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Effects of Dimilin on Larval Development of the Portinid Crab *Liocarcinus corrugatus*

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주름꽃게의 유생발생에 미치는 Dimilin 의 영향

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ABSTRACT

Laboratory experiments were conducted to determine the effects of Dimilin, an insect growth regulator which acts to inhibit chitin synthesis, during the larval development of *Liocarcinus corrugatus* Pennant.

The larvae was exposed to control (10 ppb acetone sea water and untreated sea water solution) and five concentrations 0.1, 1.0, 5.0, 10.0 and 25.0 ppb of both TG and WP-25 formulations of Dimilin from the hatching to the megalopal stage, and the effect of Dimilin on development of the larvae were determined. Two formulations (TG and WP-25) had different effect on the different stages in *L. corrugatus*, and early stage larvae of *L. corrugatus* were more sensitive to TG than to WP-25. Concentrations of diflubenzuron $>$5.0 ppb are lethal to *L. corrugatus* larvae upon chronic exposure. Lethal concentrations are defined here as those in which less than 10% of the larvae survived to the megalopal stage. However, Dimilin (TG and WP-25) showed no significant effects on developmental time of *L. corrugatus* larvae.

Key words: Dimilin, Diflubenzuron, *Liocarcinus corrugatus*, Crab, Larvae.

INTRODUCTION

Dimilin or diflubenzuron (1-[4-chlorophenyl]-3[2, 6-difluorobenzoyl]-urea) was designed to control insect infestation by interfering with cuticle deposition during insect growth through moulting or ecdysis (Jacob 1973).

At the biochemical level, Dimilin and related compounds are believed to inhibit development of one or more steps in the chitin biosynthetic pathway (Post *et al.* 1974, Deul *et al.* 1978). On the other hand, Ishaaya and Casida (1974) found an increase in chitinase activity and a simultaneous decrease in chitin in the cuticle of Dimilin-treated housefly larvae.

Since the larvae of insects are similar to crustaceans in chitin formation and molting behaviors, the effects of Dimilin on crustacean have also been studied for the last few years. Christiansen *et al.* (1978) found that Dimilin has severe effects on larvae of two estuarine crabs. Costlow (1979) observed that zoeal stages of *Menippe mercenaria* could not survive at concentration of Dimilin 0.5 ppb. Christiansen and Costlow (1980) found that levels of Dimilin which are toxic to larvae of insects are also toxic to zoeae of crabs. Nimmo *et al.* (1979) showed that Dimilin was acutely and chronically toxic to the mysid *Mysidopsis bahis* at 2.1 ppb and 1.24 ppb, respectively.

Concern about the environmental impact of Dimilin led to extensive studies of the effect of Dimilin on aquatic crustaceans and other organisms (Antia *et al.* 1985, Kim and Jang 1987). Kim and Lee (1987) suggested that *Balanus albicostatus* larvae exposed a higher concentration of more than 50 ppb of Dimilin would not survive to the cyprid stage. Kim (1992) and Choi *et al.* (1997) recorded the lethal concentrations of Dimilin on the larvae of *Caridina denticulata denticulata* and *Tigriopus japonicus*, respectively.

Dimilin is an ododless white crystaline solid, however it is available in two major formulations: the technical grade (TG) and the wettable powder (WP-25). TG is the former which contains pure form of diflubenzuron (DFB) and is very insoluble in water, while WP-25 contains additives enhancing the solubility or dispersion of diflubenzuron in water. These additive or "formulation factors" can increase or decrease the toxicity of the pesticide directly by altering its chemical nature or the solubility, which in turn could influence the absorption of the toxicant into the biological system.

The present study determined the effects of different concentrations of Dimilin on survival, developmental duration and different toxicity of TG and WP−25 to larvae of portinid crab *Liocarcinus corrugatus*.

