Toxicity of Formalin, Calcium Hypochlorite and Copper Sulfate to *Chaetoceros calcitrans*

Yont Musig[1], Jamaree Rakbanglaem[2] and Arunee Rodloy[2]

ABSTRACT

The toxicity effects of formalin, calcium hypochlorite and copper sulfate on *Chaetoceros calcitrans*, a marine diatom, were studied by monitoring changes in its growth in terms of cell population using short term bioassays. Inhibition of cell growth of *Chaetoceros calcitrans* was observed at concentrations of 2.27, 2.00 and 0.35 ppm of formalin, calcium hypochlorite and copper sulfate, respectively. Low concentrations of formalin (1.00 ppm) and copper sulfate (0.10 ppm) had no effect on the growth of *C. calcitrans*. Lethal effect was observed at concentrations of 26.46 and 60.00 ppm of formalin, wherein algal cell number decreased continuously from initial cell concentrations. Total mortality of *C. calcitrans* was observed at 48 h at 60.00 ppm of formalin. Algal cell concentration also continually decreased at calcium hypochlorite concentrations of 9.10, 15.09, 25.00 and 50.00 ppm. Total mortalities were observed at 48 and 72 h with 50.00 and 25.00 ppm calcium hypochlorite, respectively. Effective Concentration values at 96 hours (96h-EC_{50}) of calcium hypochlorite, copper sulfate and formalin for *Chaetoceros calcitrans* were 2.60, 4.30 and 8.00 ppm, respectively.

Keywords: calcium hypochlorite, copper sulfate, formalin, *Chaetoceros calcitrans*

INTRODUCTION

Chaetoceros calcitrans is a unialgal marine diatom commonly found in coastal waters and brackish water ponds. In aquaculture, *C. calcitrans* is used as a feed for shrimp and bivalve larva. Formalin (37% formaldehyde) has been used as a therapeutant to control ectoparasites and aquatic fungi disease occurrence in fish culture facilities. It is widely used for the control of fungi on fish eggs and external parasites on fish and shrimp. Its toxicity to fish and shrimp is relatively low thus it is often applied directly into aquaculture ponds (Schnick, 1991), using concentrations between 25 and 50 ppm (Boonyarattapalin, 1981). On the other hand, calcium hypochlorite has been used as a microbiocide, algaecide and for water treatment. In aquaculture farms, calcium hypochlorite is used to treat supply water, tanks and ponds and other equipment for the prevention and control of diseases and parasites (Huguenin and Colt, 1989; Lawson, 1995).

[1] Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand
[2] Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok, Thailand

Concentrations of calcium hypochlorite used for disinfection of supply water are between 6 and 30 mg. l^{-1} (Musig, 1987). Copper sulfate has been widely used for the control of filamentous algae and phytoplankton in ponds. Application rates of copper sulfate vary from 0.025 to 2 mg. l^{-1} depending on alkalinity of pond water (Bartsch, 1954; Jackson, 1974). In some shrimp farms, these chemicals are applied into ponds directly for phytoplankton control. In this study, short term toxicities of formalin, calcium hypochlorite and copper sulfate to *Chaetoceros calcitrans* were investigated.

MATERIALS AND METHODS

Chaetoceros calcitrans used for the experiment was obtained from the stock culture of the Algal Laboratory of the Faculty of Fisheries, Kasetsart University. Exponentialy-growing axenic culture of *C. calcitrans* was used for the experiments. Algal cell density was determined and 100 ml of the stock culture of *C. calcitrans* were transferred into 250 ml Erlenmeyer flasks. Test flasks were continuously illuminated by cool-white fluorescent bulbs throughout the experimental period. Light intensity on the algal media surface was 1800 lux. Aeration was provided to keep the algae in suspension and to facilitate transfer of CO_2. Sato and Serikawa medium (Sato and Serikawa, 1978) was used for algal culture. The algal media was prepared using 30 ppt filtered seawater. Temperature varied between 26 and 29°C. Laboratory grade formalin (CH_2O), calcium hypochlorite ($Ca(OCl)_2$) and copper sulfate (pentahydrate) ($CuSO_4.5H_2O$) were used for the experiments. Range finding tests were conducted to obtain the concentration of each chemical that had no observed effect on the growth of algae, and the concentration that caused total mortality of the algae. These ranges of concentrations were divided into six consecutive concentrations in logarithmic series and were used for the full scale tests. Algal cell concentration in each flask was determined at 24, 48, 72, and 96 hours after treating with chemicals. Algal cells were counted under microscope using haematocytometer. Three replications were set for each treatment. The mean values of the cell concentration for each test substance concentration and for control were plotted against time to produce growth curves. The concentration effect relationship was determined by comparing the areas under the growth curves calculated according to the following formula:

$$A = (N_1-N_0).t_1/2 + (N_1+N_2-2N_0)(t_1-t_2)/2 + (N_{n-1} + N_n - 2N_0)(t_{1n}-t_{n-1})/2$$

