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Laboratory Studies on Chemical Control of Red Tide Phytoplankton (*Chattonella marina* and *Heterosigma akashiwo*) for Black Tiger Shrimp (*Penaeus monodon*) Culture

Piansiri Piyatiratitivorakul[a,*], Thaithaworn Lirdwitayaprasit[b], Joonjara Thooithaisong[c]
[a] Department of Zoology, Faculty of Science, Kasetsart University, Bangkhen, Bangkok, Thailand.
[b] Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.
[c] Rachapat Institute of Udornthani, Udornthani, Thailand.
* Corresponding author, E-mail: fsciprp@nontri.ku.ac.th

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ABSTRACT Laboratory cultures of red tide organisms, *Chattonella marina* and *Heterosigma akashiwo*, were maintained under a light intensity of 2,000 luxes at 28 °C and salinity of 30 ppt. Growth rates of *C. marina and H. akashiwo* were 0.792 and 0.872 divisions/day, respectively. Calcium hypochlorite (66 ppm), benzalkonium chloride (0.3 ppm) and hydrogen peroxide (39 ppm) completely disintegrated 50% (24-h LC_{50}) of cultured *C. marina* within 24 hours. The 24-h LC_{50}values of *H. akashiwo* were 35, 0.1 and 15 ppm, respectively. Acute toxicities (24-h LC_{50}) of these chemicals to post larvae (PL-30) of black tiger shrimp in the *C. marina* treatment were 52, 0.2 and 42 ppm, respectively, while in the *H. akashiwo* treatment were 51, 0.2 and 62 ppm, respectively. The chemical that gave the best control of both phytoplankton species was hydrogen peroxide. During the chemical treatment procedures, water quality showed little variation.

KEYWORDS: tiger shrimp, chemical control, red tide phytoplankton.

INTRODUCTION

Chattonella marina and *Heterosigma akashiwo*, belonging to the Class Raphidophyceae, are important red tide phytoplankton, especially in Japan. They have caused tremendous mortality of the farmed yellowtail (*Seriola quinqueradiata*)[1-2] because of their detrimental effects to the fish associated with reduction of dissolved oxygen and obstruction of oxygen exchange in gills. In addition, they can also excrete neurotoxins which result in hemagglutination and hemolysis in fish blood.[3-4]

The shrimp *Penaues monodon* is one of the most economically important marine species in Thailand with an export value of more than one thousand million baht per year. Commercial culture has become widespread in Thailand in the last decade. However, there are still some serious problems associated with shrimp diseases, red tide phytoplankton blooms, wide use of chemicals to control diseases, degraded water quality and environmental pollution.[5] The present study was conducted to determine the growth rates of *C. marima* and *H. akashiwo* and the advantages or disadvantages of chemical treatments that might be used to control them in *P.monodon* culture .

MATERIALS AND METHODS

C. marina and *H. akashiwo* were isolated from a black tiger shrimp pond in Chanthaburi province. They were cultured aseptically at an initial concentration of 4.37×10^3 and 8.53×10^3 cell/ml, respectively in modified Erschreiber Seawater medium [6] and at ambient temperature (28 ± 1 °C) under a cool white fluorescent light of 2000 lux intensity with a 12 :12 h light: dark cycle. *P. monodon* post larvae (PL30) were acclimated in the laboratory for at least three days before experiments. Algal growth rates were calculated from sample counts taken every two days .[7-8] Stock solutions of 50% calcium hypochlorite, benzalkonium chloride, and 35% hydrogen peroxide in distilled water were diluted with sterile sea water to different concentrations and tested on phytoplankton aged 6-8 days (in stationary growth phase) for 24,48,72 and 96 hr periods. Serial toxicity tests were performed with the shrimp exposed to the test chemicals at five sequential concentrations (3 replications) in plastic aquaria containing either *C. marina* and *H. akashiwo* at a concentration of 40,000 cells/ml. Shrimp survival was determined every 24 hours for 4 days. Water quality parameters were analyzed by methods of

Stickland & Parsons.[9] The 24 hour median lethal concentrations of chemicals on *P. monodon* were determined following Finney.[10] A completely randomized design (CRD) analysis of variance and Duncan's new multiple range test were carried out on data for water quality, plankton cell numbers and experimental variables. All data were interpreted by the statistical analysis system (SAS).