MATERIALS AND METHODS

TG (Technical grade) contains 98.4% and WP-25 (Wettable powder) contains 25% active ingredient of diflubenzuron. Both formulations were obtained from Thompson-Hayward Chemical Company, Kansas, U. S.A.. The TG formulation which is white crystalline solid has extremely low solubility of approximately 0.2 ℃ water. Acetone was used as a carrier or solvent for TG in the present experiment, because TG is moderately soluble in organic solvents. WP-25 formulation, on the other hand, contained talc (a solid diluent) and other ingredients (dispersing agents) which enhance the solubility of diflubenzuron in water.

Stock solutions of 1.0 ppt were prepared with acetone for TG, and with filtered seawater (33.3‰) for WP-25, and both were stored at 4 ℃. One ml of the stock solution of 1ppt Dimilin was added to 1L of 33.3‰ sea water for making stock solution. This working stock solution of 1ppm Dimilin was prepared and mixed daily and was diluted with 33.3‰ sea water to make different Dimilin concentrations of 0.1, 1.0, 5.0, 10.0, and 25.0 ppb. Ten ppb acetone seawater solution and 33.3‰ filtered seawater were served as controls for the TG and WP-25 formulations, respectively.

Ovigerous female crabs *L. corrugatus* having 5 zoeal and megalopal stages were collected from fisherman in Kijang, Kyungsangnam-do between June and July, 1992. The crabs were placed individually in a glass container of 300 mm diameter by 200 mm depth, filled with seawater of 33.3 ‰ and keep there till hatching.

Ten newly hatched larvae were kept in a 9 glass bowls containing approximately 80 ml solution per bowl, totaling 90 larvae per test concentration. The larvae were kept at 23℃ in a culture chamber with a photoperiod of 14:10 hr (L : D). This experiment was repeated three times. The numbers of dead larvae, the exuviae, and the developmental stage were recorded every day. The living larvae were transferred to clean bowls containing fresh solutions and fed newly hatched *Artemia nauplii*.

RESULTS

The survival of *L. corrugatus* larvae during the first 10 days to the megalopal stage was reduced with increasing concentrations of both TG and WP-25. (Figs. 1 and 3). In TG formulation, no larvae survived to day 10 at 5.0, 10.0, and 25.0 ppb

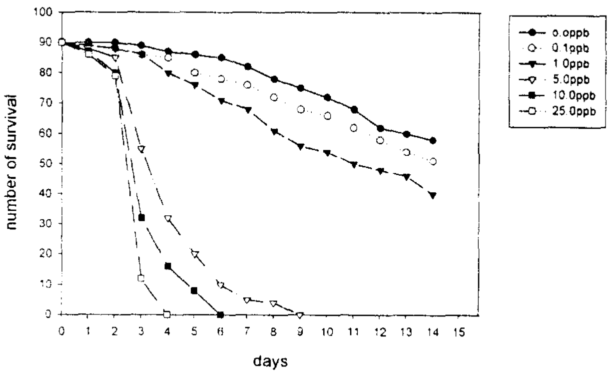

Fig. 1. Survival of *L. corrugatus* larvae in aceton control and 5 different concentrations of TG.

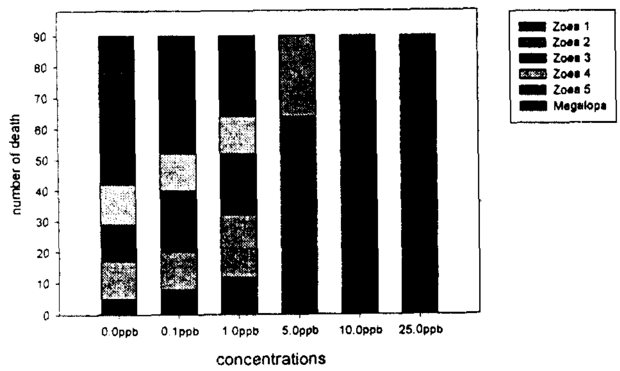

Fig. 2. Number of death of *L. corrugatus* larvae at each stage in aceton control and 5 different concentrations of TG.

concentrations and 100 percent mortality occurred on days 10, 7, and 5 for larvae exposed to the same concentrations, respectively.

