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Effects of the Insect Growth Regulator Dimilin on the Survival Rate of Larvae, Adults, and Egg Viability of *Tigriopus japonicus* Mori (Copepoda ; Harpacticoida)

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The effects of insect growth regulator Dimilin which interfere with the synthesis of chitin in the cuticle of insect larvae were investigated at various concentrations using the copepod larvae of *Tigriopus japonicus* Mori. The larvae were cultured at control, 1, 5, 10, 20, 50, and 100 ppb Dimilin solutions and three replicate experiment were carried out to give correct analysis. Lethal effects of Dimilin on larvae of *T. japonicus* occurred above 1 ppb Dimilin solution after 8 days. LC_{50} of larvae was 50 ppb Dimilin on the 4th day. Lethal effects of Dimilin on adults of *T. japonicus* occurred above 20 ppb Dimilin solution after 13 days. LC_{50} of adults was 50 ppb Dimilin on the 12 days. Egg viability has little relation to Dimilin solution concentrations.

key words : dimilin, larvae, egg viability, *Tigriopus japonicus*, copepoda

1. INTRODUCTION

The insect growth regulator Dimilin (1-[4-chlorophenyl]-3 [2,6-difluorobenzoyl]-urea) is a experimental insecticide which acts to inhibit chitin synthesis. Dimilin which has an influence to the target species only, was produced in Thomson-Hayward Chemical Company, U. S. A. (1974) and utilized to get rid of the harmful insects, such as gypsy moths, cotten bollweevils and folia feeders on soy beans causing unfavorable harvest, and also to control freshwater mosquitoes (Jakob, 1973 ; Tester and Costlow, 1981 ; Cunningham, 1982 ; Mulder and Gijswijt, 1973 ; Yu and Terriere, 1977).

Mulder and Gijswijt (1973) revealed the effect of Dimilin which disturbs endocuticular deposition in insects with histological examination. Yu and Terriere (1977) showed that Dimilin in house fly larvae results in decrease in the metabolizing ability of the enzyme β-ecdysone and disrupts chitin formation by inhibiting the metabolism of ecdysone.

Since the larvae of insects and crustaceans are similar in the viewpoint of chitin formation and molting behaviors, the effects of Dimilin on crustacean have also been studied in several laboratories. Cunningham (1976) indicated that *Artemia* sp. nauplii exposed to concentrations greater than 10 ppb would not survive beyond 3 days. Christiansen *et al.* (1978) found severe effects on larvae of two estuarine crabs when exposed to Dimilin. Costlow (1979) observed that zoeal larvae of *Menippe mercenaria*, the stone crab, could not survive at 0.5 ppb Dimilin. Christiansen and Costlow (1980) found that levels of Dimilin which were toxic to insects were also toxic to zoeae of crabs. Nimmo *et al* (1979)

showed that Dimilin was acutely and chronically toxic to the estuarine mysid *Mysidopsis bahis* at such low levels as 2.1 ppb and 1.24 ppb respectively. Kim and Lee (1987) suggested that *Balanus albicostatus* nauplii exposed to higher concentration than 50ppb would not survive to the cyprid stages. Kim (1992) recorded that the lethal concentrations of dimilin, alachlor and atrazin on the larvae of *caridina denticulata denticulate*. Tester and Costlow (1981) reported the effects of Dimilin on fecundity and egg viability of the marine copepod *Acartia tonsa*. Anita *et al.* (1985) also reported the influence of Dimilin on the growth of *Tigriopus californicus*. *Tigriopus japonicus* Mori has wide tolerance of temperature and salinity, which is commonly found in the estuarine waters of Korea, Japan and China (Ito, 1969). Therefore, both larvae and adults of this species always exposed to the pesticides and pollutants discharged from land and rivers. The present study is investigated to determine the effects of insect growth regulator, Dimilin, on the survival of larvae, adult, and egg viability of *T. japonicus*.

