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Effects of the Insect Growth Regulator Dimilin on Larval Development of *Hemigrapsus sanguineus* (Crustacea, Brachyura) Reared in the Laboratory

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ABSTRACT

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 crab larvae of *Hemigrapsus sanguineus*. The larvae were cultured at control, 0.5, 1, 5 and 10 μgL^{-1} Dimilin solutions and three replicate experiments were carried out to give correct analysis. Significant differences in percent mortality have occurred between control and 10 μgL^{-1} when the larvae were exposed to Dimilin whereas no differences were found between 5 and 10 μgL^{-1} Dimilin concentrations.

If lethal concentration is defined as concentration at which less than 10 percent of crab larvae reach to the last zoeal stage from hatching it can be concluded that insect growth regulator Dimilin is lethal to the larvae of *Hemigrapsus sanguineus* at 5 and 10 μgL^{-1} Dimilin.

INTRODUCTION

Many investigations on the pesticides have been conducted for the last three decades so as to produce ingredients which inhibit only target species without severe influence to the ecosystem (Pickering *et al.*, 1962; Mulla *et al.*, 1974; Miura and Takahashi, 1975; Bookhout *et al.*, 1984). The insect growth regulator Dimilin, which has an influence to the only target species, was produced in Thompson-Hayward Chemical Company, U.S.A. (1974) and was utilized to get rid of the harmful insects gypsy moths, cotton bollweevils and foliar feeders on soy beans causing unfavorable harvest, and also to control freshwater mosquitoes (Tester and Costlow, 1981; Cunningham, 1982).

Histological examination by Mulder and Gijswijt (1973) revealed the effect of Dimilin which intrudes the deposition of endocuticle in insects. It was suggested that Dimilin and other related chemicals interfere with the formation of chitin by inhibiting the activity of the enzyme chitin

synthetase (Post *et al.*, 1974). Yu and Terriere (1977) showed that the insect growth regulator Dimilin in house fly larvae results in reducing in the metabolizing ability of the enzyme β-ecdysone and disrupts chitin 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 insect growth regulator Dimilin on crustaceans have also been studied for the last few years.

Christiansen *et al.* (1978) revealed that concentration of insect growth regulator Dimilin being toxic to insects are also toxic to the zoeal larvae of crabs. Kim and Lee (1987) suggested that *Balanus albicostatus* nauplii exposed to higher concentration than 50 μgL^{-1} would not survive to the cyprid stage. Thus the present study was conducted to investigate the effects of the insect growth regulator Dimilin on the crab larvae of *Hemigrapsus sanguineus*. The reason why the larvae of *H. sanguineus* was selected as experiment material was that this species commonly inhabits the coastal areas of Korea, and the method for larval rearing and developmental pattern in the laboratory have also been well established (Kim and Moon, 1987).

MATERIALS AND METHODS

Technical grade (TG) Dimilin (1-[4-chlorophenyl]-3 [2.6-diflubenzoyl]-urea) which is commonly called diflubenzuron was obtained from Thompson-Hayward Chemical Company, Kansas, U.S.A. Dimilin which is white crystalline solid has extremely low solubility of almost 0.2 mgL^{-1} at 20°C water (Fig. 1). Aceton was used as a carrier or solvent in the present experiment because it is soluble in organic solvents.

One gL^{-1} Dimilin solution was prepared as stock solution by dissolving appropriate amounts in ACS aceton and was stored at about 4°C. From this stock solution, working solution of 1 mgL^{-1} Dimilin was made daily in the filtered seawater of 33 parts per thousand. To this working solution, the filtered seawater was added to make 4 different Dimilin concentrations of 0.5, 1, 5 and 10 μgL^{-1} and the Dimilin solutions of each concentration were used as culture solution for crab larvae.

Ovigerous crabs *H. sanguineus* were collected from the intertidal zone of Haeundae near Pusan, Korea from July through September 1989. The crabs were placed in circular aquariums and the flesh of clams *Tapes philippinarum* were fed. The examination for the maturity of egg mass was made under a stereomicroscope. After the microscopic inspection, crabs with matured eggs were put into 1,000 ml beaker, which has been filled with the filtered seawater containing antibiotic.

Adult crabs were kept under room temperature. The crab larvae were cultured in cabinets at a photoperiod of 14 h light and 10 h dark and a constant temperature of 25°C shortly after hatching as zoeal larvae. The crab larvae were fed brine shrimp *Artemia* nauplii daily. The larvae were reared in the Carolina Culture bowls with inner diameter of 8 cm and 2.5 cm in depth. Ten larvae were reared in each bowl containing about 60 ml solution and the number of total larvae at each concentration was 150 from the same hatching. This experiment was repeated 3 times. Alive, and dead larvae as well as exuviae were recorded daily. Alive larvae were transferred to the clean Dimilin solutions of each concentration containing a few drops of freshly hatched *Artemia* nauplii.

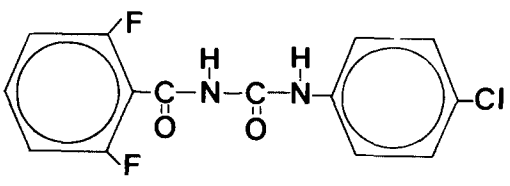

Fig. 1. Structural formula of the insect growth regulator Dimilin.