In WP-25 formulation, 4 larvae in the third zoeal stage and 3 in the second or third zoeal stages survived to day 10 at 5.0 and 10.0 ppb concentrations, respectively. After 3~5 days they died and did not develop to the next zoeal stage. While larvae survived to day 10 at 25.0 ppb concentration. One hundred percent mortality occurred at days 15, 13, and 7 for larvae exposed to the concentration of 5.0, 20.0, and 25.0 ppb, respectively. This indicates that concentrations of diflubenzuron >5.0 ppb are lethal to *L. corrugatus* larvae upon chronic exposure except for TG at 1.0 ppb where only larvae of 2.22 % molted successfully. Lethal concentrations are less than 10% of the larvae survived to the megalopal stage (Christiansen *et al.* 1978).

For the two diflubenzuron formulations, the larvae of 26-28% that survived to day 10 eventually molted successfully to the megalopal stage at 0.1 and 1.0 ppb concentrations. Except for TG at 1.0 ppb, only 2% molted successfully. There are some differences between two diflubenzuron formulations at 5.0 ppb concentration. Only larvae of 29% of the first zoeae survived to the second zoeal stage and no larvae molted to the third zoeal stage in TG, whereas 88% of the first zoeae survived to the second zoeal stage and eventually 2 zoeal larvae molted to the fourth zoeal stage in WP-25 (Figs. 1~4). Larvae of 100%

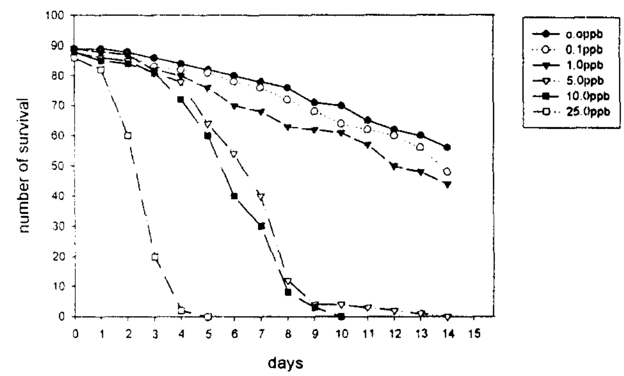

Fig. 3. Survival of *L. corrugatus* larvae in sea water control and 5 different concentrations of WP-25.

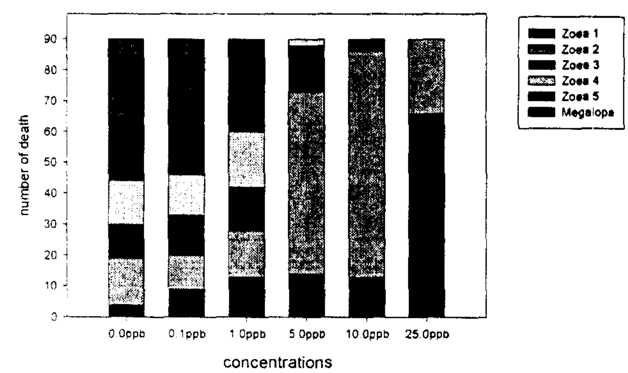

Fig. 4. Number of death of *L. corrugatus* larvae at each stage in sea water control and 5 different concentrations of WP-25.

did not molt to the second zoeal stage with 10.0 and 25.0 ppb concentrations of TG. Whereas larvae of 86% and 4% larvae each molted to the second and third zoeal stages, respectively, at 10.0 concentration of WP-25, and larvae of 28% survived to the second zoeal stage without molting to the next zoeal stage with concentrations of 25. 0 ppb of WP-25 (Figs. 2 and 4).

