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Toxicity of Organophosphorous Insecticides to Three Cyanobacterial and Five Green Algal Species

J. Ma,[1,2,*] P. Wang,[2] C. Huang,[2] N. Lu,[1] W. Qin,[2] Y. Wang[1]

[1] Department of Plant Protection, Henan Institute of Science and Technology, Xinxiang, 453003, People's Republic of China
[2] School of Life Sciences, Zhejiang Forestry College, Lin-an, 311300, People's Republic of China

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Organophosphorous (OP) insecticides were introduced as replacements for the persistent organochlorine insecticides, such as DDT. The increased use of OPs, originally seen as less of a threat to the environment due to their low persistence, has led to a different range of ecotoxicological problems associated with their high acute toxicity (Galloway and Handy 2003). However, their use may allow them to enter freshwater ecosystems by spray drift, leaching, run-off, or accidental spills and present potential risks for aquatic flora (Van der Brink and Ter Braak 1999). Alterations of the species composition of an aquatic community as a result of toxic stress may affect the structure and the functioning of the whole ecosystem (Verdisson et al. 2001). Green algae and cyanobacteria (blue-green algae) are known to be comparatively sensitive to many chemicals (Real et al. 2003). Their ecological position at the base of most aquatic food webs and the essential roles in the nutrient and phosphorus cycling are critical to ecosystems (Sabater and Carrasco 2001). Some information about the toxicological aspects of pesticides on green algae has been obtained (Ma et al. 2003; 2004a,b,c). However, little is known about the toxicological aspects of pesticides on cyanobacteria (Abou-Waly et al. 1991). Cyanobacteria can produce algal toxins, which have important implications for humans and aquatic organisms (An and Kampbell 2003). Tests on a certain species of algae are of limited applicability in assessing the effects of environmental contaminants on algal communities, which are composed of an array of species with different sensitivities (Ma et al. 2004b, c). A lot of works have been published about the comparative sensitivity of pesticides toward various green algae (Junghans et al. 2003; Ma et al. 2003; 2004a). Yet there are few reports concerning the differential response of various cyanobacteria and green algae (Bhaskar et al. 2004). In order to compare the differential sensitivity of OP insecticides to cyanobacteria and green algae, in this study, 4 such insecticides were tested to examine their effects on 3 cyanobacteria *Anabaena flos-aquae*, *Microcystis flos-aquae*, *Mirocystis aeruginosa* and the 5 green algae *Raphidocelis subcapitata*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*.

Correspondence to: J. Ma

MATERIALS AND METHODS

All of the tested OP insecticides were obtained from Zhejiang Yongnong Chemical Industry and Liben Agro-chemical Co., Ltd, People's Republic of China. Their formulation and CAS Number are shown in Table 1. The tested insecticides were dissolved by 99.5% acetone. The concentration of the acetone in the medium was less than 0.05%. The US Environmental Protection Agency recommends the allowable maximal limits of 0.05% solvent for acute tests and 0.01% for chronic tests, this level was not significant with regard to toxicity (Ma et al. 2003). The toxicity tests were carried out with the freshwater cyanobacteria *A. flos-aquae*, *M. flos-aquae*, *M. aeruginosa*, and green algae *R. subcapitata*, *S. quadricauda*, *S. obliquus*, *C. vulgaris*, *C. pyrenoidosa*, which were obtained from the Institute of Hydrobiology, the Chinese Academy of Science. The media for cyanobacteria and green alga growth inhibition tests were HGZ and HB-4 respectively (Kuang et al. 2003). The culture media were sterilized at 121℃, 1.05 kg cm^{-2} for 30 min (Ma and Chen 2005).