RESULTS

Water quality in all experiments showed little fluctuation in acceptable ranges for aquatic life : water temperature 27-30 °C, salinity 30-32 ppt, pH 7.98-8.28 and dissolved oxygen 5.20-6.30 mg/L^{-1}

Growth rates of *C. marina* and *H. akashiwo*

C. marina and *H. akashiwo* were acclimated in culture tanks for 1-2 days and progressed to the exponential growth phase in 6 days. *C. marina* was in the stationary phase for 18 days and *H. akashiwo* for 12 days before entering the death phase. Growth rates of *C. marina* and *H. akashiwo* were 0.8 and 0.9 division/day, respectively (Fig 1). The growth rate (u) of *C. marina* was higher than that of *H. akashiwo*.

Effects of chemicals on *C. marina* and *H. akashiwo*

Within 24 hours of exposure to calcium hypochlorite, *C. marina* and *H. akashiwo* showed abnormalities including shrinkage or disruption of cell membranes, slow movement or immobility, cell dissociation and congregation, settling to the bottom and finally death. Survival of the plankton decreased gradually with increasing concentration of the chemicals over 96 hours of exposure. The 24 hour LC_{50} of calcium hypochlorite to *C. marina* and *H. akashiwo* were 66 and 35 ppm, respectively (Table 2). For benzalkonium chloride and hydrogen peroxide,

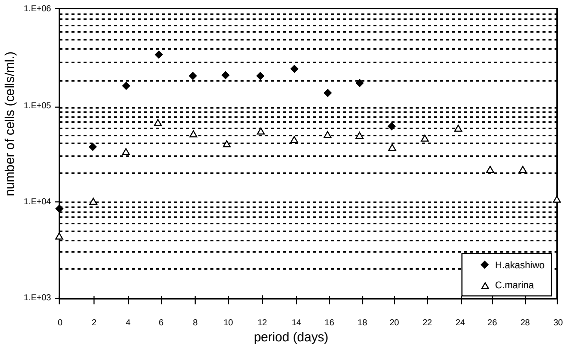

Fig 1. Growth rate of phytoplankton *Chattonella marina* and *Heterosigma akashiwa.*

Table 1. Percent accumulated mortality of the shrimp *Peneaus. monodon* (PL-30) in different concentrations of calcium hypochlorite, benzalkonium chloride and hydrogen peroxide within 96 hours.

Chemical	Concration (ppm)	% mortality							
		C. marina				H. akashiwo			
		24	48	72	96	24	48	72	96
Calcium hypochlorite	0	0	0	0	0	0	0	0	0
	20	13.33	20.00	100	100	10.00	90.00	100	100
	35	26.67	100	100	100	30.00	100	100	100
	50	40.00	100	100	100	40.00	100	100	100
	75	53.33	100	100	100	60.00	100	100	100
	100	100	100	100	100	100	100	100	100
Benzalkonium chloride	0	0	0	0	6.67	0	0	0	0
	0.1	13.33	20	93	100	20.00	30.00	90	100
	0.2	60.00	86.67	93.33	100	70.00	90.00	100	100
	0.3	86.67	86.67	100	100	70.00	90.00	100	100
	0.4	100	100	100	100	100	100	100	100
	0.5	100	100	100	100	100	100	100	100
Hydrogen peroxide	0	0	0	0	0	0	0	0	0
	10	20.00	100	100	100	20.00	90.00	100	100
	30	33.33	100	100	100	10.00	100	100	100
	50	40.00	100	100	100	30.00	100	100	100
	70	40.00	100	100	100	30.00	100	100	100
	100	66.67	100	100	100	40.00	100	100	100

C. marina showed the same cell abnormalities as with calcium hypochlorite, except that they were less severe and rapid. *H. akashiwo* showed a larger reduction in cell number than *C. marina* at the same concentration of benzalkonium chloride. With hydrogen peroxide, the two species did not dissociate completely at any concentration tested. The 24 hour LC_{50} of benzalkonium chloride and hydogen peroxide to *C. marina* and *H. akashiwo* were 0.3 and 0.1 and 39 and 15 ppm, respectively (Table 2).