2. MATERIALS AND METHODS

2.1. Materials

Tigriopus japonicus were collected from the intertidal region in the Dong-Baek Island, Haeun-dae, Pusan from June to August 1994. The collected copepods were placed in a 500 ml beaker with filtered seawater and sorted into the ovigerous and non-ovigerous females and males, respectively. The sorted copepods were transferred into separate beaker with 500 ml filtered seawater of 33.3‰. Laboratory cultures were kept at room temperature (18±4℃) while constant temperature chambers were used to maintain temperature during the experiments. All stock cultures were fed every day with yeast and diatom *Dunaliella tertiolecta* in concentration of approximately 2.0×10^4 cells. All stock cultures were kept at 20℃ in culture chamber with a light regime of 14:10 hr L : D.

2.2. Dimilin concentrations

One ml of the stock solution of 1 ppt Dimilin was added to 1 *l* of 30‰ sea water to make a working stock solution. This working stock solution of 1 ppm Dimilin was prepared daily and mixed sufficiently, which was diluted with 30‰ sea water to make concentrations of 1, 5, 10, 20, 50, and 100 ppb Dimilin, concentrations as required. Standard controls were 33.3‰ sea water.

2.3. Survival

For the measurement of larval survival rate, one ovigerous female was placed in each Petri dish with 10 ml of control, 1, 5, 10, 20, and 50 ppb Dimilin, respectively. The used seawater, media and food were replaced daily. This experiment was replicated 3 times for the control, 1, 5, 10, 20, and 50 ppb concentration. The experiment measuring larval survival rate was started from the day which nauplii of *T. japonicus* showed their first appearance. The number of living nauplii was recorded every day.

In the case of adult survival rate, ten adults of this species were placed in each of Petri dishes with 10ml of control, 5, 10, 20, 50, and 100 ppb Dimilin. Ten adults of *T. japonicus* were placed in petri dishes with 10 ml of control, 5, 10, 20, 50, and 100 ppb Dimilin, respectively. The number of living adult copepoda was recorded every day. The death of larva and adult of *T. japonicus* was confirmed under stereomicroscope.

After one ovigerous female was placed in each Petri dishes with 10 ml of control, 1, 5, 10, 20, and 50 ppb Dimilin, the number of hatched nauplius from this female was recorded for 48 hours. Because it is necessary to count the number of nauplii as soon as hatched. The number of unhatched eggs in the bottom of each Petri dish was recorded, and regarded as the died egg. Egg viability rate (E.V.R.) was calculated as follows ;

$$E.V.R. = \frac{\text{number of hatched nauplii}}{\text{total number of eggs}} \times 100(\%)$$

The presented data are mean values obtained from at least three experiments. Statistical analyses were performed using standard analysis -of-variance technique applicable to randomized block experiments (Snedecor, 1967) ;

$$X^2 = \frac{(C-O)^2}{C}$$

O, Individual number of Dimilin-treated group ;
C, Individual number of control

3. RESULTS

3.1. Effects of Dimilin on the survival rate of *Tigriopus japonicus* larvae

Effects of Dimilin on the larval survival of *T. japonicus* were similar to those of non-target organisms. Larval survival rate was not significantly affected by 1 to 4 day exposure at the concentrations of 1 to 20 ppb Dimilin. On the other hand, in 50 ppb Dimilin, survival rate was 53.85% on the 4th day, and all larvae died when exposed for 8 days (Fig. 1).

