J Appl Phycol (2010) 22:803–811
DOI 10.1007/s10811-010-9522-x

Study on the ecological safety of algacides: a comprehensive strategy for their screening

Lihong Zhou · Xuehao Chen · Tianling Zheng

Received: 19 October 2009 / Revised and accepted: 9 March 2010 / Published online: 30 March 2010

Abstract The ecological safety of 14 algacidal materials were appraised using algal growth inhibition tests as well as toxicity test for zooplankton and fish and prawn juveniles. In addition, an integrative analysis for evaluating their potential as algacides was made by comparing the 24-h LC_{50} and ratio of efficiency to safety (RES) of each material. The results showed that the growth of algae tested were not affected after 96 h of exposure to diallyl trisulfide even when the concentration was 10 mg L^{-1}; there was a similar toxicity between garlic solution and diallyl trisulfide on seven species of aquatic organisms tested. Moreover, both prawn juveniles had lower resistances to garlic solution and diallyl trisulfide than the other 12 materials. According to the RES, green tea, garlic solution, diallyl trisulfide, and the Chinese medical herbs Cortex Fraxini, Herba Houttuyniae, Semen Arecae, and Rhizoma Coptidis are better algacides than the other materials tested in this study.

Keywords Algacide · Ecological safety · Ratio of efficiency to safety · Aquatic organism · Toxicity

L. Zhou · X. Chen
Fisheries College, Institute of Aquaculture Biotechnology, Jimei University,
Xiamen 361021, China

T. Zheng (✉)
MOE of Key Laboratory for Coast and Wetland Ecosystem Research, School of Life Sciences, State Key Laboratory of Marine Environmental Science, Xiamen University,
Xiamen 361005, China
e-mail: wshwzh@xmu.edu.cn

Introduction

Algacides are considered to be emergency agents for algal bloom control, and many researchers have been trying to find effective algacides (Elder and Horne 1978; Murray-Gulde et al. 2002; Hehmann et al. 2002; Lovejoy et al. 1998; Ridge et al. 1999; Brownlee et al. 2003; Furusawa et al. 2003; Alamsjah et al. 2005; Zhou et al. 2007, 2008). In general, the inhibitory efficiency of algacides should be considered first when screening materials as potent algacides. However, the adverse effects of algacides to natural or aquacultural waters may hinder their application; thus it is also very important to study the toxicity of algacides to aquatic organisms. Recently, the toxicity of algacides to aquatic organisms has received attention, and often, an aquatic risk assessment is carried out (Klapes 1990; Boyd and Massaut 1999; Boylan and Morris 2003; Balcomb et al. 2002; Straus and Tucker 2007). Compared to a toxicity test with individual species, tests with a wide range of fish, algae, and invertebrates may evaluate the impact of algacides more comprehensively. In addition, the cost for algal bloom control should be considered. Up to now, potential algacides have mostly been selected using algal growth inhibition tests for harmful algae and toxicity tests for non-target species (Sabine and Andreas 2002; Murray-Gulde et al. 2002; Brownlee et al. 2003; Mulderij et al. 2003; Jin and Dong 2003; Ferrier et al. 2005; Jancula et al. 2007, 2008; Schrader et al. 2007). Obviously, it is not enough to evaluate algacidal materials using only a single factor, especially when they have different advantages such as high effect, low toxicity, or low cost. However, no model has been reported to screen algacides using integrated parameters. This study set out to find such parameters for algacide screening and may provide an all-around strategy in the research into algal bloom control.

Material and methods

Algacides

Eleven types of Chinese medicinal herbs, Rhizoma Coptidis (dry root and stem of *Coptis chinensis* Franch., *Coptis deltoidea* C. Y. Cheng et Hsiao, or *Coptis teeta* Wall.), Semen Arecae (dry ripe nut of *Areca catechu* Linn.), Radix Isatidis (dry root of *Isatis indigotica* Fort.), Radix Sophorae Flavescentis (dry root of *Sophora flavescens* Ait.), Herba Houttuyniae (dry *Houttuynia cordata* Thunb.), Radix et Rhizoma Rhei (dry root and stems of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf., or *Rheum officinale* Baill.), Semen Torreyae (dry ripe seed of *Torreya grandis* Fort.), Fructus Carpesii (dry ripe fruit of *Carpesium abrotanoides* L.), Cortex Fraxini (dry cortices of *Fraxinus rhynchophylla* Hance, *Fraxinus chinensis* Roxb., *Fraxinus szaboana* Lingelsh., or *Fraxinus stylosa* Lingelsh.), Folium Isatidis (dry leaf of *I. indigotica* Fort.), Fructus Mume(dry ripe fruit of plant *Prunus mume* (Sieb.) Sieb. et Zucc.), and one type of green tea were used. The preparations of garlic solution and diallyl trisulfide were as described by Zhou et al. (2008).

Test organisms and culture conditions

A total of ten species of aquatic organisms were used. Three species of algae, *Chlorella* sp. (green alga), *Isochrysis galbana* (chrysophyte), and *Chaetoceros muelleri* (diatom) used for the test of algal growth inhibition, were obtained from the Fisheries College of Jimei University. They were cultivated at 20±0.5°C in a 12:12-h day/night cycle at a light intensity of 52 μmol photons m^{-2} s^{-1}, in F/2 medium (Guillard and Ryther 1962) prepared with sterile filtered seawater. Three species of zooplankton, two species of prawn juveniles, and two fish juveniles were obtained from the Fish and Prawn Hatchery in Xiamen and reared for 1–2 days in the laboratory at 28±0.5°C for the zooplankton *Brachionus plicatilis* and *Moina mongolica daday*, 25±0.5°C for *Artemia parthenogenetica* (hatched 5 days) and 20±0.5°C for the juvenile prawns *Litopenaeus vannamei* (body length 4.08±0.23 mm) and *Penaeus monodon* (body length 6.35±0.53 mm) and for the juvenile fish *Pagrosomus major* (body length 5.09±0.18 cm) and *Plectorhinchus cinctus* (body length 3.83±0.16 cm). To maintain uniformity of age, only hatched larvae after a 1-day interval for *B. plicatilis* and 5 days for *A. parthenogenetica* were used in this study. The test organisms were fed with *Chlorella* sp. for the zooplankton and with artificial diets for juvenile prawn and fish during temporary rearing, but all of them were not fed during the experiments. The salinity of natural filtered seawater was 21 psu except in the case of *L. vannamei* where seawater of lower salinity (12 psu) was used.

