Effects of Benthiocarb Herbicide on Growth of Planktonic Organisms, Chlorella saccharophila and Brachionus plicatilis

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Effects of Benthiocarb Herbicide on Growth of Planktonic Organisms, *Chlorella saccharophila* and *Brachionus plicatilis*

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Abstract

The effects of benthiocarb herbicide on the population growth of planktonic organisms were examined to evaluate its toxicity in natural rivers as run-off from rice fields. The organisms used in this experiment were *Chlorella saccharophila* and *Brachionus plicatilis*. Concentrations of the herbicide were regulated to sub-acute levels at: 0.0 ppm as control, 0.003 ppm, 0.01 ppm, 0.03 ppm, 0.1 ppm, and 0.3 ppm. Ethanol was used to dilute the original concentrated benthiocarb. The effects of ethanol on the growth of plankton were also observed.

There were no significant differences found among the culture experiments conducted for the growth of *C. saccharophila* and *B. plicatilis* below the herbicide concentration of 0.3 ppm. However, in the ethanol experiments, the growth rate of plankton was less than that at a 0.3 ppm herbicide concentration. Careful utilization of ethanol for dilution is suggested for practical use.

Chemical weed control has an important role in modernized rice production. Benthiocarb is one of the chemical herbicides used extensively for rice cultivation in the paddy fields around the world[1]. Herbicide residues have been reported in rice paddy soil and river waters draining from rice field[2]. Minimization of such water pollution is among the most essential problems in aquatic biology[3].

The biological significance of benthiocarb herbicide on planktonic organisms, however, has not been thoroughly investigated. The present experiments were then conducted to evaluate its toxicity to the plankton by simulating herbicide concentrations in natural rivers and by using two representative species of planktonic organisms. Ethanol was employed for dilution of the original concentrated liquid benthiocarb. The effects of ethanol on the growth of plankton were also observed.

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Materials and Methods

The organisms used in the present experiments were the phytoplankton, *Chlorella saccharophila* var. *saccharophila*, and the zooplankton, *Brachionus plicatilis*. Both originated from the Yashima strains[4]. They are commonly cultured in hatcheries as diet for fish and crustacean larvae. *B. plicatilis* used in the experiment were fed with *C. saccharophila* cultured in Yashima medium[5]. The experiments consisted of two parts: Exp. I for *C. saccharophila*, and Exp. II for *B. plicatilis*.

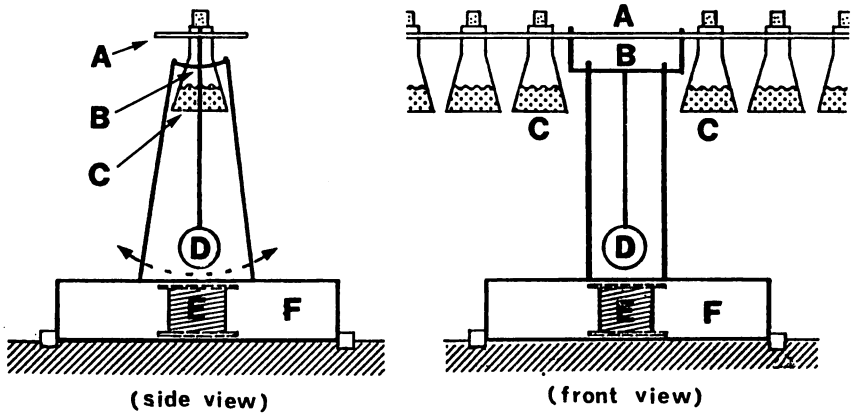

Fig. 1. Schematic diagram showing swing-culture apparatus for cultivation of planktonic organisms. Eight 30ml flasks with 10ml of medium were employed. A = flask hanger, B = fulcrum, C = flask, D = pendulum with permanent magnet, E = electro magnet (about 100 rpm), F = stand table.

Concentrations of benthiocarb in each test were regulated to sub-acute toxicity levels at: 0.0 ppm as control in Flask A, 0.003 ppm in Flask B, 0.01 ppm in Flask C, 0.03 ppm in Flask D, 0.1 ppm in Flask E, and 0.3 ppm in Flask F. These concentrations were based on the report of Yusa and Ishikawa[2] as observed in natural rivers.