It takes 12 days to death for all larvae in 1 ppb Dimilin ; 11 days in 5 ppb Dimilin, 9 days in 10 and 20 ppb Dimilin. The larval survival rate was declined so steeply in relation to the

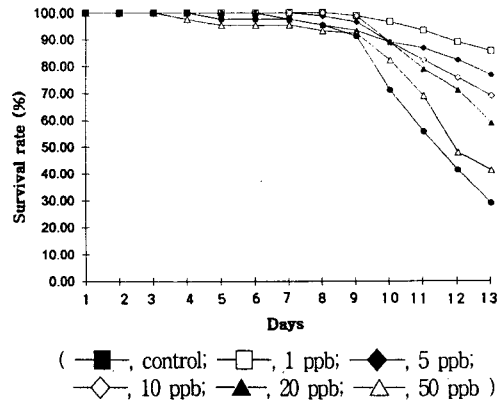

Fig. 1. The survival number of larvae of *Tigriopus japonicus* at various concentrations of Dimilin. Each value is the average of three replicate experiments.

increment of Dimilin. All tested larvae were died after 12 days of treatment, except the control (Fig. 1). All larvae survived in control for 8 days, then, 95.00% of larvae survived to the last in toxic materials (Fig. 1). Lethal effects of Dimilin on the larvae of *T. japonicus* were shown above 1 ppb Dimilin after 8 days. When LC_{50} was defined as those in which 50% death of the larvae, LC_{50} of larvae *T. japonicus* was 50 ppb Dimilin on the 4th day.

Table 1. The X^2 value of the survival rate of the larvae of *Tigriopus japonicus*.

	X^2 value				
days	1ppb	5ppb	10ppb	20ppb	50ppb
1	0	0	0	0	0
2	0	0	0	0	2.25
3	0	0	0	0	9.61*
4	0	0	0	0	21.16*
5	0	0	0.36	2.24	29.16*
6	0.36	0	1.14	14.44*	59.29*
7	1.44	16*	8.41*	14.44*	84.64*
8	3.24	16*	57.76*	72.25*	100.00*
9	30.69*	59.21*	95.00*	95.00*	95.00*
10	62.41*	70.78*	95.00*	95.00*	95.00*
11	72.52*	95.00*	95.00*	95.00*	95.00*
12	95.00*	95.00*	95.00*	95.00*	95.00*
13	95.00*	95.00*	95.00*	95.00*	95.00*

X^2 values with asterisk show significant difference ($p<0.05$).

The X^2(chi square)-value of the larval survival rate appears in Table 1. On the 9th day, the survival rates of all the Dimilin-treated groups differed significantly ($p < 0.05$) from the survival rate of the control group.

3.2. Effect of Dimilin on the survival rate of *Tigriopus japonicus* adults

The survival rate of adult of *T. japonicus* ranged from 91.11 % (100 ppb dimilin) to 98.89 % (control) for 9 days (Fig. 2). Dimilin had no significant effects on the adult survival rate up to the 5th day even at the highest concentration of 100 ppb. The survival rate of adult was declined slightly after 9 days (Fig. 2). Death of adult was observed in most of the groups since then, mortality of adult copepod increased significantly as the concentration of Dimilin increased (Fig. 2).

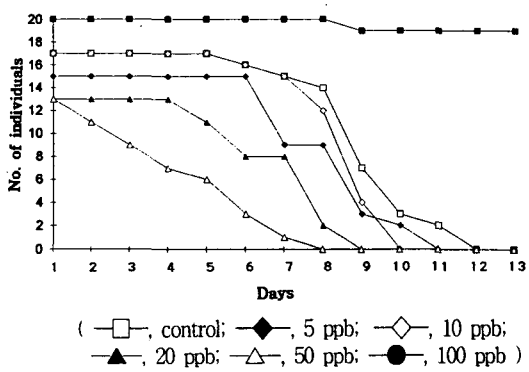

Fig. 2. The survival rate of adults of *Tigriopus japonicus* at various concentrations of Dimilin. Each value is the average of three replicate experiments.

The survival rate of adult in 50 and 100 ppb Dimilin, especially, showed significant differences from those of the control on the 12th day. The survival rate of adult was 47.78% in 50 ppb Dimilin, 41.11% in 100 ppb Dimilin and 88.89% in the control (Fig. 2). LC_{50} of adult was the 50 ppb on the 12th day.