RESULTS AND DISCUSSION

The sensitivity of the crab, *Hemigrapsus sanguineus*, larvae to Dimilin of several concentrations was varied at various zoeal stages but significant differences were found between control group and 5 and 10 μgL^{-1} Dimilin treatment group in this species. At 0.5 μgL^{-1} Dimilin concentration, mortality was not considerably higher than control group whereas almost 100 percent of the zoeal larvae did not survive at 5 and 10 μgL^{-1} Dimilin treatments.

Percent survival of the crab larvae reared in the laboratory showed that the larval development of the crab was influenced by variations of Dimilin concentrations. However, significant differences in the survival did not occur between 5 μgL^{-1} and 10 μgL^{-1} Dimilin concentrations even though these experiments were repeated three times for all the Dimilin concentrations. Among the crab larvae exposed to 10 μgL^{-1} Dimilin shortly after hatching, about 12 percent larvae molted to the succeeding stage whereas 96 to 97 percent larvae exuviated in the control (Fig. 2).

A number of zoeal larvae died in the course of molting behavior to the subsequent stage and deformed swimming setae, rostral spine and dorsal spine in the experimental groups. Therefore many larvae could not reach to the next stage and showed abnormality, especially at 5 μgL^{-1} and 10 μgL^{-1} Dimilin (Table 1). According to the result of the present study, there was a considerable delay in the larval development of *H. sanguineus* when the zoeal larvae were treated with Dimilin of 0.5 μgL^{-1} and 1 μgL^{-1} concentrations. Christiansen *et al.* (1978) also observed significant delay in the developmental duration of crab *Rhithropanopeus harrisii* by the time the larvae were exposed to 0.01 ppm and 0.1 ppm methoprene from hatching. Thus it could be explained that there could be a cumulative effect of the chemical agent in low rather than in high concentrations.

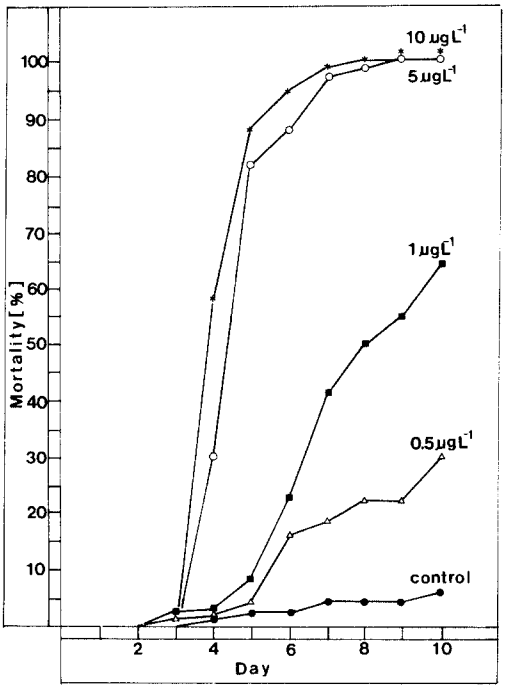

Fig. 2. Survival of zoeal larvae exposed to 0.5 μgL^{-1}, 1 μgL^{-1}, 5 μgL^{-1} and 10 μgL^{-1} Dimilin concentration (average of three replicates).

Table 1. The number and percentages of molting at 4 concentrations of Dimilin. Initial zoeal population was 150 in each concentration (average of three replicates)
–: no data, Z: stage of zoea

Concentration (μgL^{-1})	Z1–Z2		Z2–Z3		Z3–Z4	
	No.	%	No.	%	No.	%
control	135	90.0	123	83.0	45	30.0
0.5	125	83.3	88	58.7	43	28.7
1	115	76.7	67	44.7	30	20.0
5	27	18.0	17	11.3	—	—
10	18	12.0	—	—	—	—

Thompson-Hayward Chemical Company (1974) reported that the chitin synthesis of a number of insect species could be inhibited by the insect growth regulator Dimilin. For instance, when *Aedes taeniorhynchus* larvae were exposed to this chemical agent, 96 to 100 percent larvae died at 1 μgL^{-1} Dimilin, whereas mosquito *Culex pipiens* caused 95 percent mortality at 6 μgL^{-1} and 20 μgL^{-1} Dimiln treatments for the second and the fourth instars, respectively. The result of experiment carried out by Cunningham (1976) indicated that brine shrimp *Artemia* nauplii, nontarget species, exposed to Dimilin greater than 10 μgL^{-1} would not survive more than 3 days. The experiment using crab larvae as test material by Christiansen *et al.* (1978) showed that the first zoeal larvae of crab, *Rhithropanopeus harrisii,* exposed to 10 μgL^{-1} Dimilin for various days during intermolt period appeared greater than 95 percent mortality and the larvae were more sensitive to this chemical agent late than early in the period. According to the result of the present study, mortality of *H. sanguineus* larvae was found to be increased with increasing dosages, but significant effects were not shown up to 4 days even at the highest treatment of 10 μgL^{-1} concentration (Fig. 3).