Pure benthiocarb herbicide was diluted with 90% ethanol. Final concentrations of ethanol in the culture medium were adjusted to 0.1% in each experiment. Ethanol blanks without benthiocarb were conducted in Exp. I, using Flask I-A', in order to determine the effects of ethanol effects.

A swing-culture apparatus was devised for culture experiments in Exp. I. *C. saccharophila* were inoculated into eight 30ml flasks. The flasks were swung by the principle of an electro-magnetic pendulum clock as shown in Fig. 1. The apparatus was placed in an incubator, and the temperature maintained at about 23°C. Photoperiod was set at 15L:9D with a white beam fluorescent lamp. The light intensity was about 1000 lux in the incubator when the lamp was on during the day. Aeration was not supplied.

The culture experiments in Exp. II were conducted with six 3-liter flasks; I-A ~ F. Aeration was appropriately supplied into the medium. The flasks were set in a cultivation room where the temperature was maintained at about 23°C by an air-conditioner. Photoperiod and light intensity were adjusted to the same conditions as in Exp. I.

Each experiment was repeated three times. The population densities of the organisms were observed daily by microscope. Temperature and pH values of the culture water were also measured.

Results and Discussion

Chlorella test Exp. I

Results obtained in Exp. I are shown in Table 1 and Fig. 2. Population densities at the beginning of the experiment were adjusted to about 0.5×10^6 cells·ml^{-1} in each flask. The final mean population density in the controls, Flask A, was 3.75×10^6 cells·ml^{-1}. The daily growth rate was calculated to be 1.09×10^6 cells·ml^{-1}·day^{-1} (Table 1). Therefore, it is considered that the growth

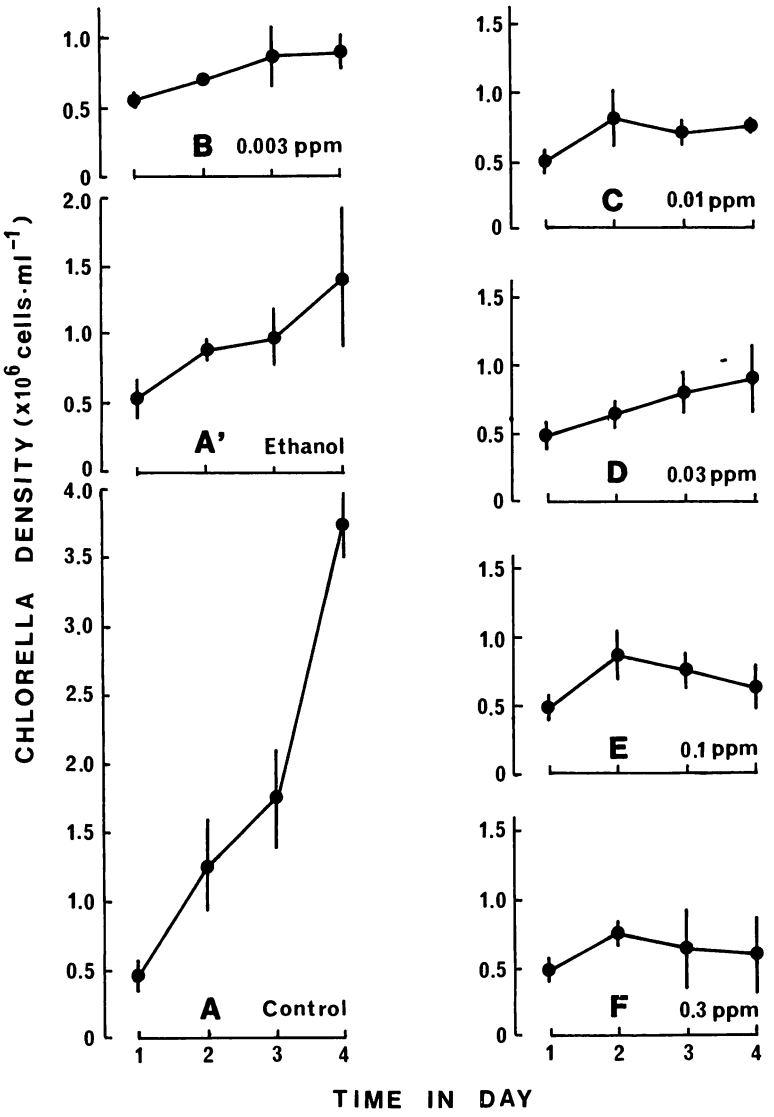

Fig. 2. Population growth of *Chlorella saccharophila* under different concentrations of benthiocarb herbicide, including ethanol blank test in diagram A'.