Table 2 shows the X^2-value (chi square) of the survival rate of adult. On the 13th day, the survival rates in 20, 50 and 100 ppb Dimilin differed significantly ($p < 0.05$) from the survival rate of the control.

Table 2. The X^2 value of the survival rate of adult of *Tigriopus japonicus*.

	X^2 value					
days	1ppb	5ppb	10ppb	20ppb	50ppb	100ppb
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0.04	0
5	0	0	0	0	0.16	0.04
6	0	0	0	0	0.16	0.04
7	0	0	0	0.04	0.16	0.04
8	0	0.01	0	0.16	0.49	0.16
9	0	0.04	0	0.36	0.49	0.65
10	0	0.66	0.66	0.66	2.32	6.97*
11	0	0.39	1.30	2.11	6.19*	14.72*
12	0	0.55	1.62	3.64	18.89*	25.89*
13	0	0.94	3.36	8.48*	23.55*	37.78*

3.3. Effect of Dimilin on the egg viability of *Tigriopus japonicus* eggs

The experiment on the effect of Dimilin on the eggs of female *T. japonicus* exposed to 1, 5, 10, 20 and 50 ppb Dimilin for 48 hours did not represent a significant differences (Fig. 3). A female of *Tigriopus japonicus* laid 15 eggs, 14 eggs of them were hatched on the average. The mean of hatching rate was 92.37 %. The lowest hatching rate was about 80.02% in 5ppb Dimilin, and all eggs were hatched in 1 ppb Dimilin and control (Fig. 3). The hatching rate tended to decrease slightly as Dimilin concentration increased (Fig. 3).

The egg hatching time varied with different Dimilin concentrations. When each ovigerous

female was kept in control and 1 ppb Dimilin, the egg hatching was completed within 3-4 hours. The eggs, however, in 5, 10, 20 and 50 ppb Dimilin began to hatch after 9-11 hours.

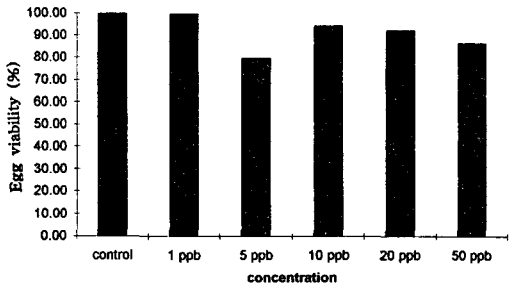

Fig. 3. The egg viability of *Tigriopus japonicus* at various concentrations of Dimilin.

4. DISCUSSIONS

The effect of Dimilin on non-target organism and crustacean larvae have been referred to many papers. (Henzell *et al.*, 1976 ; Chaffoy *et al.*, 1978 ; Ascher *et al.*, 1980 ; Christiansen *et al.*, 1982).

In the present study, Dimilin was clearly injurious to larvae of *Tigriopus japonicus* at concentration as low as 1 ppb. Acute toxicity (96 hour LC_{50}) was found to be 50 ppb Dimilin. Christiansen *et al.* (1978) reported that the survival rate of *Rhithropanopheus harrisii* larvae was affected significantly at concentration of 1 and 3 ppb, and survival rate of *Sesarma reticulatum* larvae was significantly lower than in the control. It is worth comparing the effects of Dimilin in the species mentioned above with the effects of Dimilin in target species such as mosquitoes. When the fourth instars of the mosquito *Culex pipiens quinquefasciatus* were exposed to Dimilin, 50 % survival rate was occurred at 0.6 ppb and 10 % survival rate at 1.3 ppb (Mulla *et al.*, 1974). According to the Thompson-Hayward Chemical Company (1974), black salt-mosquito *Aedes taeniorhynchus* larvae at concentrations of 1 and 3 ppb Dimilin were controlled to 96 to 100 %, respectively. Thus the lethal concentration for the larvae of mosquitoes might be lethal for the larvae of several crustaceans. One should, therefore, be extremely caution to use Dimilin in areas where the larvae of these crustacean occur.

