalgal photosynthesis, growth, enzyme activity, and respiration. The effective concentration of toxicants that inhibits 50% microalgal growth at 96 h (96 h EC50) is widely used as an index of toxicity (Hornstrom, 1990). It has been reported that microalgal production of glutathione, thiols, or superoxide dismutase (SOD) activity can be stimulated by heavy metals, which may also be a defense mechanism against heavy metal toxicity (Rijstenbil et al., 1998; Leal et al., 1999). Pigments have often been used as biomarkers of exposure to herbicides in plants including algae (Couderchet and Vernet, 2003). We report in this paper the effects of cypermethrin on *S. obliquus*.

2. Methods

S. obliquus FACHB39 was obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences, cultivated in 100 mL liquid HB-4 medium (Li et al., 1959) in 250-mL flasks, and illuminated with cool-white fluorescent lights ($70\ \mu E/m^2\ s$) at 12:12 LD cycle (EPA, 1971). Temperature was maintained in an air-conditioned growth chamber at 28°C. Cells in the exponential phase of

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growth were collected from stock cultures and used as the inocula for experiments. Cypermethrin, technical grade (96.4%), was obtained from the National Center for Monitoring Pesticides, Institute of Hubei Agricultural Sciences. All operations were carried out under sterile conditions to avoid contamination from bacteria or other algae.

Cypermethrin was diluted in acetone, since Stratton (1989) has shown that acetone is also toxic to *S. obliquus*. So, it was necessary to evaluate the no observed effect concentration (NOEC) of acetone. First, *S. obliquus* were cultivated in series concentration of acetone: 0.1%, 0.15%, 0.20%, 0.50%, and 1%. The results indicated that treatment of 0.1% stimulated the algal growth and treatments of 0.5% and 1% are inhibitory. Using Student's *t* tests, analyses of significant differences showed that the NOEC of acetone was 0.15%. Therefore, the concentration of acetone in the experiment was held below 0.15%.

The cypermethrin was added to the culture medium sterilized at 121°C for 20 min, at the beginning of the experiment. The cypermethrin concentrations were at 0.0, 50.0, 100.0, 150.0, 200.0, and 250.0 mg/L. Algal cells were treated with cypermethrin for 96 h. Each test was replicated three times. The data presented here were the average values of three parallel samples, and their relative standard deviations were less than 10%.

From day 0 to day 4, 0.5 mL algal cells were taken daily and cell numbers were counted with a Petroff–Hausser counting chamber under a microscope to determine the growth rate and inhibition percentage.

After the EC50 was determined, the algae were treated with the concentration of EC50. At the end of the experiment (96 h), 10 mL algae cells was collected on an acid fiber filter ($\varnothing$ 0.8 μm). The fibers were cut and transferred to a centrifuge tube, and 5 mL 80% acetone was added, mixed well, and extracted in the dark for 24 h. The extracts were then centrifuged at 4000 rpm for 5 min; the supernatant was analyzed for optical density at 350–700 nm light wavelength continuously (Shimado, UV-1601) (Zhu, 1990).

The effect of toxic agents on the photosynthesis of algae could be measured by using pigments such as chlorophyll and carotenoids. The contents of chlorophyll-*a* and -*b*, total chlorophyll, and carotenoids could be calculated according to Jeffrey and Humphrey (1975) by

$$C_A = 12.7OD_{663} - 2.69OD_{645},$$
$$C_B = 22.9OD_{645} - 4.68OD_{663},$$
$$C_T = C_A + C_B = 20.2OD_{645} + 8.02OD_{663}, \text{ and}$$
$$C_K = 4.70OD_{440} - 0.27C_{A+B},$$

where C_A is the content of chlorophyll-*a* (mg/L), C_B the content of chlorophyll-*b*, C_T the content of total chlorophyll, and C_K is the content of carotenoids.

For enzyme activity assessment, the algal cells were collected and extracted with 1 mL of Tris/borate (0.1 M/0.3 M, pH 7.5, 5 mM EDTA, and 7 nM β-mercaptoethanol) buffer on ice for 10 min. The extract was then centrifuged at 10,000 rpm for 20 min at 4°C; the extracts were stored at −58°C until the enzyme activity was measured (Kong and Sang, 1999).

