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Differential Toxicity of Agricultural Fungicides Toward Three Cyanobacterial and Five Green Algal Species

MA JIANYI[1,2], TONG SENMIAO[2], WANG PINWEI[2] and CHEN JIANMENG[1,*]

[1]College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, P.R. China
[2]School of Forestry and Biotechnology, Zhejiang A & F University, Linan 311300, P.R. China

*Corresponding author: Fax: +86 57163732747; E-mail: jchen@zjut.edu.cn

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Six fungicides were tested to examine toxicity to cyanobacteria and green-algae. The results indicated that: (1) the decreasing order of the toxicity was: chlorothalonil > hexaconazole > cymoxanil > benalaxyl > fosetyl-aluminum > metalaxyl; (2) the sensitivity of various species of algae exposed to benalaxyl, cymoxanil and hexaconazole varied by one order of magnitude and those exposed to metalaxyl and chlorothalonil varied by two orders of magnitude; (3) cyanobacteria were less sensitive to metalaxyl, chlorothalonil, cymoxanil and fosetyl-aluminum than were green algae. The decreasing order of ecosystem risk was fosetyl-aluminum > chlorothalonil > metalaxyl > cymoxanil > hexaconazole > benalaxyl.

Key Words: Agricultural fungicides, Toxicity, Sensitivity, Cyanobacteria, Green algae.

INTRODUCTION

Fungicides, which are often used in agriculture to reduce, kill or control fungal pests are commonly responsible for such aquatic problems. Alterations of the species composition of an aquatic community as a result of toxic stress may affect the structure and functioning of the whole ecosystem. Green algae and cyanobacteria are known to be comparatively sensitive to many chemicals[1]. Their ecological position at the base of most aquatic food webs and their essential roles in the nutrient cycling and oxygen production, are critical to all ecosystems[2]. A great deal of information regarding the toxicological effects of pesticides on green algae has been reported[3]. However, little is known about the toxicological effects of pesticides on cyanobacteria[4]. Tests on a certain species of algae are of limited applicability in assessing the effects of environmental contaminants on algal communities, as such communities are composed of an array of species with different sensitivities[2]. A few reports have been published about the comparative sensitivities of pesticides of various green algae[5]. However, there are few reports concerning the differential responses of various cyanobacteria and green algae. In order to compare the differential sensitivities of agricultural fungicides to cyanobacteria and green algae, the acute toxicity test has been devised. In the present study, six agricultural fungicides were tested to examine their effects on three cyanobacteria (*Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*) and five green algae (*Selenastrum capricornutun*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Chlorella vulgaris* and *Chlorella pyrenoidosa*).

EXPERIMENTAL

All of the tested agricultural fungicides were purchased from the People's Republic of China; their CAS, REG. NO. and formulation are shown in Table-1.

TABLE-1
SELECTED FUNGICIDES: CHEMICAL CLASSES AND MODE OF ACTION

Active ingredient	Reg. No.	Site of action	Formulation* (%)
Benalaxyl	71626-11-4	Affect RNA synthesis	95 TC
Metalaxyl	57837-19-1	Affect RNA synthesis	98 TC
Chlorothalonil	1897-45-6	Multi-site activity	98 TC
Cymoxanil	57966-95-7	Unknown	97 TC
Hexaconazole	79983-71-4	Inhibition of sterol synthesis	93 TC
Fosetyl-aluminum	39148-24-8	Unknown	90 TC

*TC: Technical product.

The tested fungicides were dissolved in a small amount of acetone. The concentration of the acetone in the medium was kept minimal in response to the solubility of the tested fungicides. 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 and this level was not significant with regards to toxicity[6]. The toxicity tests were carried out with *A. flos-aquae*, *M. flos-aquae* and *M. aeruginosa* and *S. capricornutun*, *S. quadricauda*, *S. obliquus*, *C. vulgaris* and *C. pyrenoidosa*, which were obtained from the Institute of Wuhan Hydrobiology, the Chinese Academy of Science.