Acute toxicity tests

The classic algal bottle test was used in the algal growth inhibitory test, and the optical density of the algae was measured after 1, 2, and 3 days. The wavelengths used were 440 nm for *Chlorella* sp. and 300 nm for *I. galbana* and *C. muelleri*. The relationships between the cell density and the optical density of the algal cultures obtained before the experiment were $Y = 327.74 \times \mathrm{OD} \times 10^5$, R^2=0.9822 for *Chlorella* sp., $Y = 296.72 \times \mathrm{OD} \times 10^4$, R^2=0.9599 for *I. galbana*, and $Y = 39.66 \times \mathrm{OD} \times 10^5$, R^2=0.9951 for *C. muelleri.* Algae were cultured in different concentrations of diallyl trisulfide (0–10.0 mg L^{-1}), and triplicate treatments were cultivated under the same conditions as described above.

For testing the acute toxicity of algacides on the zooplankton, a bioassay was developed utilizing cell culture plates: 24-well, 12-well, and six-well plates for *B. plicatilis*, *M. mongolica daday*, and *A. parthenogenetica*, respectively. Each well contained ten *B. plicatilis* or *M. mongolica daday* or 20 *A. parthenogenetica*. Acute toxicity tests were conducted with six or more algacidal concentrations (having conducted range finding tests for all species prior to the formal tests). These were 0.02–1.28% (*w*/*v*) garlic solution (concentration of 1.28% not tested for *B. plicatilis*) and 1–25 mg L^{-1} diallyl trisulfide (concentration of 25 mg L^{-1} not tested for *A. parthenogenetica*). Controls were conducted in natural filtered seawater without algacide. Both controls and the tests were carried out in triplicate. Culture conditions were the same as described above. Mortality was recorded over 1 day.

For acute toxicity testing on the prawns, ten juveniles of *L. vannamei* were randomly selected and introduced into a 100-mL glass beaker containing 50 mL of filtered seawater without aeration and feeding. The nominal concentrations for the prawn tests were 0.02–0.64% (*w*/*v*) garlic solution, 1–10 mg L^{-1} diallyl trisulfide, 0.32–5.60 g L^{-1} green tea, 0.1 g L^{-1} Rhizoma Coptidis, and 0.63–6.30 g L^{-1} Semen Arecae. Controls were carried out in natural filtered seawater without any algacide during the same period. The exposure time was 1 day with three replicates for each concentration tested and for the control. Mortality was recorded every 24 h. The procedure followed for the juveniles of *P. monodon* was almost the same as that for *L. vannamei*, but at higher algacide concentrations. The nominal concentrations of algacidal materials were 0.02–0.64% (*w*/*v*) garlic solution, 0.25–10 mg L^{-1} diallyl trisulfide, 0.18–5.60 g L^{-1} green tea, 0.1 g L^{-1} Rhizoma Coptidis, 0.25–6.30 g L^{-1} Semen Arecae, 0.40–2.50 g L^{-1} Herba Houttuyniae, and 0.63–6.30 g L^{-1} Cortex Fraxini. The concentrations of both Radix Isatidis and Radix Sophorae Flavescentis were 0.063–0.630 g L^{-1}. Those of both Semen Torreyae and Fructus Carpesii were 0.10–

0.63 g L^{-1}, and those of Radix et Rhizoma Rhei, Fructus Mume, and Folium Isatidis were 0.063–0.400 g L^{-1}.

For acute toxicity testing on juveniles of *P. major* and *P. cinctus*, glass tanks (35-cm diameter, 30-cm height) were filled with 3 L of natural filtered seawater, and ten juvenile fish were used in each. These tests were with aeration but without feeding. The nominal concentrations of algacidal materials for these two fish species were 0.04–0.64% (*w*/*v*) garlic solution and 1–25 mg L^{-1} diallyl trisulfide (concentration of 25 mg L^{-1} not tested for *P. cinctus*). A group without algacide was used as a control. Both the control and the tests were in triplicate and mortality was recorded after 24 h.

Data analysis

The 24-h LC_{50} values were calculated to evaluate the toxicities of the various algacidal materials. The LC_{50} values and their 95% confidence limits for 24-h intervals were estimated by linear regression: $y=a+b\ x$ where x is the logarithm of the concentration, y the probit unit of mortality, a the intercept, and b the slope. All the correlation coefficients of the regression equation were tested for significance using a t test. A value of $P<0.05$ indicates a relationship and $P<0.01$ a significant relationship.

Results

Effects of algacidal materials on benign algae

For the cell densities of *Chlorella* sp., *I. galbana*, and *C. muelleri*, there was no significant difference between treatment and control groups ($P>0.05$), and no significant difference between different concentrations of diallyl trisulfide ($P>0.05$, Fig. 1).