SOD activity was determined by NBT test (Giannoplities and Ries, 1977). Protein concentrations in the algal cells were determined by the method of Lowry (1951) using bovine serum albumin as standard. Enzyme activity was expressed in U/mg soluble protein.

The growth rate was calculated according to the equation (Guillard, 1973)

$$U = (\mathrm{Ln}\, N_t - \mathrm{Ln}\, N_0)/(t - t_0), \quad (1)$$

where U is the growth rate, N_t the cell number at t time, N_0 the cell number at 0 time, t the sample time for counting cell number, and t_0 is the origin time of the treatment.

The effects of cypermethrin on the growth rate of algae was calculated as

$$\%\text{Inhibition} = (U_{ck} - U_{tox})/U_{ck} \cdot 100, \quad (2)$$

where U_{tox} is the growth rate in the presence of cypermethrin, and U_{ck} is the growth rate in the control.

The percentages of growth inhibition were compared with the concentration data in a regression analysis "logarithmic-probit" from a computer program (Statistica software), and the exact value of 96 h EC50 was determined. The dose response equation was χ^2 tested with 95% confidence.

3. Results

Cultured in different concentrations of cypermethrin, the algae growth curve as shown in Fig. 1. It can be seen that the cypermethrin consistently inhibited the algal population growth in concentrations from 50 to 250 mg/L. The "dose–response" or "concentration–effect" relationship was very significant by comparing the data obtained for EC50 with the assayed cypermethrin concentrations. Moreover, the algal cells changed morphologically when observed under the optical microscope. Some cells were swollen and cell divisions were found abnormal, when the maternal cell divided, the descendant cells remained attached. This indicated that the cypermethrin had a potential of mutagenic effects. This was consistent with the phenomenon observed by Xia and Kuang (1993) while studying the effect of heat and wastewater on *S. obliquus*.

Using the statistic program described above, the EC50 of different intervals (24, 48, 72, and 96 h) could be calculated as shown in Table 1. It was clear that with the progression of the test, the EC50 increased at the