Effects of chemicals on *P. monodon* cultured with *C. marina* and *H. akashiwo*

When exposed to high concentration of the tested chemicals, *P.monodon* post larvae (PL-30) showed symptoms of erratic swimming, jumping from the water surface, sinking and crawling on the bottom of the container followed by immobility and finally death. The 24 hour - LC_{50} of the shrimp reared together with *C. marina* and *H. akashiwo* and exposed to calcium hypochlorite, benzalkonium chloride and hydrogen peroxide are shown in Table 1 and 3. Water quality parameters such as ammonia, dissolved oxygen, and pH, displayed some variation but remained at safe levels in every treatment. Salinity and temperature exhibited little fluctuation during the experiments.

Discussion

Growth rates of *C. marina* and *H. akashiwo* were similar to rates reported for other unicellular algae. Stages of growth for the two revealed the same pattern proposed by Fogg & Thake[7] , that is, a lag or induction phase in the first two days, followed by an exponential or logarithmic phase. Growth curves for *C. marina* were comparable to those reported by Ahmed et al.[11] despite dissimilar culture conditions. Growth of *H. akashiwo* was comparable to that reported by Sato and Fujii.[12]

In normal shrimp pond management practice, aerators are turned off before phytoplankton treatment. As a result, the algae accumulate near the water surface at the downwind side or corner of the pond where treatment chemicals can be added at relatively high concentration. Shrimp flee the treatment area and the chemicals are substantially diluted when dispersed after aerators are subsequently restarted. Our results showed that when *C. marina* and *H. akashiwo* were treated with calcium hypochlorite, benzalkonium chloride and hydrogen peroxide, cell abnormalities and reduction occurred more rapidly in *H. akashiwo* than in *C. marina*, possibly because the cells of *C. marina* are larger than those of *H. akashiwo.* From our experiments, we found that the highest concentration that had no effect on *C. marina* and *H. akashiwo* was less than 10 ppm, as reported by Murata et al.[13] Ichikawa et al[14] used hydrogen peroxide at 100 mg/l to destroy *C. marina* cysts within 96 hours while motile cells were killed within 30 minutes at 15 mg/l indicating that motile cells were more susceptible to hydrogen peroxide than cysts.

In chemical treatments of *C. marina* and *H. akashiwo* reared with *P. monodon*, we found that

Table 2. Comparison of acute toxicity (LC_{50},ppm) of chemicals on *Chattonella marina* and *Heterosigma akashiwo* within 24 hours.

chemicals	24-h LC_{50}		95% confidence	
	C. marina	*H. akashiwo*	*C. marina*	*H. akashiwo*
calcium hypochlorite(ppm)	66	35	-	25-43
benzalkonium chloride(ppm)	0.3	0.1	-	0.10-0.14
hydrogen peroxide(ppm)	39	15	23-55	12-18

Table 3. Comparison of acute toxicity (LC_{50}, ppm) of chemicals on the shrimp *Penaeus monodon* cultured with phytoplankton *Chattonella marina* and *Heterosigma akashiwo* within 24 hours.

Chemicals	*Penaeus monodon* 24-h LC_{50}		95%confidence		Chi-square(X^2)		d.f.
	C. marina	*H. akashiwo*	*C. marina*	*H. akashiwo*	*C. marina*	*H. akashiwo*	
calcium hypochlorite	52	51	31-97	30-89	2	2.4	3
benzalkonium chloride	0.2	0.2	0.1-0.2	0.1-0.2	0.3 1.6	3	
hydrogen peroxide	42	65	7-235	28-393	1.1	0.8	3

calcium hypochorite may be better to control *H. akashiwo* than *C. marina*. The reason was that the concentration of the chemical needed to control *C. marina* was lethal to *P. monodon(* PL- 30). Benzalkonium chloride was also inappropriate to control both phytoplankton species reared with *P.monodon*. This result was different from those of Wongwiwatanawuht and Darunchoo[15] reported using as high as 60% calcium hypochlorite and 1-5 ppm. benzalkonium chloride and Su et al[16] who reported using 1-2 ppm. benzalkonium chloride to successfully control phytoplankton in *P. monodon* ponds, respectively. By contrast, hydrogen peroxide was appropriate to control *H. akashiwo* in our laboratory shrimp culture since there as a spread of 50 ppm. Between the lethal concentration for the shrimp and the alga. Although hydrogen peroxide showed little effect on water quality, and degraded easily, but it may also have adverse effects on the shrimp.

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