Table 1. Selected insecticides, formulation and CAS Number

Insecticides	CAS Number	Formulation[a]
Methidathion	950-37-8	96.1%TC
Thionazin	297-97-2	90.0%TC
Diazinon	333-41-5	95.0%TC
Phoxim	14816-18-3	90.0%TC

[a]TC denote technical product

Both cyanobacteria and green algae were cultivated in a 250 mL Erlenmeyer flask containing 100 mL liquid medium and kept on a rotator shaker (100 rpm) at 24℃, illuminated with cool-white fluorescent lights at a continuous light intensity of 450μmol m^{-2} s^{-1} (Ma et al. 2004b). 20 mL medium containing cyanobacterial or green algal cells (initial concentration OD_{680nm} = 0.008) were distributed to sterile 50 mL Erlenmeyer flasks, respectively. A wide range of concentrations was examined in a previous test in order to find the adequate range of toxicity for each insecticide. Then, adequate concentrations (0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 mg/L) were tested according to the results of the previous test (Ma et al. 2005). The media were then treated with various insecticide concentrations, and incubated for 96 h on an orbital shaker (100 rpm) at a temperature of 24℃ and a continuous light intensity of 450μmol m^{-2} s^{-1} (Ma 2005). Biomass was correlated with absorbance over time for 96 h on a Shimadzu UV-2401PC spectrophotometer. The most suitable wavelength for monitoring culture growth was 680 nm. Good linear relationships between dry weight concentration (DWC) or chlorophyll-a (ChlaA) content of algal cultures and OD_{680nm} were obtained. Therefore, the growth of cyanobacterial and green algal biomass were calculated indirectly using spectrophotometric data. Three replicates were made for each insecticide concentration and control. An appropriate control containing no insecticide was included in each experiment. Control and treated cultures grew under the same conditions as the stock cultures. In each experiment, the percent inhibition, relative to growth in control systems, was calculated using spectrophotometric data (Ma et al. 2005).

EC_{50} values were calculated using linear regression analysis of transformed insecticide concentration as natural logarithm data versus percent inhibition (Ma et al. 2004a). Weighted analysis of variance was used, followed by a one-sided Dunnett's test using a 5% significance level to obtain the lowest observable effect concentration (LOEC). The no observable effect concentration (NOEC) was taken to be the test concentration immediately below the LOEC, while the chronic value (CV) was the geometric mean of the NOEC and LOEC (Ma 2005).

RESULTS AND DISCUSSION

The acute toxicity of the four insecticides to the three cyanobacteria *A. flos-aquae*, *M. flos-aquae*, *M. aeruginosa*, and five green algae *R. subcapitata*, *S. quadricauda*, *S. obliquus*, *C. vulgaris*, *C. pyrenoidosa* are shown in Table 2. The 96 h EC_{50}, LOEC and NOEC values of thionazin to cyanobacteria and green algae varied from 2.6-5.3 mg/L and 14.4-46.5 mg/L, 1-2 mg/L and 2-5 mg/L, 0.5-1 mg/L and 1-2 mg/L, respectively. The 96 h EC_{50}, LOEC and NOEC values of methidathion varied from 6.2-67.5mg/L and 7.5-57.0 mg/L, 5-25 mg/L and 1-10 mg/L, 2-10 mg/L and 0.5-5 mg/L, respectively. The average toxicity of methidathion to cyanobacteria and green algae was lower than that of thionazin. The 96 h EC_{50}, LOEC and NOEC values of phoxim varied 0.2-12.2 mg/L and 1.9-30.1 mg/L, 0.2-2 mg/L and 1-10 mg/L, 0.1-1 mg/L and 0.5-5 mg/L, respectively. The average acute toxicity of phoxim to cyanobacteria and green algae was the highest among all tested insecticides. The 96 h EC_{50}, LOEC and NOEC values of diazinon varied around 11.5-22.2mg/L and 10.8-15.4 mg/L, 2-20 mg/L and 1-20 mg/L, 1-10 mg/L and 0.5-10 mg/L, respectively. The average acute toxicity of diazinon to cyanobacteria and green algae was the lowest among all tested insecticides. The decreasing order of the average acute toxicity to cyanobacteria and green algae of four dissimilar organophosphates insecticides was: phoxim > thionazin > methidathion > diazinon.