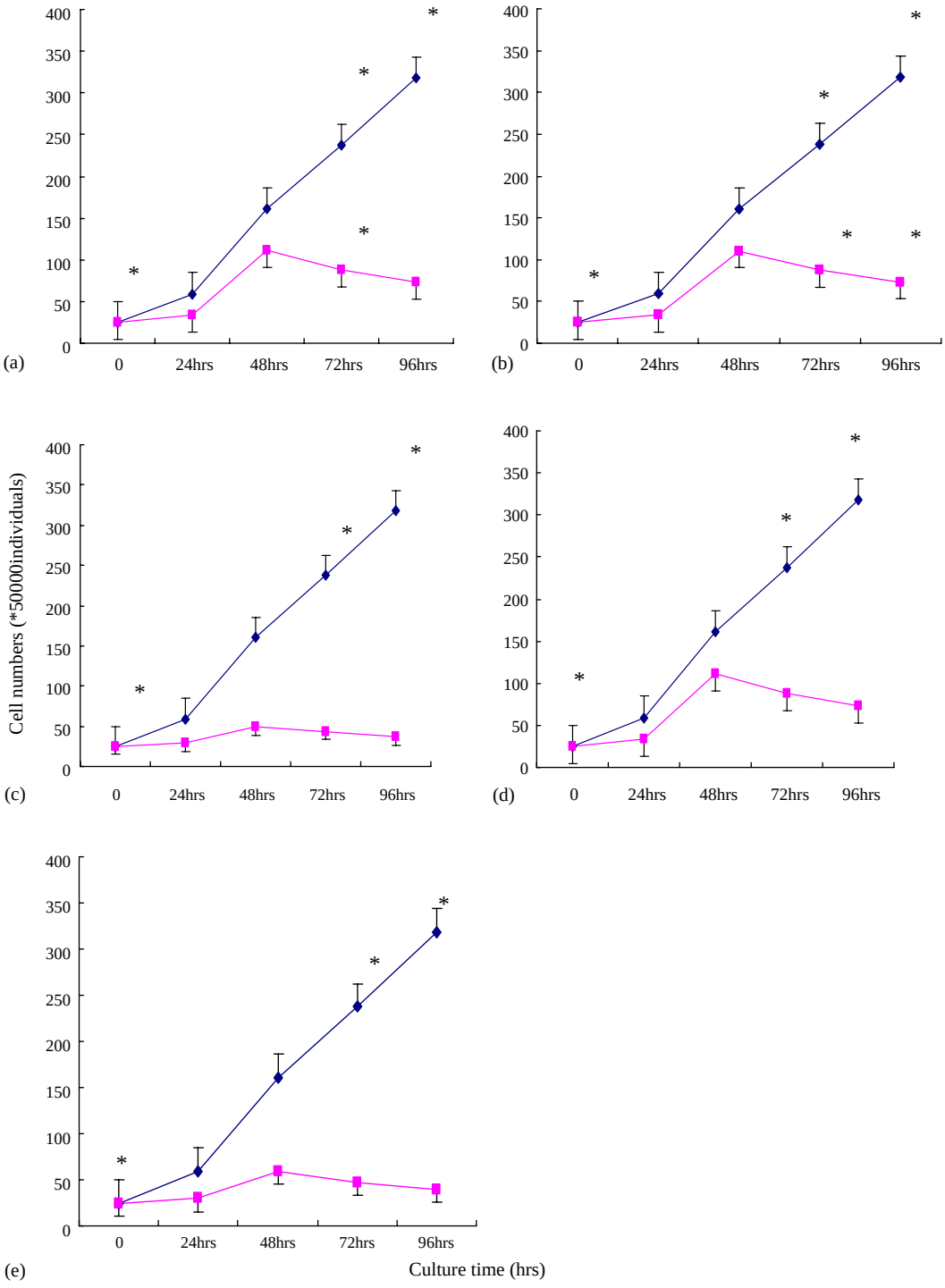

Fig. 1. Effects of different concentrations of Cypermethrin on growth of *S. obliquus*. (1a) (◆) control; (■) 50 mg/L; (1b) (■) 100 mg/L; (1c) (■) 150 mg/L; (1d) (■) 200 mg/L; (1e) (■). 250 mg/l; $^{*}P<0.05$.

beginning and then decreased, indicating that the *S. obliquus* recovered partly. The 96 h EC50 of cypermethrin was 112.45 mg/L, while in other reports (Fairchild and Ruessler, 1998) the 96 h EC50s of atrazine, metribuzin, alachlor, and metolachlor to *S. quadricauda* were 0.169, 0.152, 1.328, and >3.0 mg/L, respectively. This could be due to (1) the sensitivities of various species was different or (2) the cypermethrin was less toxic to algae as nontarget organisms.

The effect of cypermethrin on the pigments is shown in Table 2. It was obvious that cypermethrin decreased the contents of chlorophyll and carotenoids, but not so strongly the chlorophyll-*a*/*b* ratio, suggesting that the biomass of algae was affected by cypermethrin much

Table 1
Toxicity of cypermethrin to *S. obliquus*

	Regression formula	EC50 (mg/L)±SD	*r*
24 h	$y = 0.6346x + 2.4547$	55.20±10.36	0.976
48 h	$y = 0.9795x - 0.1304$	188.25±40.34	0.994
72 h	$y = 1.0894x - 0.2193$	120.42±14.58	0.994
96 h	$y = 1.1545x - 0.4521$	112.45±9.79	0.998

Table 2
Effect of cypermethrin on the pigments in *S. obliquus*

	Control (mg/L)	Cypermethrin (mg/L)	Inhibition percentage (%)
C-*a*	3.74	1.59	57
C-*b*	1.32	0.66	50
C-*a*+*b*	5.07	2.25	57
C-*k*	1.73	0.92	48
C-*a*/C-*b*	2.83	2.41	16

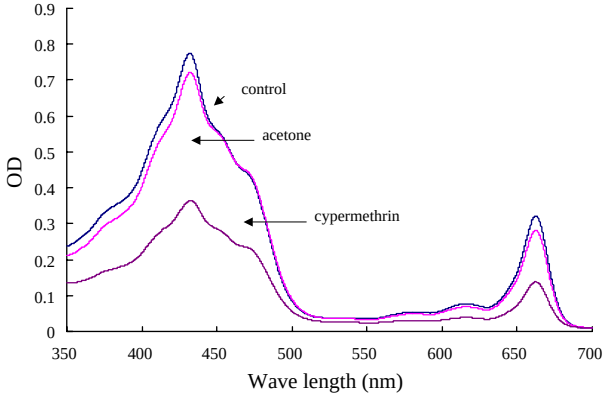

Fig. 2. Absorption spectrum of *S. obliquus* after 96 h.