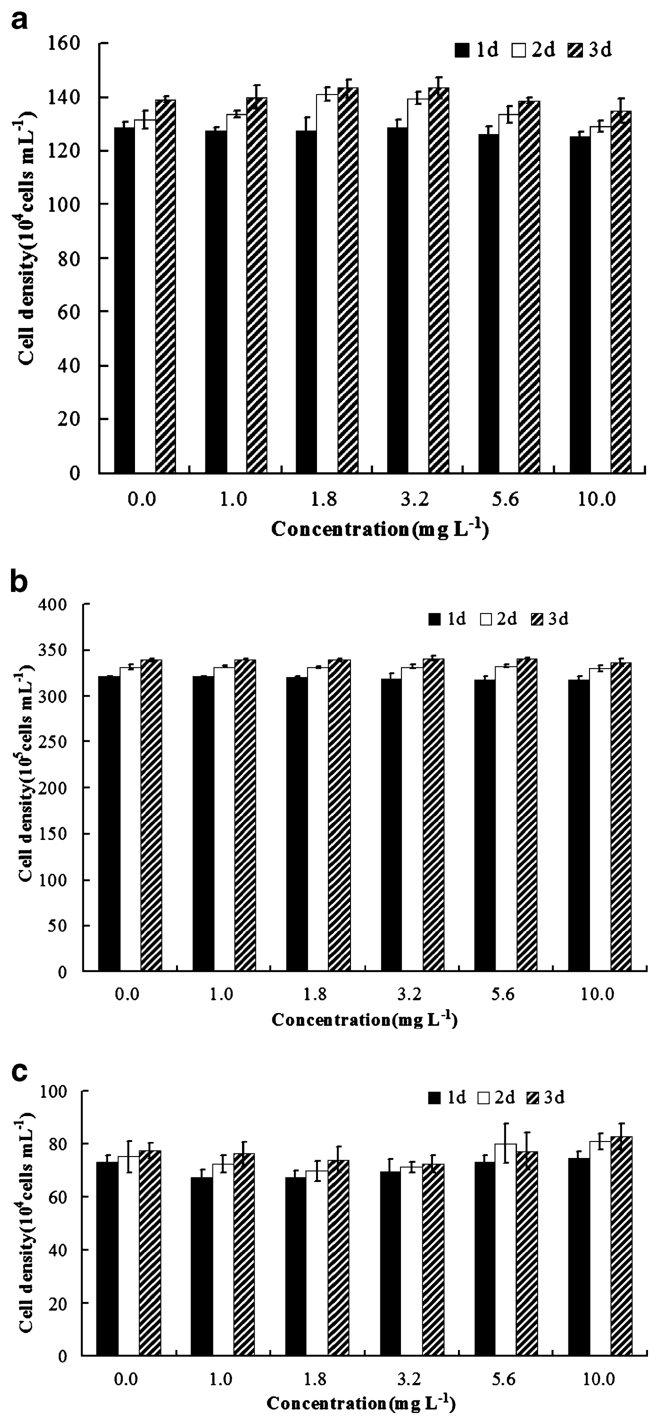

Fig. 1 Effects of diallyl trisulfide on algal cell densities. **a** *C. muelleri*, **b** *Chlorella* sp., and **c** *Isochrystis galbana*

Acute toxicity of algacidal materials on aquatic organisms

The major test results are summarized in Table 1 and Figs. 2, 3, 4, 5, and 6. Both garlic solution and diallyl trisulfide of certain concentrations were harmful to the zooplankton and the juvenile prawns and fish tested. In general, the mortality increased significantly with increasing concentration of garlic solution or diallyl trisulfide. As for *B. plicatilis*, the survival rate for 24 h was 100% when the concentration of garlic solution was 0.02% or lower and that of diallyl trisulfide 1 mg L^{-1}. At 0.08%, 0.16%, 0.32%, and 0.64% of garlic solution and 2.5, 4.0, 6.30, 10.0, 16.0, and 25.0 mg L^{-1} of diallyl trisulfide, the mortality showed significant differences when compared with the control group ($P<0.01$, Figs. 2 and 3). As for *A. parthenogenetica,* 1.28% garlic solution and 16 mg L^{-1} diallyl trisulfide were lethal with all individuals in 24 h (Figs. 2 and 3), and the 24-h LC_{50} values were 0.11% garlic solution and 5.06 mg L^{-1} diallyl trisulfide (Table 1). Similarly, 100% mortality of *M. mongolica daday* was observed in 1.28% garlic solution, but the lethal concentration of diallyl trisulfide was higher than that for *A. parthenogenetica*,

Table 1 Single species acute toxicity values with various algacidal materials

Algacidal material	Test species	24h LC_{50}	95% confidence intervals
Garlic solution	*B. plicatilis*	0.17%	0.11–0.25%
	A. parthenogenetica	0.11%	0.10–0.13%
	M. mongolica daday	0.33%	0.23–0.46%
	L. vannamei	0.07%	0.06–0.08%
	P. monodon	0.08%	0.08–0.09%
	P. major	>0.32%[a]	cnc[a]
	P. cinctus	0.26%	0.18–0.39%
Diallyl trisulfide	*B. plicatilis*	10.69 mg L^{-1}	6.59–17.33 mg L^{-1}
	A. parthenogenetica	5.06 mg L^{-1}	4.53–5.64 mg L^{-1}
	M. mongolica daday	10.77 mg L^{-1}	8.45–13.73 mg L^{-1}
	L. vannamei	1.38 mg L^{-1}	1.21–1.57 mg L^{-1}
	P. monodon	2.41 mg L^{-1}	2.15–2.70 mg L^{-1}
	P. major	12.28 mg L^{-1}	10.42–14.46 mg L^{-1}
	P. cinctus	7.31 mg L^{-1}	6.36–8.40 mg L^{-1}
Green tea	*L. vannamei*	3.66 g L^{-1}	3.18–4.21 g L^{-1}
	P. monodon	1.54 g L^{-1}	1.35–1.76 g L^{-1}
Rhizoma Coptidis	*L. vannamei*	1.07 g L^{-1}	0.81–1.42 g L^{-1}
	P. monodon	1.60 g L^{-1}	1.28–1.99 g L^{-1}
Semen Arecae	*L. vannamei*	1.27 g L^{-1}	1.17–1.38 g L^{-1}
	P. monodon	2.01 g L^{-1}	1.79–2.26 g L^{-1}
Radix Isatidis	*P. monodon*	0.31 g L^{-1}	0.28–0.33 g L^{-1}
Herba Houttuyniae	*P. monodon*	4.24 g L^{-1}	3.47–5.19 g L^{-1}
Radix Sophorae Flavescentis	*P. monodon*	0.20 g L^{-1}	0.18–0.22 g L^{-1}
Radix et Rhizoma Rhei	*P. monodon*	0.15 g L^{-1}	0.13–0.16 g L^{-1}
Fructus Mume	*P. monodon*	0.26 g L^{-1}	0.24–0.27 g L^{-1}
Semen Torreyae	*P. monodon*	0.73 g L^{-1}	0.64–0.83 g L^{-1}
Cortex Fraxini	*P. monodon*	6.63 g L^{-1}	5.35–8.21 g L^{-1}
Fructus Carpesii	*P. monodon*	0.76 g L^{-1}	0.66–0.87 g L^{-1}
Folium Isatidis	*P. monodon*	>0.40 g L^{-1}[a]	cnc[a]