Wide variations were found occurring in response to the tested insecticides among individual species of the three cyanobacteria and five green algae. Compared to the magnitude of the EC_{50}, within the three cyanobacterial species, the decreasing sensitivity order of the tested insecticides was: phoxim > methidathion > thionazin / diazinon (see Table 3). Within the five algal species, the decreasing

Table 2. The effect of various insecticides on eight cyanobacteria and green algae

Insecticides	Regression equation*	Coefficient correlation	Significance level	EC_{50} (mg/L)	LOEC (mg/L)	NOEC (mg/L)
Methidathion	(1)Y=5.1258+0.4817X	0.9874	0.0020	67.5210	25	10
	(2)Y=3.0494+0.2128X	0.9531	0.0033	6.2780	5	2
	(3)Y=4.1608+0.3413X	0.9915	0.0009	21.9523	10	5
	(4)Y=2.7550+0.1911X	0.9654	0.0010	7.5038	1	0.5
	(5)Y=2.4997+0.1843X	0.9980	0.0001	19.3999	10	5
	(6)Y=2.1515+0.1690X	0.9752	0.0001	57.0154	10	5
	(7)Y=2.7732+0.2259X	0.9721	0.0001	42.6340	10	5
	(8)Y=4.0848+0.3566X	0.9803	0.0030	43.6844	5	2
Thionazin	(1)Y=3.4592+0.2302X	0.9528	0.0437	2.6077	1	0.5
	(2)Y=2.5797+0.1702X	0.9799	0.0001	4.9305	1	0.5
	(3)Y=3.3204+0.2321X	0.9821	0.0029	5.2841	2	1
	(4)Y=2.2502+0.1754X	0.9996	0.0001	46.5052	5	2
	(5)Y=2.0683+0.1482X	0.9839	0.0001	25.3604	2	1
	(6)Y=2.5534+0.1843X	0.9980	0.0001	14.4963	2	1
	(7)Y=2.4314+0.1815X	0.9607	0.0010	23.9074	2	1
	(8)Y=2.8025+0.2107X	0.9793	0.0010	17.9511	2	1
Diazinon	(1)Y=2.0435+0.1440X	0.9965	0.0001	22.1262	2	1
	(2)Y=5.5668+0.4710X	0.9792	0.0208	21.2929	20	10
	(3)Y=4.5583+0.3570X	0.9855	0.0021	11.5441	20	10
	(4)Y=2.6452+0.1935X	0.9628	0.0010	15.3207	2	1
	(5)Y=2.5873+0.1935X	0.9860	0.0001	20.6483	1	0.5
	(6)Y=1.7367+0.1246X	0.9581	0.0010	48.9182	2	1
	(7)Y=2.5005+0.1983X	0.9830	0.0010	41.5651	20	10
	(8)Y=2.6519+0.1882X	0.9995	0.0001	10.8200	2	1
Phoxim	(1)Y=5.0652+0.3110X	0.9594	0.0098	0.4214	0.5	0.2
	(2)Y=1.9824+0.1310X	0.9843	0.0004	12.1543	2	1
	(3)Y=5.1234+0.3027X	0.9957	0.0043	0.2329	0.2	0.1
	(4)Y=2.1397+0.1575X	0.9788	0.0002	30.1056	5	2
	(5)Y=2.3476+0.1722X	0.9649	0.0001	21.9503	2	1
	(6)Y=2.4196+0.1767X	0.9757	0.0002	19.1423	10	5
	(7)Y=2.0495+0.1439X	0.9788	0.0001	21.0651	2	1
	(8)Y=1.4981+0.0760X	0.9576	0.0420	1.9790	1	0.5

Y and X stand for percent inhibition and natural logarithm of concentration respectively.

(1) *A. flos-aquae*; (2) *M. aeruginosa*; (3) *M. flos-aquae*; (4) *R. subcapitata*; (5) *S. quadricauda*; (6) *S. obliquus*; (7) *C. vulgaris*; 8) *C. pyrenoidosa*

order was phoxim > methidathion > diazinon / thionazin. Between the three cyanobacterial and the five green algal species, the decreasing order was phoxim > thionazin > methidathion > diazinon. Compared using the CV, within three cyanobacterial species, the decreasing order of the sensitivity to the tested insecticides was: phoxim / diazinon > methidathion > thionazin. Within five algal species, the decreasing order was the same. Between three cyanobacterial and five green algal species, the decreasing order was phoxim > methidathion / diazinon > thionazin (see Table 3). Therefore, the decreasing order of the ecological risk was phoxim > diazinon / methidathion > thionazin.