more strongly than the structure of the chlorophyll body. Moreover, the content of carotenoids was highly inhibited, indicating that it could be used as a sensitive biomarker in monitoring aquatic contaminants. Our data were in accordance with those of Rai et al. (1991), who reported that zinc induced a drastic decrease on chl-*b* and that carotenoid production was more sensitive to zinc than chlorophyll in *S. obliquus*. Fisher and Jones (1981) found that low zinc levels enhanced the total chlorophyll in *Asterionella japonica*. In contrast, carotenoid production was more resistant to cypermethrin toxicity in *S. obliquus* which suggested that it may enhance the oxidative steps and inhibit the reductive steps in the biosynthesis pathway of these pigments.

The record of the absorption spectrum in Fig. 2 showed that the shapes of the curves were very similar, but the values of OD were quite different. When algae was cultured only in acetone with concentration of 0.15%, its OD values were quite similar to those of the control, but when cultured in cypermethrin, the result

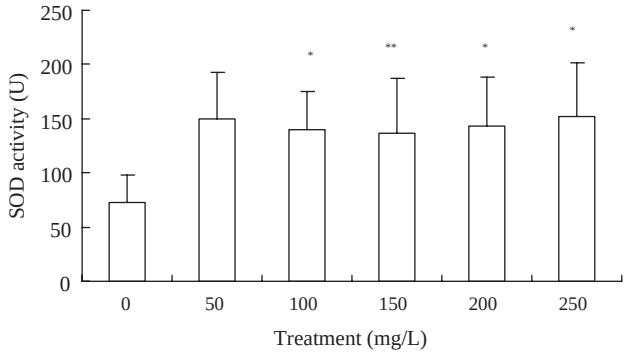

Fig. 3. SOD activity of algae cultured in cypermethrin. $^{*}P<0.05$, $^{**}P<0.01$.

was quite different. This was in agreement with the results of the contents of pigment.

The results of SOD activity are shown in Fig. 3. It was clear that the SOD activity was stimulated by the treatment of cypermethrin. The SOD activities of all the cypermethrin-treated cultures were much higher than those of the control, regardless of the concentrations of cypermethrin.

4. Discussion

According to this study, the 96 h EC50 of cypermethrin on *S. obliquus* was 112.45 mg/L, while in other reports, the EC50s of insecticides on *Scenedesmus* sp. were rather low; for example the 96 h EC50s of lindan and *N′*-(2,4-dimethylphenyl)-*N*-methylformamidine were 2.5 and 6.5 mg/L, respectively, and the 72 h EC50 of parathion methyl was 15 mg/L (Schäfer et al., 1994). Compared with these insecticides, the toxicity of cypermethrin was much lower and so it seemed rather safe to algae. In another words, *S. obliquus* was less sensitive to cypermethrin.

However, the growth of algae was highly affected by cypermethrin. First, the number of cells was decreased significantly when treated with cypermethrin. Second, the content of pigments, especially carotenoid production, and the activity of SOD were highly affected by cypermethrin. The change of SOD activity started earlier than the change of growth. This indicated that the change of SOD activity was very sensitive to the environment. Since Kong and Sang (1999) had shown that SOD was one of the key enzymes to eliminate active oxygen in algal cells once the content of pollutants increases, the cellular detoxification system was stimulated and the synthesis of SOD was started. Because these changes took place at the molecular level in the cells, they happened much earlier than growth or reproduction (Rabinowich and Fridovich, 1985). Therefore, SOD and carotenoids could serve as very sensitive biomarkers and be used for early warning of pollution.

5. Conclusion

From the conducted experiment it can be concluded that (1) the 96 h EC50 of cypermethrin to *S. obliquus* is 112 ± 9 mg/L (compared with other insecticides, cypermethrin is of low toxicity to *S. obliquus*), (2) the growth and photosynthetic pigments are highly inhibited by higher concentrations of cypermethrin and carotenoid production is more sensitive to cypermethrin than chlorophyll (this is consistent with the findings of Omar, 2002), and (3) the algal SOD activity is stimulated by cypermethrin treatment so that SOD and carotenoids can be used as sensitive biomarkers for early warning of pollution.

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