The medium for the green algal and cyanobacterial growth inhibition test was HB-4 and HGZ media, respectively. Which, was adapted for suitability for incubating green algae and cyanobacteria, were composed of distilled water and the chemical ingredients[5]. A total of 20 mL HGZ or HB-4 medium containing cyanobacterial or green algal cells (initial concentration = 0.008) were distributed to sterile 50 mL Erlenmeyer flasks, respectively. A wide range of concentrations were examined in a previous test in order to find the adequate range of toxicity for each fungicide. Following this, adequate double concentration gradients were tested according to the results of the previous tests[7]. The media was then treated with various fungicide concentrations and incubated for 96 h at the same temperature and light intensity. The most suitable wavelength for monitoring culture growth was 680 nm on a Shimadzu UV-2401PC spectrophotometer. Good linear relationships between dry weight concentration (DWC) or chlorophyll-a (Chl-a) content of algal cultures and A 680 nm were found. Three replicates were made for each fungicide concentration and control. Appropriate control systems containing no fungicide were included in each experiment. Control and treated cultures grew under the same conditions as the stock cultures. In each experiment, percentage inhibition values, relative to the growth in the control systems, were calculated by using OD680 data[8]. Statistical analysis was using SPSS version 11.0.

RESULTS AND DISCUSSION

The acute toxicity of six fungicides to the three cyanobacteria (*A. flos-aquae*, *M. flos-aquae* and *M. aeruginosa*) and five green algae (*S. capricornutun*, *S. quadricauda*, *S. obliquus*, *C. vulgaris* and *C. pyrenoidosa*) is shown in Table-2. The 96 h EC_{50}, LOEC and NOEC values of benalaxyl to cyanobacteria and green algae varied as follows: 6.6-17.8, 5.3-53.8, 2-5, 0.5-10, 1-2 and 0.1-5 mg/L, respectively. The 96 h EC_{50}, LOEC and NOEC values of metalaxyl to cyanobacteria and green algae varied as follows: 35.9-941.6, 82.8-367.4, 10-500, 5-50, 5-200 and 2-20 mg/L, respectively. The average acute toxicity of metalaxyl to cyanobacteria and green algae was the lowest among all of the tested six fungicides. However, benalaxyl and metalaxyl have the same chemical group (acylamines) and the same mode/target site of action (affect RNA synthesis). The EC_{50}, LOEC and NOEC values of chlorothalonil varied as follows: 0.09-0.29, 0.001-0.385, 0.002-0.1, 0.0005-0.05, 0.001-0.05 and 0.0002-0.02 mg/L, respectively. The average toxicity of chlorothalonil to algae was the highest; the mode/target site of action of chlorothalonil is a multi-site activity. The EC_{50}, LOEC and NOEC values of cymoxanil to algae varied as follows: 6.9-22.9, 2.0-30.2, 1-2, 0.5-5, 0.5-1 and 0.1-2 mg/L, respectively. The toxicity of cymoxanil was higher than that of benalaxyl. The EC_{50}, LOEC and NOEC values of hexaconazole varied as follows: 0.2-7.2, 0.8-2.9, 0.05-2, 0.2-0.5, 0.02-1 and 0.1-0.2 mg/L, respectively. Its toxicity was higher than that of cymoxanil. The mode/target site of action of cymoxanil is inhibition of sterol synthesis. Fosetyl-aluminum varied as follows: 38.9-121.7, 1.5-31.6, 20-100, 0.2-5, 10-50 and 0.1-2 mg/L, respectively. The toxicity of fosetyl-aluminum was lower than that of benalaxyl. The decreasing order of the average acute toxicity to cyanobacteria and green algae of the six agricultural fungicides was: chlorothalonil > hexaconazole > cymoxanil > benalaxyl > fosetyl-aluminum > metalaxyl.

Wide variations were found in response to the tested fungicides among individual species of eight algae. For benalaxyl, according to the magnitude of EC_{50} values, the decreasing order of sensitivity to cyanobacteria and green algae was *C. vulgaris* > *S. quadricauda* > *M. flos-aquae* > *M. aeruginosa* > *S. capricornutun* > *S. obliquus* > *A. flos-aquae* > *C. pyrenoidosa*. In addition, the sensitivity of various species of algae-*C. vulgaris* and *C. pyrenoidosa*-when exposed to benalaxy varied by one order of magnitude. According to the magnitude of CV, the decreasing order of sensitivity was: *S. capricornutun* > *C. vulgaris*/*S. quadricauda* > *M. aeruginosa*/*M. flos-aquae* > *S. obliquus*, *A. flos-aquae* > *C. pyrenoidosa*. In addition, the sensitivity of various species of algae-between *S. capricornutun* and *A. flos-aquae*/*S. obliquus*/*C. pyrenoidosa*-when exposed to benalaxy, varied by one order of magnitude.