In the case of the survival rate of adults of *T. japonicus* showed littles changes for a days : 91.11 to 98.89 % at 100 ppb Dimilin and control, respectively (Fig. 2). The survival rate of adults, was slightly decreased with the increment of exposure time to Dimilin from 1 to 9 days : on the 12th day, 41 to 89 %, and on the 13th day, 29 to 86 % at 100 ppb and control, respectively. From the results of the present study, it was found that Dimilin also was lethal to adults of *T. japonicus*. According to Antia (1985), when adults of *T. californicus* were exposed to Dimilin for 71 days, the survival rate of adults decreased slowly at low concentration of 1 ppb Dimilin : on the 28th and 36th day, 71.4 % and 28.6 %, respectively. On the other hand, the survival rate of the Dimilin treated adults of *Acartia tonsa* was similar to the survival rate of the control for 5 days (Tester and Costlow, 1981). According to Mulder and Gijswijt (1973) and other authors, death caused by Dimilin appeared to be invariably connected with ecdysis in insects. Treated larvae of *Pieris brassicae* at all stages remained seemingly unaffected till the process of apolysis (i. e., the separation of the old cuticle from the underlying epidermis), which precedes the actual shedding of the exuvia, but the molting process stagnated somewhere during this state. The insecticidal activity manifests itself as a failure of moulting or pupation, resulting in

death. Thus Dimilin on the any arthropod experienced a terminal molt have little effects on survival. However, a few species of crustaceans such as copepods and crabs, continue to moult for growth during adult period. It may be expected that the survival rates of adults of copepodes decrease if adults were exposed to Dimilin for a long time.

Tolerance on Dimilin between two species of copepods and two species of crabs was compared with 10 ppb Dimilin (Fig. 4). Both *T. japonicus* and *T. californicus* had more tolerance than *R. harrisii* and *S. reticulatum*, through they inhabit same estuarine regions.

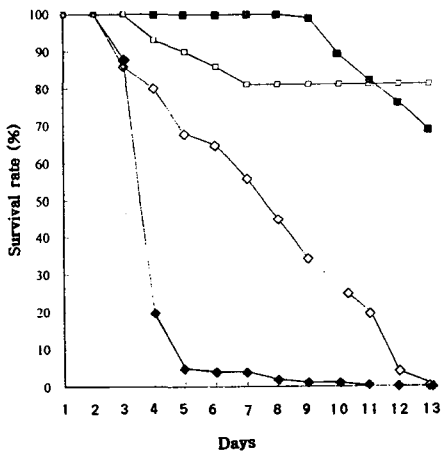

Fig. 4. Comparison of the survival rate of adult of *Tigriopus japonicus* with the other crustaceans at 10 ppb Dimilin.

The eggs from non-treated female *T. japonicus* which were placed in various concentrations of Dimilin hatched normally (Fig. 3), because these eggs were already formed. The egg viability rates in each concentration of Dimilin were similar to those in control. Tester and Costlow (1981) showed the contrasting study that the eggs produced by female *A. tonsa* exposed to 1 and 10 ppb Dimilin for 12 to 60 hours did not hatch ; nauplii were observed to move inside the egg membranes. Dimilin delayed hatching time in *T. japonicus*. It took about two hours for all eggs in an egg sac to finish hatching in all Dimilin treated groups and the control. These results were different from those of *A. tonsa* eggs in various concentrations of Dimilin. Most rapid development of eggs of *A. tonsa* were associated with the highest concentration of Dimilin, and abnormally developed nauplii from the eggs were not observed. With respect to the effects of Dimilin in *T. japonicus*, these results indicate that toxic effect must be fully considered. Although Dimilin was designed to function as a safe insecticide, it is injurious to valuable crustacean and may be harmful to other unexamined arthropods.

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