Table. 1 Population densities and daily growth rates of *Chlorella saccharophila* in each trial. Units in (a), (b), (c), and (d) are represented as follows: (a) & (b) = cells·ml^{-1}, (c) = days, (d) = cells·ml^{-1}·day^{-1}. Unit ppm indicates the concentrations of benthiocarb examined.

Trial	Flask	Population density		Culture period	Daily growth rate
		Initial (a)	Final (b)	(c)	(d = (b − a)/c)
Control	I-A	0.49	3.75	3	1.09
Alcohol	I-A'	0.53	1.40	3	0.29
0.003 ppm	I-B	0.55	0.91	3	0.12
0.01 ppm	I-C	0.49	0.73	3	0.08
0.03 ppm	I-D	0.49	0.91	3	0.14
0.1 ppm	I-E	0.49	0.61	3	0.04
0.3 ppm	I-F	0.49	0.58	3	0.03

Table 2. Statistical testing for difference between two means among each trial. The numbers indicate confidence limits in percent. Unit ppm indicates concentrations of benthiocarb examined.

	Alcohol	0.003 ppm	0.01 ppm	0.03 ppm	0.1 ppm	0.3 ppm
Control	95*	95*	95*	95*	95*	95*
Alcohol	—	80	90	80	90	90
0.003 ppm	—	—	90	80	90	85
0.01 ppm	—	—	—	80	80	80
0.03 ppm	—	—	—	—	85	85
0.1 ppm	—	—	—	—	—	80

* significant at 95% probability level.

rates of *C. saccharophila* in the control flasks were similar to other reports[6-7]. While the final population density in the ethanol treatment, Flask I-A', was only 1.4×10^6 cells·ml^{-1}, and the growth rate was 0.29×10^6 cells·ml^{-1}·day^{-1}. This growth rate was about 1/4 that of the control flasks. Therefore, there was a significant difference between control and ethanol treatments.

Daily growth rates of *C. saccharophila* in benthiocarb treatments with ethanol dilution, Flasks I-B ~ F, ranged from 0.03 to 0.14 cells·ml^{-1}·day^{-1}. The statistical analysis for the difference between two means of each trial is presented in Table 1. Significant differences were found between the control and other treatments. No significant differences, however, were observed among each benthiocarb treatment.

From the results mentioned above, it could be concluded that the growth of *C. saccharophila* was affected by ethanol which was used for the dilution of the concentrated benthiocarb. The algal growth rates were also affected by benthiocarb herbicede at 0.1 ppm and 0.3 ppm, but this was not statistically significant.

Daily growth rates of *C. saccharophila* in benthiocarb treatments, Flasks I-B ~ F, ranged from 0.03 to 0.14 cells·ml^{-1}·day^{-1}. The results showed rather irregular distribution, and there were no significant differences with the control or ethanol treatments. Slightly lower values were found in the case of 0.1 ppm and 0.3 ppm benthiocarb treatments. Growth rates in Flask E (0.1 ppm) and Flask F (0.3 ppm) were relatively lower than those of the other treatments.

Brachionus test Exp. II

Population growth rates, feeding rate, egg production rate, and pH variation in each trial are shown in Table 3 and Fig. 3.

Daily population growth rates observed in Exp. II ranged between 172.6%·day^{-1} in Flask II-E (0.1 ppm) and 201.1%·day^{-1} in the control flasks. No significant differences were obtained in the growth rates by statistical analysis. This means that the population increased 1.7 times per day in Flask E and 2.0 times per day in the control flasks.

The same trend was also observed in feeding rates of *B. plicatilis* in Exp. II; that is, the feeding rates in the experiments ranged from 260 to 301 × 10^3 cells·ind^{-1}·day^{-1}. The maximum rate was found in Flask II-F which contained the highest benthiocarb concentration. The minimum rate was observed in Flask B with regulated herbicide at 0.003 ppm. The mean feeding rate in the control flasks was calculated to be 282 × 10^3 cells·ind^{-1}·day^{-1}.