[a] *cnc* could not calculate 24 h LC_{50} and confidence intervals, value approximate

being 25 mg L^{-1}. However, the mortality of *M. mongolica daday* increased more slowly than that of *A. parthenogenetica* with increasing concentration of both algacides (Figs. 2 and 3).

In 0.64% garlic solution, *L. vannamei* juveniles soon could not move, and the mortality was up to 96.7% two hours later. In the groups at higher concentrations (≥0.16%), all the juveniles died within 5 h. The mortality increased with increasing concentration of garlic solution

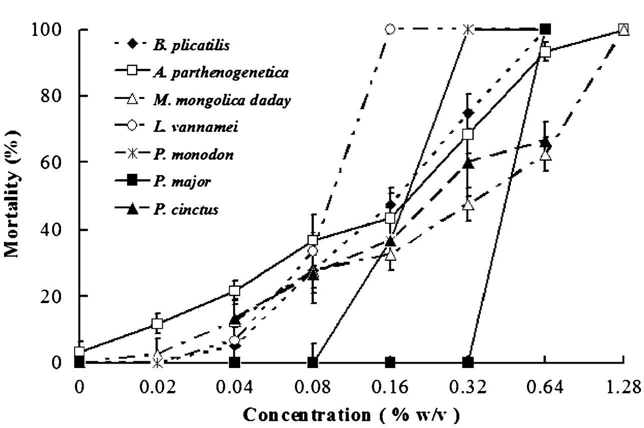

Fig. 2 Mortalities of various aquatic organisms treated with different concentrations of garlic solution for 24 h

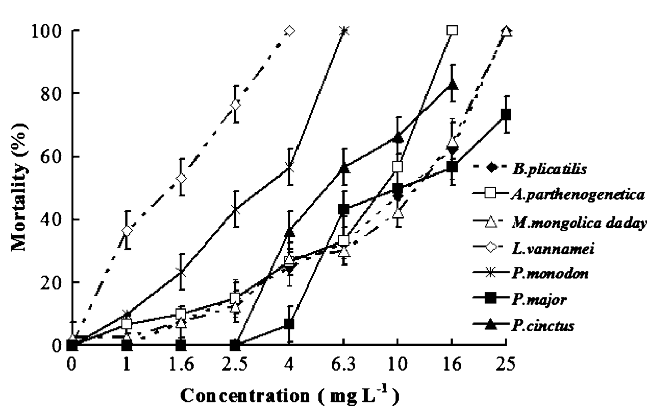

Fig. 3 Mortalities of various aquatic organisms treated with different concentrations of diallyl trisulfide for 24 h

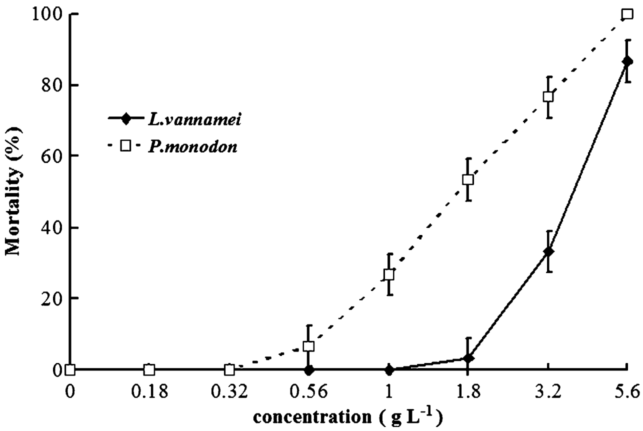

Fig. 4 Mortalities of *L. vannamei* and *P. monodan* treated with different concentrations of green tea for 24 h

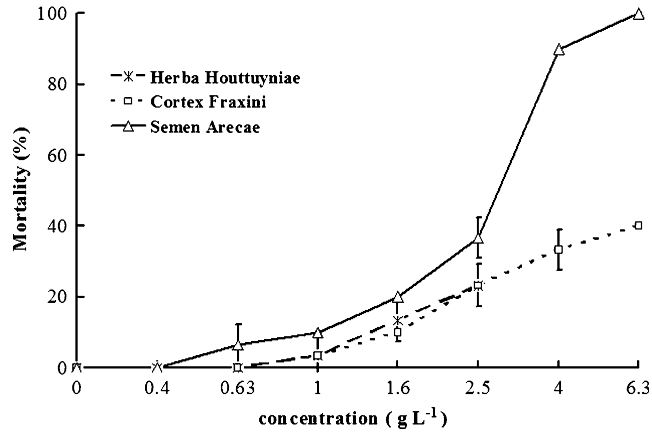

Fig. 6 Mortalities of *P. monodon* treated with three species of Chinese herbs for 24 h

and extended experimental time. When the tested material was diallyl trisulfide, the results were similar to the above, and at 10 mg L^{-1}, about half the juveniles (46.67%) died in 2 h, and all the juveniles in treatment concentrations above 4 mg L^{-1} died within 24 h (Figs. 2 and 3).