As for metalaxyl, according to EC_{50} values, the decreasing order of sensitivity was: *M. flos-aquae* > *C. vulgaris* > *S. obliquus* > *S. capricornutun* > *S. quadricauda* > *C. pyrenoidosa* > *M. aeruginosa* > *A. flos-aquae*. In addition, the sensitivity between *C. vulgaris* and *A. flos-aquae*/*M. aeruginosa* and between *M. aeruginosa* and *A. flos-aquae*/*M. aeruginosa*/*C. pyrenoidosa*, varied by one order of magnitude. In terms of the CV, it was as follows: *S. quadricauda* > *M. flos-aquae*/*S. capricornutun*/*S. obliquus* > *C. vulgaris*/*C. pyrenoidosa* > *A. flos-aquae* > *M. aeruginosa*. The sensitivity between *S. quadricauda* and *A. flos-aquae* and between *M. flos-aquae* and *S. capricornutun*/*S. obliquus*, varied by one order of magnitude; that between *S. quadricauda* and *M. aeruginosa* varied by two orders of magnitude-that is, the sensitivity of the five green algae is far higher than that of the three cyanobacteria.

As for chlorothalonil, in accordance with EC_{50} values, the decreasing order of sensitivity was: *C. pyrenoidosa* > *S. quadricauda* > *S. obliquus* > *A. flos-aquae* > *M. flos-aquae*/*S. capricornutun* > *M. aeruginosa*/*C. vulgaris*. The sensitivity between *C. pyrenoidosa* and *M. flos-aquae*/*S. capricornutun* varied by one order of magnitude and that between *C. pyrenoidosa* and *M. aeruginosa*/*C. vulgaris* varied by two orders of magnitude. The CV was: *C. pyrenoidosa* > *S. obliquus* > *A. flos-aquae* > *M. flos-aquae*, *C. vulgaris* > *S. quadricauda* > *S. capricornutun* > *M. aeruginosa*. The sensitivity between *C. pyrenoidosa* and *M. flos-aquae*/*C. vulgaris*/*S. quadricauda* varied by one order of magnitude and that between *C.*