Ultrastructural study on the formation of larval cuticle of Dimilin-treated nontarget species *R. harrisii* by Christiansen and Costlow (1982) revealed that the larvae deformed endocuticle and exocuticle while the formation of epicuticle was not affected. Therefore, they explained that the

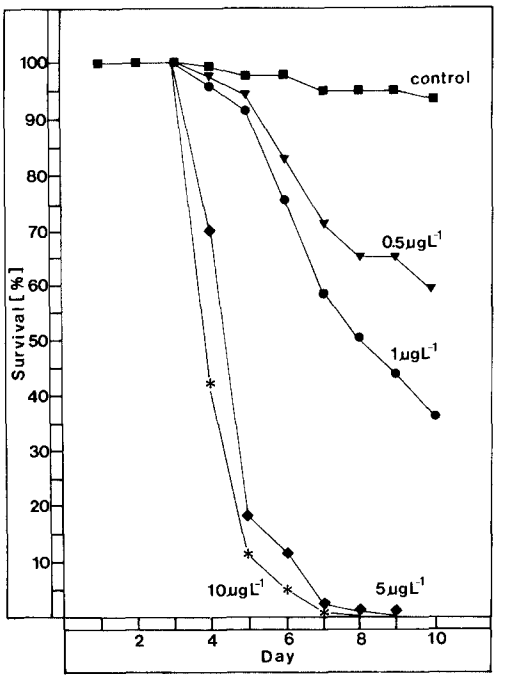

Fig. 3. Cumulative mortality at various concentrations of Dimilin (average of 3 replicates at each concentration).

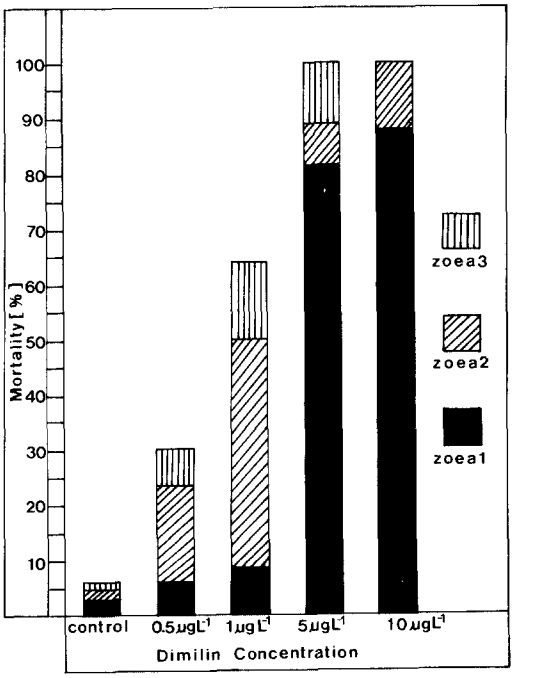

Fig. 4. Mortality of each larval stage at various concentrations of Dimilin (average of three replicates). Total number at each concentration was 150 individuals.

major reason the larvae were not alive was due to not resisting the muscular contraction and the increased tugor which occurred during molting procedure. Similar results also occurred to the crab larvae of the present study exposed to various Dimilin concentrations. At 5 and 10 μgL^{-1} Dimilin, clearly 82 and 88 percent larvae respectively failed to molt to the second zoeal stage from hatching because of the muscular contraction and the incomplete cuticle formation, and almost 100 percent larvae could not arrive at the third zoeal stage at 10 μgL^{-1} Dimilin concentration (Fig. 4).

Although several investigators have studied effects of Dimilin and other related chemical agents on marine invertebrates and insect larvae (Jacob, 1973; Mulla *et al.*, 1974; Tester and Costlow, 1981), they have not figured out the influence of the actual pesticide concentrations in the field and estuarine areas where zooplankton occurs. Therefore it is difficult to predict the influence of the insect growth regulator Dimilin on the environment correctly. However, we should be cautious in using Dimilin for insect control in estuarine field where crab larvae inhabit.

적 요

곤충 유충에 있어 각피의 키틴합성을 저해하는 곤충성장억제제인 Dimilin의 영향에 대해 무늬발게 유생을 실험종으로 하여 여러 농도에서 실험하였다. 무늬발게 유생은 대조군과 0.5, 1,5 그리고 10 μgL^{-1} 농도에서 사육되었으며, 본 연구의 정확한 분석을 위하여 실험은 3회 반복 시행되었다. 대조군과 10 μgL^{-1} 농도에 있어 사망율은 현저한 차이가 났으나 5 μgL^{-1} 농도와 10 μgL^{-1} 농도에서는 현저한 차이점을 발견할 수 없었다. 치사농도를 10% 이하의 게유생이 부화후 마지막 zoea 유생기에 도달하는 것으로 정의한다면 곤충성장억제제인 Dimilin은 5 μgL^{-1}와 10 μgL^{-1} 농도에서 무늬발게 유생에 대해 치사영향을 준다고 할 수 있다.

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