During the experiments, 0.02% garlic solution did not harm *P. monodon* juveniles, and the activity of the tested prawns was the same as the controls. The toxicity to the animals increased significantly as the concentration of garlic solution went above 0.08%, and after 2 h, only about 47% of the juveniles were alive in the 0.64% concentration, while the live prawns moved only a little with their trembling pereiopods in the beakers. At the same time, the prawns in all concentrations of diallyl trisulfide moved normally, except for a small number in 10 mg L^{-1} diallyl trisulfide. Within the range 6.3–10.0 mg L^{-1} diallyl trisulfide, the mortality over 24 h was 100% (Figs. 2 and 3).

The most important and visible effect of garlic solution and diallyl trisulfide on the two species of juvenile fish was hyperactivity. This happened immediately after they were placed in certain concentrations. In the aquaria, *P. major*

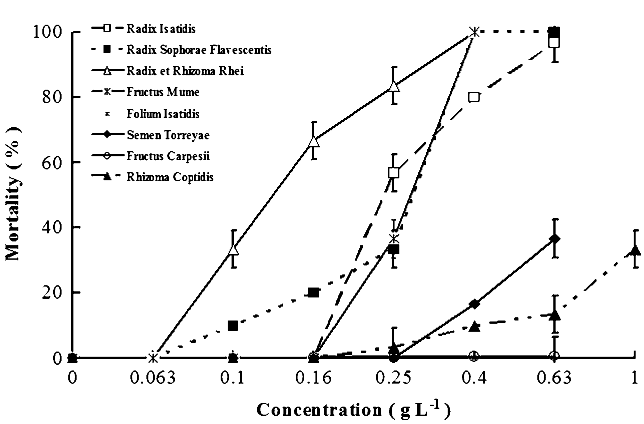

Fig. 5 Mortality of *P. monodon* treated with different concentrations of various Chinese herbs for 24 h

juveniles massed at the water surface in 0.16% garlic solution. With increasing concentration, more and more fish massed at the surface and started to jump fiercely into the air. In the highest concentration (0.64% garlic solution), the mortality was 16.7% within 2 h, and all the fish died within 4 h. For the lower concentrations (0.02–0.08% garlic solution), the fish were as peaceful as the control during the 2 h. In the 0.16% and 0.32% treatments, the water became turbid and the fish swam slowly at the bottom of the aquaria. All fish, except those in the 0.64% garlic solution, survived after 24 h. The *P. major* juveniles became restless and swam fast in the water as soon as they were placed in the diallyl trisulfide solution, but they became peaceful 10 min later. However, within the range 6.3–25.0 mg L^{-1} diallyl trisulfide, the fish jumped all the time. In 25.0 mg L^{-1} diallyl trisulfide, mortality was 13.3% in 1 h and half the fish died within 15 h, with the mortality increasing sharply with extended time. Lower concentrations of diallyl trisulfide (≤2.5 mg L^{-1}) did not cause death of *P. major* juveniles or abnormal activities (Figs. 2 and 3).

The results for *P. cinctus* were similar to those for *P. major*: the fish swam more and also faster in the 0.32% and 0.64% garlic solutions than those in the control group, but 10 min later, they became tranquil and moved to the bottom of the aquaria. Within the range 6.3–16.0 mg L^{-1} diallyl trisulfide, the fish jumped fiercely at the water surface during the first 30 min. However, the fish in the other treatments (1.0–4.0 mg L^{-1} diallyl trisulfide) were as peaceful as the control.

The 24-h LC_{50} values for garlic solution ranged from 0.07 (*L. vannamei*) to 0.33% (*M. mongolica daday*; Table 1). Within taxa, acute toxicity values ranged from 0.11% to 0.33% for the zooplankton species, 0.07% to 0.08% for prawn juveniles, and 0.26% to >0.32% for fish juveniles (Table 1). The 24-h LC_{50} values for diallyl trisulfide ranged from 1.38 (*L. vannamei*) to 12.28 (*P. major*) mg L^{-1} (Table 1). Within taxa, acute toxicity values ranged from 5.06 to 10.77 mg L^{-1} diallyl trisulfide for the zooplankton species, 1.38 to 2.41 mg L^{-1} diallyl trisulfide for prawn juveniles, and 7.31 to 12.28 mg L^{-1} diallyl trisulfide for fish juveniles (Table 1).

In the experiments, there was no mortality in the control groups, except 3.33% in *A. parthenogenetica* in the garlic experiment and 2.5% for *M. mongolica daday* in the diallyl trisulfide experiment.

Comparison between the control and green tea treatments revealed comparability in prawn activity during the first 5 h. Thereafter, mortality increased with higher concentrations, while the prawns in the 0.32, 0.56, and 1.0 g L^{-1} green tea groups had a similar activity level, but were less mobile than those in the control. The mortality of *L. vannamei* was lower than that of *P. monodon* at the same treatment concentrations (Fig. 4), and the 24-h LC_{50} values were 3.66 and 1.54 g L^{-1} for *L. vannamei* and *P. monodon* juveniles, respectively (Table 1). The results for Rhizoma Coptidis were similar to green tea, within the range 0.10–1 g L^{-1}, and the survival rate for 5 h was 100% in the two prawn species. Similar mobility was observed among prawns of the treatment groups and the control. However, the mortality increased with extended time and increasing concentrations (Fig. 5). There was no prawn mortality in the different concentrations of Semen Arecae in the first 5 h. In addition, no difference in the mobility of the prawns was observed between treatment and the control, except prawns in 6.30 g L^{-1} Semen Arecae which were much less mobile. However, high mortality appeared at higher concentrations (2.5–6.3 g L^{-1} for *L. vannamei* and 4.0–6.3 g L^{-1} for *P. monodon*) over 24 h and ranged from 90% to 100% (Table 2 and Fig. 6).