Where

A = area
N_0 = nominal number of cells. ml^{-1} at time t_0
N_1 = measured number of cells. ml^{-1} at t_1
N_n = measured number of cells. ml^{-1} at t_n
t_1 = time of first measurement after beginning of the test
t_n = time of n_{th} measurement after beginning of the test

The percentage inhibition of the cell growth at each test substance concentration (I_A) is calculated as the difference between the area under the control growth curve (A_c) and the area under the growth curve at each test substance concentration (A_t) as:

$$I_A = 100(A_c - A_t)/A_c$$

IA values were plotted on semi-logarithmic probit paper against the corresponding concentrations. The points were fitted by a straight line by eye and EC_{50} was obtained from the point of the interception of regression line with the parallel drawn to the abscissa at I_A = 50% (OECD, 1984: APHA *et al*., 1992).

RESULTS AND DISCUSSION

Short term toxicity of copper sulfate to *Chaetoceros calcitrans*

At the concentration of 0.10 ppm copper sulfate had no effect on the growth of *Chaetoceros calcitrans* in terms of cell population. Reduction in growth of the algae was clearly observed at copper sulfate concentrations of 0.35 ppm and higher. The percent inhibition of algal cell growth were 24.5, 29.0, 47.5 and 76.6% at copper sulfate concentrations of 0.35, 1.20, 4.16 and 14.43 ppm, respectively. At the concentration of 50.00 ppm, copper sulfate was acutely toxic to *C. calcitrans*, resulting in the decrease in algal cell density from an initial cell concentration of 0.73 x 10^6 cells. ml^{-1} to 0.54 x 10^6 cells. ml^{-1} within 24 h. At 48 h, total mortality of *C. calcitrans* was observed at this concentration (50.00 ppm) (Table1). Effective $Concentration_{50}$ value at 96 hours (96h-EC_{50}) of copper sulfate for *C. calcitrans* was 4.30 ppm. According to Kemp *et al*. (1966), the application of 2 ppm copper sulfate was toxic to *Chlamidomonas*, *Chlorella*, *Gomphonema*, *Navicula*, *Oocystis* and *Pandorina*, resulting in no growth of test algae during 28 days experimental period. At the concentration level of 4ppm, copper sulfate was partially toxic to *Calothrix*, *Closterium*, *Nostoc* and *Plectonema*, resulting in limited growth of algae during the later part of the experimental period (Kemp *et al*., 1966). Fitzgerald and Faust (1963) reported the toxicity of copper sulfate to *Chlorella*, *Microcystis*, *Glaetrichia* and *Anabaena* at concentration levels of 2.0, 0.5, 0.5 and 0.1 ppm, respectively.

Table 1. Effect of different concentrations of copper sulfate on the growth of *Chaetoceros calcitrans* in terms of cell population

Concentration of copper sulfate (ppm)	Number of algal cells X 10^6. ml^{-1} at different time intervals				
	Initial	24 h	48 h	72 h	96 h
control	0.73	1.11	1.60	2.08	3.83
0.10	0.73	1.11	1.60	2.17	3.80
0.35	0.73	1.06	1.42	1.83	2.76
1.20	0.73	1.25	1.23	1.73	2.58
4.16	0.73	1.17	0.96	1.35	2.51
14.43	0.73	0.90	0.83	1.17	1.25
50.00	0.73	0.54	0	0	0

Short term toxicity of formalin to *Chaetoceros calcitrans*

At the concentration of 1.00 ppm formalin had no effect on the growth of *Chaetoceros calcitrans*. Reduction in algal growth was clearly observed at formalin concentrations of 2.27 ppm and higher. Formalin concentrations of 2.27, 5.14 and 11.67 ppm resulted in percent inhibition of algal cell growth of 16.4, 24.6 and 32.1%, respectively. Formalin at concentrations of 26.46 and 60.00 ppm were acutely toxic to *C. calcitrans*. The application of formalin at 26.46 ppm resulted in a decrease in algal cell concentration from the initial cell concentration of 1.02 x10^6 cells. ml^{-1} to 0.39 x 10^6 cells. ml^{-1} within 96 h. At the concentration of 60.00 ppm, total mortality of *C. calcitrans* was observed at 48 h (Table2). Effective $Concentration_{50}$ value at 96 hours (96h-EC_{50}) of formalin for *C. calcitrans* was 8.00 ppm. There has been no report on the toxicity of formalin to phytoplankton. However, Chiyavareesajja and Boyd (1993) found decreased amounts of chlorophyll *a* after the application of formalin to freshwater ponds at the rate of 10 mg. l^{-1}. The 48 h-TLm values of formalin on giant tiger prawn (*Penaeus monodon* Fabricius) were 7.1, 128.8 and 110.8 ppm for postlarva 1, postlarva 6 and postlarva 11, respectively (Maeroh, *et al.*, 1987).