Table 3. Differential sensitivities of algal species to selected insecticides

Insecticides*	Within Cy-EC	Within Ga-EC	Between CyGa-EC	Within Cy-CV	Within Ga-CV	Between CyGa-CV
Methidathion	10.76	7.60	9.08	5.00	10.00	22.36
Thionazin	2.03	3.21	17.83	2.00	2.24	4.472
Diazinon	1.92	4.52	4.24	10.00	20.00	20.00
Phoxim	52.18	15.21	129.26	10.00	20.00	50.00

* Within Cy-EC, Within Ga-EC and between CyGa-EC denote the ratio of maximal and minimal EC_{50} value of the three cyanobacterial species, that of the five green algal species and that between cyanobacterial and green algal species respectively. The same ratio was used for, within Cy-CV, within Ga-CV and between CyGa-CV with respect to chronic value.

The decreasing order of the sensitivity to various algal species was: *M. aeruginosa* > *R. subcapitata* > *S. quadricauda* > *M. flos-aquae* > *C. vulgaris* / *C. pyrenoidosa* > *S. obliquus* / *A. flos-aquae*, when exposed to methidathion. For thionazin, the decreasing order was *A. flos-aquae* / *M. aeruginosa* > *M. flos-aquae* > *S. obliquus* > *C. pyrenoidosa* > *C. vulgaris* > *S. quadricauda* > *R. subcapitata*. With respect to diazinon, the decreasing order was: *C. pyrenoidosa* > *M. flos-aquae* > *R. subcapitata* > *S. quadricauda* / *A. flos-aquae* / *M. aeruginosa* > *C. vulgaris* / *S. obliquus*. As to phoxim, the decreasing order was: *M. flos-aquae* > *A. flos-aquae* > *C. pyrenoidosa* > *M. aeruginosa* > *R. subcapitata* / *S. quadricauda* / *S. obliquus* / *C. vulgaris*.

If green algae and cyanobacteria have greater differential sensitivity, especially when the sensitivity of cyanobacteria is much lower than that of green algae, the contamination may result in a shift of green algal and cyanobacterial group structure, i.e. from dominance by green algae to dominance by cyanobacteria. This may sustain cyanobacterial blooms during this period, thus, the contamination would present higher ecological risk (Ma et al. 2005). The results indicate that the decreasing order of the average toxicity of the four pesticides to the eight algae was: phoxim > thionazin > methidathion > diazinon. However, the decreasing order of having greater differential sensitivity between green algae and cyanobacteria was: phoxim > diazinon / methidathion > thionazin. There was some dissimilarity between magnitude of toxicity and magnitude of differential sensitivity. However, whether there exists more potential aquatic ecological risk owing to the greater differential sensitivity between green algae and cyanobacteria remains to be further studied and proved by more experimental data.

The aquatic ecological system is very complicated. Single-species toxicity tests have historically been the source of biological data for hazard evaluation. Yet it has been discussed as to whether the information from these standard tests alone is enough for predicting the effects at the ecosystem level (Boutin and Rogers 2000). Multiple-species toxicity tests such as microcosm and field tests enable observation of the indirect effects of chemicals caused by interactions among species. But conducting such tests to assess the impact of chemicals on the ecosystem involves skilled labor and it is time-consuming and expensive, and not easy to interpret (Boxall et al. 2002). Accordingly, it is difficult to require applicants to assess the potential ecological risk of their pesticide products using multiple-species in pesticide registration schemes. However, single-species tests are of limited applicability in assessing the effects of environmental contaminants on algal communities, which are composed of an array of species with different sensitivities. Therefore it needs to be fulfilled that the response of selected organisms should correspond to those of a larger array of organisms in natural systems and the chosen endpoints should be more sensitive than any other at any level of organization (Boxall et al. 2002). If single-species toxicity tests indicate that a pesticide is likely to pose a potential risk to the environment, then, impacts can be determined using multiple-species toxicity tests. In the present work, the five green algae are "official species" (all are chlorophyta) but the three cyanobacteria are not. Moreover, the toxicity of pesticides to cyanobacterial species has been generally ignored by researchers. Cyanophyta, chlorophyta, cryptophyta and bacillariophyta may be good organisms for toxicity tests due to the character of natural aquatic systems.

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