The toxicity effect on *P. monodon* juveniles differed for the different algacides. Among these (except diallyl trisulfide), Radix et Rhizoma Rhei was the most toxic for *P. monodon*, with a minimum 24-h LC_{50} of 0.15 g L^{-1} and a maximum 24-h LC_{50} was 6.63 g L^{-1} with Cortex Fraxini (Table 1). One hundred percent mortality was observed after 24 h in 0.4 g L^{-1} Radix et Rhizoma Rhei or Fructus Mume (Fig. 5), whereas all prawns survived 0.63 g L^{-1} Cortex Fraxini (Fig. 6). The other algacides having higher toxicity for *P. monodon* juveniles were Radix Isatidis, Fructus Mume, Radix Sophorae Flavescentis, Semen Torreyae, and Fructus Carpesii, with low 24-h LC_{50} values (0.26–0.76 g L^{-1}; Table 1). The survival rate was 100% in 24 h in the experiment with different concentrations of Folium Isatidis (Fig. 5).

Table 2 Mortality of *L. vannamei* in different concentrations of Rhizoma Coptidis and Semen Arecae for 24 h

Concentrations (g L^{-1})	Mortality (%)	
	Rhizoma Coptidis	Semen Arecae
Control	0±0	0±0
0.10	3.33±5.77	
0.16	10.00±0	
0.25	13.33±5.77	
0.40	26.67±5.77	
0.63	33.33±5.77	3.33±5.77
1.0	46.67±5.77	20.00±0
1.6		56.67±5.77
2.5		100±0

Values expressed as mean ± standard error of the mean

Discussion

Algacides are worthy of further investigation as an important algal bloom-controlling measure. The principles for developing an algacide should be high effectiveness, low toxicity, economic feasibility, and convenience. Toxicity is one of the key factors that influence the use of an algacide. Thus, special attention should be paid to their ecological safety (Elisabeth et al. 1996). The algacidal materials referred to in this paper have been tested in the laboratory and shown to be effective for algal inhibition (Zhou et al. 2007, 2008), and many of them have been applied extensively in medicine (such as diallyl trisulfide and the Chinese herbs), aquaculture, stock raising, as well as for human food (such as garlic and green tea) because of their antibacterial, antiviral, and nutritional functions. Toxicity studies on these materials have focused mostly on humans and other mammals, and few reports are available related to fish. Considering cost and dilution by currents, we think that it is more reasonable to apply algacides in aquaculture water than in the open sea. Therefore, the toxicities of algacidal materials on aquatic organisms, particularly those common in aquaculture water, should be studied first to evaluate any potential negative effects. In addition, it is also very important to select algacides which are cost-effective, user-friendly, and environmentally sound even in large quantities.

The results showed that the toxic effects of an algacidal material differed with the species of aquatic organisms involved. Thus, it is necessary to use more species in evaluating the effect of algacides on aquatic ecosystems. Algae are the primary producers and play a very important role in ecosystem balance. The three species of algae tested are commonly distributed in the sea and belong to the Chlorophyta (*Chlorella* sp.), Bacillariophyta (*C. muelleri*), and Chrysophyta (*I. galbana*). The results indicated that there was no significant effect on the growth of these algae at 10 mg L^{-1} diallyl trisulfide after 96 h, nor was there any significant difference between different concentrations.

Table 3 RES of garlic solution and diallyl trisulfide for various aquatic organisms

Species	RES	
	Garlic solution	Diallyl trisulfide
M. mongolica daday	0.143 (1)	0.108 (2)
P. major	<0.147 (2)	0.095 (1)
P. cinctus	0.181 (3)	0.159 (4)
B. plicatilis	0.285 (4)	0.109 (3)
A. parthenogenetica	0.428 (5)	0.230 (5)
P. monodon	0.567 (6)	0.483 (6)
L. vannamei	0.672 (7)	0.844 (7)
Mean	0.346	0.290

The number in parenthesis is the rank order of RES

According to Zhou et al. (2008), 0.32% garlic solution does not influence the growth of *C. muelleri*. Thus, it can be seen that these benign algae could still grow normally even when the concentrations of garlic solution and diallyl trisulfide were much higher than their inhibitory concentrations on the toxic bloom-forming alga *Alexandrium tamarense*, which are shown to be 0.04% for garlic solution and 1 mg L^{-1} for diallyl trisulfide (Zhou et al. 2008). This means that it may be safe for the non-target algae if these two materials are used for controlling algal blooms caused by *A. tamarense*.

In marine ecosystems, zooplanktons as the primary consumers play a very important role as the link between producers and consumers. Among them, Daphniidae have been used widely in ecotoxicological studies because of their fast reproduction, short life cycle, convenient cultivation, and, especially, sensitivity to many toxic materials. In addition, Daphniidae, rotifers, and brine shrimps are the diet of many aquaculture fish, so their quantity in the sea may influence the latter both during growth and development. Because of their importance as economic aquatic animals and major aquaculture organisms, prawns and fish were chosen to evaluate the potential effects of algacides in aquaculture. However, before applying algacides in inshore or aquaculture waters, these materials were tested on the prawns and fish under laboratory conditions. The juveniles were used for the test because they are more sensitive to toxins than the adult. According to 24-h LC_{50} values, the toxicity of garlic solution to the aquatic organisms can be ranked from weak to strong in *P. major* juveniles, *M. mongolica daday*, *P. cinctus* juveniles, *B. plicatilis*, 5-day *A. parthenogenetica*, *P. monodon* juveniles, *L. vannamei* juveniles, and the ranking for diallyl trisulfide was *P. major* juveniles, *M. mongolica daday*, *B. plicatilis*, *P. cinctus* juveniles, 5-day *A. parthenogenetica*, *P. monodon* juveniles, *L. vannamei* juveniles. Thus, in this study, prawn juveniles were shown to be more sensitive to garlic solution and diallyl trisulfide than the other organisms. Therefore, caution should be applied to the use of these materials in waters for prawn breeding.