Table 2. Effect of different concentrations of formalin on the growth of *Chaetoceros calcitrans* in terms of cell population

Concentration of formalin (ppm)	Number of algal cells X 10^6. ml^{-1} at different time intervals				
	Initial	24 h	48 h	72 h	96 h
control	1.02	1.91	3.15	4.31	4.78
1.00	1.02	1.94	3.12	4.31	4.88
2.27	1.02	1.88	2.96	3.57	4.01
5.14	1.02	1.68	2.54	3.54	3.97
11.67	1.02	1.56	2.43	3.38	3.52
26.46	1.02	0.75	0.77	0.61	0.39
60.00	1.02	0.23	0	0	0

Short term toxicity of calcium hypochorite to *Chaetoceros calcitrans*

Reduction in growth of *Chaetoceros calcitrans* was clearly observed at calcium hypochlorite concentration of 2.00 ppm and higher. The percent inhibition of algal cell growth was 18.0 and 83.4% at calcium hypochlorite concentrations of 2.00 and 3.31 ppm, respectively. Calcium hypochlorite at concentrations of 9.10 ppm and higher was acutely toxic to *C. calcitrans*. Application of calcium hypochlorite at concentrations of 9.10 and 15.09 ppm resulted in decreased algal cell concentration from an initial

concentration of 1.02 X10^6 cells. ml^{-1} to 0.45 x 10^6 and then 0.35 x 10^6 cells. ml^{-1} within 96 h. At 25.00 ppm calcium hypochlorite, total mortality of *C. calcitrans* was observed at 96 h (Table3). Effective $Concentration_{50}$ value at 96 hours (96h-EC_{50}) of calcium hypochlorite for *C. calcitrans* was 2.60 ppm. Honig, *et al.,* (1980) reported that the 48h-EC_{50} value of calcium hypochlorite for the Cryptomonad, *Chiromonas paramecium*, was 0.3 mg. l^{-1}. According to Kemp *et al.,* (1966), the application of 2 ppm calcium hypochlorite was toxic to *Anacystis*, *Cylindrospermum*, *Gomphonema*, *Navicula*, *Oocystis* and *Pandorina*, resulting in no algal growth during 28 days of experimental period. At the concentration level of 4 ppm, calcium hypochlorite was partially toxic to *Calothrix*, *Chlorella*, *Closterium, Nostoc*, *Plectonema* and *Scenedesmus*, resulting in limited algal growth during the later stages of the experimental period (Kemp *et al.*, 1966).

Table 3. Effect of different concentrations of calcium hypochlorite on the growth of *Chaetoceros calcitrans*

Concentration of calcium hypochlorite (ppm)	Number of algal cells X 10^6. ml^{-1} at different time intervals				
	Initial	24 h	48 h	72 h	96 h
control	5.60	6.25	7.08	8.75	8.45
2.00 ppm	5.60	5.75	6.60	8.35	8.60
3.31 ppm	5.60	4.55	5.75	6.40	7.15
5.49 ppm	5.60	3.95	3.95	4.85	5.75
9.10 ppm	5.60	3.40	1.70	1.20	0.45
15.09 ppm	5.60	2.20	0.60	0.40	0.35
25.00 ppm	5.60	1.70	0.25	0.05	0

CONCLUSION

Formalin, calcium hypochlorite and copper sulfate are toxic to *Chaetoceros calcitrans*. Inhibition of algal cell growth was clearly observed at the concentrations of 2.27, 2.00 and 0.35 ppm of formalin, calcium hypochlorite and copper sulfate, respectively. Lethal effect on *C. calcitrans* which resulted in continuous decrease in algal cell concentration from the initial cell count was observed at concentrations of 26.46 and 60.00 ppm for formalin, 9.10, 15.09 and 25.00 ppm for calcium hypochlorite, and 50.00 ppm for copper sulfate. Total mortality of *C. calcitrans* was observed at 48 h at 60.00 ppm formalin and 50.00 ppm copper sulfate, and at 72 h at 25.00 ppm calcium hypochlorite. Effective $Concentration_{50}$ values at 96 hours (96h-EC_{50}) of calcium hypochlorite, copper sulfate and formalin were 2.60, 4.30 and 8.00 ppm, respectively. Low concentrations of 1.00 ppm formalin and 0.10 ppm copper sulfate had no effect on the growth of *C. calcitrans*.

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