Egg production rates and pH variations in the control flasks showed a relatively higher average

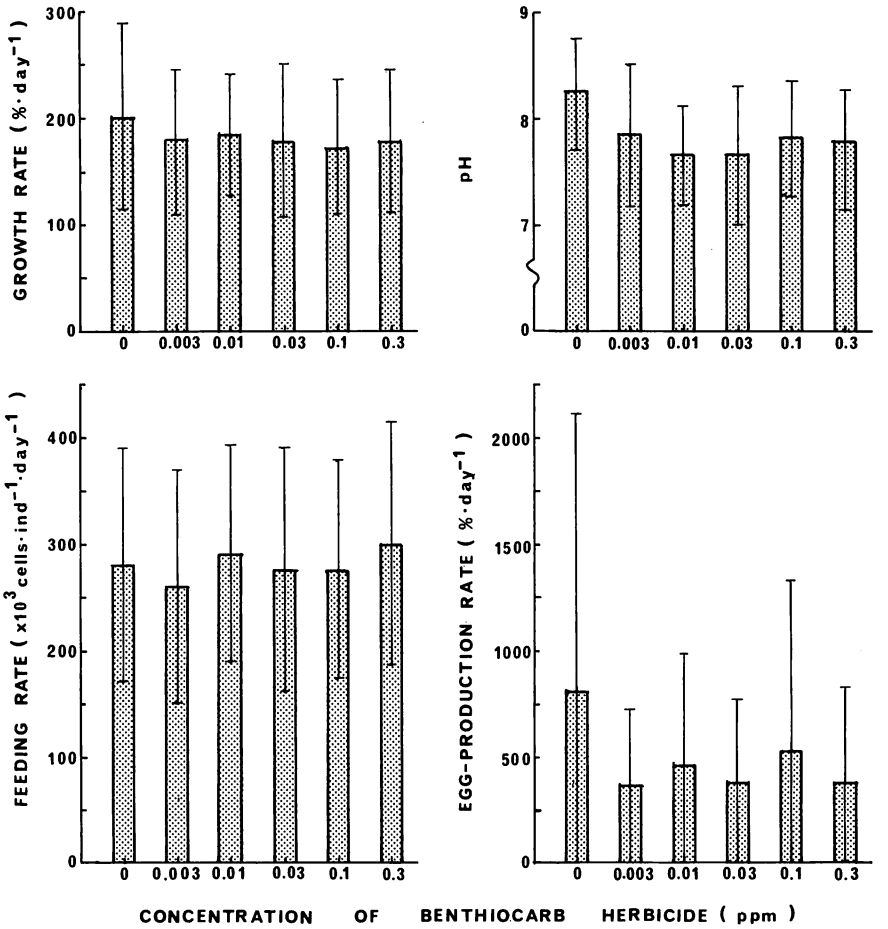

Fig. 3. Population growth, feeding, and egg production rates of *Brachionus plicatilis* and pH variation under different concentrations of benthiocarb herbicide.

Table 3. Population growth, feeding, and egg production rates of *Brachionus plicatilis* and pH variation in culture water under different concentrations of benthiocarb herbicide. Unit ppm indicates the herbicide concentration.

Trial	Flask	Growth rate ($\%\cdot day^{-1}$)	Feeding rate ($\times 10^3$ cells$\cdot ind^{-1}\cdot day^{-1}$)	Egg production rate ($\%\cdot day^{-1}$)	pH
Control	II-A	201.1	282	805	8.25
0.003 ppm	II-B	180.8	260	359	7.85
0.01 ppm	II-C	182.9	291	453	7.66
0.03 ppm	II-D	177.8	276	363	7.66
0.1 ppm	II-E	172.6	275	523	7.81
0.3 ppm	II-F	178.4	301	374	7.70

than those in the herbicide treatments. There were, however, no significant differences found among the herbicide treatments in Flasks B~F. Therefore, the difference between control and herbicide treatments (Flasks II-B~F) might have been caused by the ethanol effect as observed in Exp. I.

From the results mentioned above, it can be concluded that benthiocarb concentration below 0.3 ppm has no adverse effect on population growth of *B. plicatilis*. Some effects of ethanol used for dilution of the herbicide were found in the egg production of *B. plicatilis* and in pH variations in the water. Caution in the utilization of ethanol for dilution is suggested for practical use. Effects of pH are also considered in examining the toxicity[8-9].

The standard deviations calculated for average determinations were rather high in each experiment. This might have been due to unstable culture techniques[3]. In order to minimize the standard deviations, methods should be modified to determine the exact performance of the planktonic organisms during the culture experiments.

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