The 24-h LC_{50} is also a useful parameter for evaluating the tolerance of aquatic organisms to various materials. In this study, *P. monodon* and *L. vannamei* juveniles were used to test various algacidal materials. The results showed that the tolerance of *L. vannamei* juveniles to these algacidal materials can be ranked from strong to weak as: green tea, Semen Arecae, Rhizoma Coptidis, diallyl trisulfide, and the ranking for *P. monodon* juveniles was Cortex Fraxini, Herba Houttuyniae, Semen Arecae, Rhizoma Coptidis, green tea, Fructus Carpesii, Semen Torreyae, Radix Isatidis, Fructus Mume, Radix Sophorae Flavescentis, Radix et Rhizoma Rhei, diallyl trisulfide.

Strategy for the selection of an algacide

The practicability of using an algacide depends on its algal inhibition effect, safety to other aquatic organisms, cost of disposal, and so on; therefore, it is difficult to assess an algacide according to only one of these aspects. Considering this, we used the ratio of efficiency to safety (RES) to evaluate the practicability of each algacide.

$$\mathrm{RES} = \frac{C \div 24\mathrm{hIR}}{24\mathrm{hLC}_{50}}$$

where C is the effective concentration of algacidal inhibition and 24h IR is the algal inhibitory rate in 24 h.

Table 4 RES of various algacidal materials for two prawn species juveniles

Algacidal materials	RES	
	P. monodon	*L. vannamei*
Cortex Fraxini	0.229 (1)	–
Herba Houttuyniae	0.297 (2)	–
Diallyl trisulfide	0.483 (3)	0.844 (4)
Semen Arecae	0.531 (4)	0.840 (3)
Garlic solution	0.567 (5)	0.672 (2)
Rhizoma Coptidis	0.641 (6)	0.956 (5)
Green tea	0.689 (7)	0.290 (1)
Fructus Carpesii	1.768 (8)	–
Semen Torreyae	2.569 (9)	–
Folium Isatidis	<2.775 (10)	–
Radix Isatidis	3.747 (11)	–
Fructus Mume	3.891 (12)	–
Radix Sophorae Flavescentis	5.866 (13)	–
Radix et Rhizoma Rhei	7.135 (14)	–

The number in parenthesis is the rank order of RES

The effective concentrations and 24-h IR values were obtained from another study on algal growth inhibition tests, some of the results of which have been reported and others will be covered in another paper. The effective concentrations are 0.04% for garlic solution, 1 mg L^{-1} for diallyl trisulfide (Zhou et al. 2008), and 1 g L^{-1} for green tea, Rhizoma Coptidis, Semen Arecae, Radix Isatidis, Herba Houttuyniae, Radix Sophorae Flavescentis, Radix et Rhizoma Rhei, Fructus Mume, Folium Isatidis, Semen Torreyae, Cortex Fraxini, and Fructus Carpesii. Twenty-four-hour IR values were 0.850, 0.860, 0.942, 0.978, 0.937, 0.875, 0.794, 0.861, 0.960, 1, 0.901, 0.537, 0.659, and 0.748 for these algacidal materials in the same order as they appear above, and 24-h LC_{50} values are listed in Table 1. Obviously, a material having a low RES value is indicated as a possible source of algacide manufacture because the lower effective concentration and the higher 24-h IR and 24-h LC_{50} will cause a lower RES value. Furthermore, lower effective concentration means a lower quantity of algacide and a lower cost in application, higher 24-h IR means good algacidal effect, and higher 24-h LC_{50} value means lower toxicity of algacide on the non-target organisms. The RES values of garlic solution and diallyl trisulfide to different aquatic organisms are listed in Table 3, and the RES values of various algacidal materials to *P. monodon* and *L. vannamei* are in Table 4.

Compared to the garlic solution, diallyl trisulfide has lower RES values for most of the test species used in this study, except for *L. vannamei* juveniles. Although garlic solution had a higher algal inhibitory rate than diallyl trisulfide (Zhou et al. 2008), the latter had lower toxicity than the former. Considered over all, diallyl trisulfide is better as an algacide than garlic solution. The RES values of the prawn juveniles in both algacides were higher than those of the other test species, and the reason may be that they are more sensitive to the garlic solution and diallyl trisulfide than the other test species. Thus, care should be taken with the use of these two algacides in the water for culturing juvenile prawns.

In the case of juvenile *P. monodon*, the RES values for Cortex Fraxini were the lowest and Radix et Rhizoma Rhei was the highest in the 14 algacidal materials tested (Table 4). In terms of the algal inhibitory rate, both Radix et Rhizoma Rhei and Rhizoma Coptidis were more than 93% in 5 h, and the high IR was maintained for several days. However, the *RES* value for Rhizoma Coptidis was much lower than that for Radix et Rhizoma Rhei, and this showed that Rhizoma Coptidis is better as an algacide than Radix et Rhizoma Rhei. Although the 24-h IR for Cortex Fraxini and Herba Houttuyniae were only 65.86% and 79.48%, respectively, their lower toxicity to the aquatic organisms tested resulted in lower RES values (0.229 and 0.297, respectively), and this implies that the two materials are still good algacides. On the other hand, the higher *RES* values for Fructus Carpesii, Semen Torreyae, Folium Isatidis, Radix Isatidis, Fructus Mume, Radix Sophorae Flavescentis, and Radix et Rhizoma Rhei imply that these materials may not be a good choice for making algacides, even though they had better algacidal effects.