TABLE-2
EFFECT OF VARIOUS FUNGICIDES ON EIGHT CYANOBACTERIA AND GREEN ALGAE

Fungicides	Regression equation*	CC	SL	EC_{50}	LOEC	NOEC
Benalaxyl	(1) Y = 3.2472 + 0.2511X	0.9599	0.0401	17.740	5	2
	(2) Y = 2.7287 + 0.1890X	0.9798	0.0001	7.560	2	1
	(3) Y = 3.7766 + 0.2747X	0.9837	0.0025	6.617	2	1
	(4) Y = 4.0207 + 0.3066X	0.9897	0.0103	10.291	0.5	0.1
	(5) Y = 3.1142 + 0.2170X	0.9980	0.0000	5.862	1	0.5
	(6) Y = 3.8606 + 0.2986X	0.9547	0.0030	12.932	5	2
	(7) Y = 3.4426 + 0.2425X	0.9731	0.0000	5.371	1	0.5
	(8) Y = 2.8252 + 0.2365X	0.9899	0.0001	53.721	10	5
Metalaxyl	(1) Y = 2.2955 + 0.2577X	0.9933	0.0067	941.509	200	100
	(2) Y = 2.2658 + 0.2526X	0.9924	0.0076	919.858	500	200
	(3) Y = 2.5318 + 0.1985X	0.9869	0.0003	35.927	10	5
	(4) Y = 2.1647 + 0.1850X	0.9771	0.0008	123.872	10	5
	(5) Y = 1.5859 + 0.1245X	0.9963	0.0000	162.531	5	2
	(6) Y = 1.9253 + 0.15557X	0.9948	0.0000	104.989	10	5
	(7) Y = 2.0663 + 0.1917X	0.9338	0.0020	82.854	50	20
	(8) Y = 2.8042 + 0.2913X	0.9829	0.0000	367.332	50	20
Chlorothalonil	(1) Y = 4.2138 + 0.2296X	0.9920	0.0001	0.094	0.002	0.001
	(2) Y = 6.8560 + 0.4221X	0.9874	0.0130	0.289	0.1	0.05
	(3) Y = 5.5479 + 0.3204X	0.9752	0.0005	0.144	0.005	0.002
	(4) Y = 3.6795 + 0.2021X	0.9940	0.0000	0.147	0.05	0.02
	(5) Y = 4.5512 + 0.2423X	0.9838	0.0025	0.055	0.01	0.005
	(6) Y = 3.1340 + 0.1598X	0.9788	0.0000	0.069	0.001	0.0005
	(7) Y = 2.8263 + 0.1575X	0.9859	0.0000	0.385	0.005	0.002
	(8) Y = 2.3709 + 0.0933X	0.9772	0.0230	0.002	0.0005	0.0002
Cymoxanil	(1) Y = 3.0359 + 0.2373X	0.9844	0.0000	22.852	1	0.5
	(2) Y = 2.6276 + 0.1792X	0.9899	0.0001	6.978	1	0.5
	(3) Y = 2.8050 + 0.1942X	0.9731	0.0001	7.003	2	1
	(4) Y = 2.5527 + 0.1565X	0.9666	0.0001	2.018	0.5	0.2
	(5) Y = 3.2486 + 0.2125X	0.9933	0.0007	2.410	0.2	0.1
	(6) Y = 2.2392 + 0.1671X	0.9649	0.0000	30.186	5	2
	(7) Y = 2.5917 + 0.1677X	0.9849	0.0000	3.829	0.5	0.2
	(8) Y = 2.5132 + 0.1954X	0.9503	0.0500	33.533	5	2
Hexaconazole	(1) Y = 5.2733 + 0.4031X	0.9970	0.0003	7.200	2	1
	(2) Y = 1.7430 + 0.0807X	0.9560	0.0110	0.204	0.05	0.02
	(3) Y = 3.9896 + 0.2811X	0.9945	0.0000	4.061	1	0.5
	(4) Y = 3.0123 + 0.1903X	0.9723	0.0001	1.850	0.5	0.2
	(5) Y = 3.0591 + 0.1833X	0.9815	0.0000	0.866	0.2	0.1
	(6) Y = 2.5095 + 0.1547X	0.9566	0.0000	2.284	0.5	0.2
	(7) Y = 2.6770 + 0.1707X	0.9823	0.0000	2.893	0.5	0.2
	(8) Y = 4.7433 + 0.3077X	0.9915	0.0010	1.025	0.2	0.1
Fosetyl-aluminum	(1) Y = 3.5752 + 0.3120X	0.9975	0.0020	52.4102	20	10
	(2) Y = 3.5168 + 0.2971X	0.9889	0.0110	38.9134	20	10
	(3) Y = 7.0887 + 0.7309X	0.9894	0.0106	121.6131	100	50
	(4) Y = 3.0630 + 0.2000X	0.9836	0.0004	2.7158	0.5	0.2
	(5) Y = 2.3467 + 0.1439X	0.9840	0.0000	2.6668	0.2	0.1
	(6) Y = 2.2547 + 0.1537X	0.9737	0.0000	11.0333	1	0.5
	(7) Y = 2.7361 + 0.1669X	0.9854	0.0000	1.5184	0.2	0.1
	(8) Y = 2.7045 + 0.2127X	0.9586	0.0100	31.5366	5	2

[a]Y, X, CC and SL stand for per cent inhibition, natural logarithm of concentration, coefficient correlation significance level, respectively; (1) *A. flos-aquae*, (2) *M. Aeruginosa*, (3) *M. flos-aquae*, (4) *S. Capricornutun*, (5) *S. Quadricauda*, (6) *S. Obliquus*, (7) *C. Vulgaris*, (8) *C. Pyrenoidosa*.

pyrenoidosa and *M. aeruginosa*/*S. capricornutun* varied by two orders of magnitude.

With respect to cymoxanil, according to EC_{50}, the decreasing order was *S. capricornutun* > *S. quadricauda* > *C. vulgaris* > *M. flos-aquae*/*M. aeruginosa* > *A. flos-aquae*/*S. obliquus*/*C. pyrenoidosa*. The sensitivity between *S. capricornutun* and *A. flos-aquae*/*S. obliquus*/*C. pyrenoidosa* and between *S. quadricauda* and *S. obliquus*/*C. pyrenoidosa*, varied by one order of magnitude. In terms of conformity to CV, this was: *S. quadricauda* > *S. capricornutun*, *C. vulgaris* > *A. flos-aquae*/*M. aeruginosa* > *M. flos-aquae* > *S. obliquus*/*C. pyrenoidosa*. The sensitivity between *S. quadricauda* and *M. flos-aquae*/*S. obliquus*/*C. pyrenoidosa* and between *S. capricornutun*/*C. vulgaris* and *S. obliquus*/*C. pyrenoidosa*, varied by one order of magnitude.