Since most RES values for *L. vannamei* were higher than those for *P. monodon* using the same algacidal materials (except for green tea), the application of algacide may have caused more negative effects to *L. vannamei*.

In this study, the toxicities of 14 algacidal materials to several aquatic organisms were tested, and the *RES* values are presented here to help evaluate these algacides. According to their *RES* value, Cortex Fraxini, Herba Houttuyniae, diallyl trisulfide, Semen Arecae, garlic solution, Rhizoma Coptidis, and green tea are better than the other materials as algacides. Among these, Cortex Fraxini, Herba Houttuyniae, Semen Arecae, and Rhizoma Coptidis are common Chinese herbs, and green tea, garlic solution, and diallyl trisulfide are classed as “food, beverage, or medicine,” and so they are easily collected and stored and so very convenient for algal bloom control.

Acknowledgments This research was supported by the Special Research Funds Program of China for Public Sector(Agriculture): Research and Demonstration of eel pond culture with Sustainable Development Technology (no. nyhyzx07-043), the High-Tech Research and Development Program of China (no.2008AA09Z408), the subitem of National Science Foundation of China (no. B04005), the NSF of China (no. 40930847, 40876061), and the Program for Changjiang Scholars and Innovative Research Team in University (no. 40821063).We would like to thank Professor I. J. Hodgkiss for his assistance with English and L.N. Han for her help with the experiments.

References

Alamsjah MA, Hirao S, Ishibashi F, Fujita Y (2005) Isolation and structure determination of algicidal compounds from *Ulva fasciata*. Biosci Biotechnol Biochem 69:2186–2192

Balcomb R, Hoberg JR, Giddings JM (2002) Fate and toxicity of the algicide Irgarol 1051: a marine microcosm study. Bull Environ Contam Toxicol 69:696–703

Boyd CE, Massaut L (1999) Risks associated with the use of chemicals in pond aquaculture. Aquacult Eng 20:113–132

Boylan JD, Morris JE (2003) Limited effects of barley straw on algae and zooplankton in a midwestern pond. Lake Reserv Manage 19:265–271

Brownlee EF, Sellner SG, Sellner KG (2003) Effects of barley straw (*Hordeum vulgare*) on freshwater and brackish phytoplankton and cyanobacteria. J Appl Phycol 15:525–531

Elder J, Horne AJ (1978) Copper cycles and $CuSO_4$ algicidal capacity in two California lakes. Environ Manage 2:17–30

Elisabeth MG, Holger M, Gerhard S (1996) Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. Phytochemistry 41:133–138

Ferrier MD, Butler BR, Terlizzi DE, Lacouture R (2005) The effects of barley straw (*Hordeum vulgare*) on the growth of freshwater algae. Bioresource Technol 96:1788–1795

Furusawa G, Yoshikawa T, Yasuda A, Sakata T (2003) Algicidal activity and gilding motility of *Saprospira* sp SS98-5. Can J Microbiol 49:92–100

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can J Microbiol 8:229–239

Hehmann A, Kaya K, Watanabe MM (2002) Selective control of Microcystis using an amino acid—a laboratory assay. J Appl Phycol 14:85–89

Jancula D, Suchomelova J, Gregor J, Smutna M, Marsalek B, Taborska E (2007) Effects of aqueous extracts from five species of the family Papaveraceae on selected aquatic organisms. Environ Toxicol 22:480–486

Jancula D, Drabkova M, Cerny J, Karaskova M, Korinkova R, Rakusan J, Marsalek B (2008) Algacidal activity of phthalocyanines—screening of 31 compounds. Environ Toxicol 23:218–223

Jin Q, Dong SL (2003) Comparative studies on the allelopathic effects of two different strains of *Ulva pertusa* in *Heterosigma akashiwo* and *Alexandrium tamarense*. J Exp Mar Biol Ecol 293:41–55

Klapes NA (1990) Acute toxicity of the natural algicide, cyanobacterin, to *Daphnia magna*. Ecol Environ Safety 20:167–174

Lovejoy C, Bowman JP, Hallegraeff GM (1998) Algacidal effects of a novel marine *Pseudoalteromonas* isolate (class Proteobacteria, gamma subdivision) on harmful algal bloom species of the genera *Chattonella, Gymnodinium*, and *Heterosigma*. Appl Environ Microb 64:2806–2813

Mulderij G, Donk VE, Roelofs JGM (2003) Differential sensitivity of green algae to allelopathic substances from *Chara*. Hydrobiologia 491:261–271

Murray-Gulde CL, Heatley JE, Schwartzman AL, Rodgers JH (2002) Algicidal effectiveness of clearigate, cutrine-plus, and copper sulfate and margins of safety associated with their use. Arch Environ Contam Toxicol 43:19–27

Ridge I, Walters J, Street M (1999) Algal growth control by terrestrial leaf litter: a realistic tool? Hydrobiologia 396:173–180

Sabine K, Andreas N (2002) Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. J Phycol 38:862–871

Schrader KK, Tucker CS, Mischke CC (2007) Preliminary pond evaluation of a natural-based algicide for managing musty off-flavor in channel catfish aquaculture. North Am J Aquacult 69:360–366

Straus DL, Tucker CS (2007) Acute toxicity of copper sulfate and chelated copper to Channel catfish *Ictalurus punctatus*. J World Aquacult Soc 24:390–395

Zhou LH, Zheng TL, Wang X, Ye JL, Tian Y, Hong HS (2007) Effect of five Chinese traditional medicines on the biological activity of a red-tide causing alga—*Alexandrium tamarense*. Harmful Algae 6:354–360

Zhou LH, Zheng TL, Chen XH, Wang X, Chen SB, Tian Y, Hong HS (2008) The inhibitory effects of garlic (*Allium sativum*) and diallyl drisulfide on *Alexandrium tamarense* and other harmful algal species. J Appl Phycol 20:349–358