With regards to hexaconazole, according as EC_{50}, the decreasing order was: *M. aeruginosa* > *S. quadricauda* > *C. pyrenoidosa* > *S. capricornutun* > *S. obliquus* > *C. vulgaris* > *M. flos-aquae* > *A. flos-aquae*. The sensitivity between *M. aeruginosa* and *A. flos-aquae*/*M. flos-aquae*/*S. obliquus*/*C. vulgaris* varied by one order of magnitude. In accordance with CV, this was: *M. aeruginosa* > *S. quadricauda*/*C. pyrenoidosa* > *S. capricornutun*/*S. obliquus*/*C. vulgaris* > *M. flos-aquae* > *A. flos-aquae*. The sensitivity between *M. aeruginosa* and *S.*

capricornutun/*S. obliquus*/*C. vulgaris*/*M. flos-aquae*/*A. flos-aquae* and between *A. flos-aquae* and *S. quadricauda*/*C. pyrenoidosa* varied by one order of magnitude.

As far as fosetyl-aluminum was concerned, in terms of EC_{50}, the order was: *C. vulgaris* > *S. quadricauda*/*S. capricornutun* > *S. obliquus* > *C. pyrenoidosa* > *A. flos-aquae* > *M. flos-aquae*. The sensitivity between *C. vulgaris*/*S. capricornutun*/*S. quadricauda* and *C. pyrenoidosa*/*M. aeruginosa*/*A. flos-aquae*/*M. flos-aquae* varied by one order of magnitude. According to CV, this was: *C. vulgaris*/*S. quadricauda* > *S. capricornutun* > *S. obliquus* > *C. pyrenoidosa* > *A. flos-aquae*/*M. aeruginosa* > *M. flos-aquae*. The sensitivity between *C. pyrenoidosa* and *S. quadricauda*/*C. vulgaris*/*M. flos-aquae* varied by one order of magnitude; that between *S. quadricauda*/*C. vulgaris* and *A. flos-aquae*/*M. aeruginosa*/*M. flos-aquae* and between *S. capricornutun* and *M. flos-aquae*, varied by two orders of magnitude-that is, the sensitivity of five green algae was far higher than that of three cyanobacteria.

A simple formula (Table-2) showed that the toxicity of fungicides with multi-site activity was higher than that with single-site activity; for acute toxicity, fungicides with affected physiological activity had a higher toxicity than those with affected hereditory matter. For chronic toxicity, this may be opposite in direction; as to the sensitivity of various species of algae, fungicides with multi-site activity may have a higher sensiticity than those with single-site activity in terms of ecosystem risk. However, the mechanisms for toxicity and sensitivity are much more complicated. The simple formula may be premature at this stage and remains to be further studied and proved by more experimental data.

Conclusion

The tested five green algae were recommended for toxicity tests as ecological indicators by many countries' EPA due to their high sensitivity. In this study, six fungicides were tested to examine their effects on eight cyanobacteria and green algae. The results showed that the decreasing order of toxicity to eight algae of six fungicides was: chlorothalonil > hexaconazole > cymoxanil > benalaxyl > fosetyl-aluminum > metalaxyl. There was more than one order of magnitude difference in the toxicity of fungicides that have a similar (or same) chemical group and the same mode/target site of action as the pest control mechanism, such as benalaxyl (EC_{50} range, 5-54 mg/L) and metalaxyl (EC_{50} range, 35-942 mg/L). Wide variations occurred in response to the tested fungicides between the eight individual species, in terms of the sensitivity of various species of algae: when they were exposed to benalaxyl, cymoxanil and hexaconazole, they varied by one order of magnitude and when exposed to metalaxyl and chlorothalonil, they varied by two orders of magnitude. Cyanobacteria were less sensitive to the fungicides-metalaxyl, chlorothalonil, cymoxanil and fosetyl-aluminum-than to green algae. Those fungicides may result in a shift from dominance by green algae to dominance by cyanobacteria. According to probability the decreasing order of ecosystem risk was fosetyl-aluminum > chlorothalonil > metalaxyl > cymoxanil > hexaconazole > benalaxyl. There was a strong variance between toxicity and ecosystem risk. In addition, the sensitivity of various algal species, when exposed to fungicides with the same chemical group and mode/target site of action, varied to a large extent-for example, benalaxyl and metalaxyl (max/min EC_{50} ratio ≈10 and 30).

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