Duration of development from hatching to the megalopa for both TG and WP-25 was not affected by increasing diflubenzuron concentration (Tables 1 and 2). For each concentration, there is no difference in the effect of diflubenzuron on the duration of larval development.

DISCUSSION

The insecteide diflubenzuron (DFB) is commonly used in various places for suppression of gypsy moths in hardwood forests. Dimilin is potentially toxic to nontarget species because it can enter aquatic systems through aerial application or runoff after precipitation events (Fischer and Hall 1992).

Dimilin which has been tested in both laboratory and field experiments show relatively few adverse effects on nontarget species with the exception of some aquatic insects and small crustaceans (Mulla *et al.* 1974, Miura and Takahashi 1975). For *Aedes taenicrhynchus*, the same effect was found by Thompson-Hayward Chemical Company (1974). There was no effect on juvenile crayfish at 2.0 ppm Dimilin in a laboratory experiment, while field experiments showed that concentrations of 0.001 to 0.003 ppm Dimilin killed 96 to 100% of *A. taeniorhychus*. Also Dimilin killed cladocerans, clam shrimps, and tadpole shrimps at concentrations below 0.1 ppb or in no more than 24h to 48h of laboratory toxicity tests (Miura and Takahashi 1975). Reproduction also declined in brine shrimp, *Artemia salina*, exposed to 2.0 ppb (Cunningham 1976). Sublethal concentrations of Dimilin which were tested at 0.3 ppb affected swimming rates of larvae of the crab, *Rhithropanopeus harrisii*. Christiansen *et al.* (1978) found effects of Dimilin severe on larvae of two estuarine crabs *Rhithropanopeus harrisii* and *Sesarma reticulatum* when exposed to Dimilin. Lethal doses (7-10 ppb) for crab larvae were similar to those which are also lethal to several target species.

Dimilin is also lethal to the larvae of *L. corrugatus* at concentrations of 5.0 ppb. Lethal concentrations is when less than 10% of the larvae survive till the megalopal stage in this experiment. An increase in motality of larvae after hatching was found in the first stage (TG) and the second stage (WP-25) at 5.0, 10.0, and 25.0 ppb concentrations (Figs. 2 and 4). However, survival in 0.1 ppb concentration of both

Table 1. Developmental time of *L. corrugatus* larvae from hatch to megalopa. (－: no data)

TG Concentrations (ppb)	Zoea Ⅱ	Zoea Ⅲ	Zoea Ⅳ	Zoea Ⅴ	Megalopa
Acetone control	3.92 (0.69)	8.29 (1.35)	11.48 (1.54)	14.75(1.33)	19.89 (1.42)
0.1	4.37 (0.62)	8.47 (0.93)	12.07 (1.13)	15.53 (1.16)	20.89 (1.32)
1.0	4.41 (0.61)	9.43 (1.45)	12.95 (1.43)	16.58 (1.14)	22.00 (1.41)
5.0	4.42 (0.58)	－	－	－	－
10.0	－	－	－	－	－
25.0	－	－	－	－	－

Table 2. Developmental time of *L. corrugatus* larvae from hatch to megalopa. (－: no data)

WP-25 Concentrations (ppb)	Zoea Ⅱ	Zoea Ⅲ	Zoea Ⅳ	Zoea Ⅴ	Megalopa
Seawater control	3.77 (0.63)	8.69 (1.69)	12.32 (1.87)	15.30 (1.58)	20.24 (1.51)
0.1	5.00 (0.84)	9.47 (1.38)	12.19 (1.09)	15.84 (1.55)	20.84 (1.68)
1.0	4.86 (0.97)	9.26 (1.40)	12.92 (1.66)	16.27 (1.34)	21.58 (1.95)
5.0	4.66 (0.81)	8.76 (0.75)	13.00 (1.41)	－	－
10.0	4.40 (0.49)	8.00 (0.00)	－	－	－
25.0	4.86 (1.38)	－	－	－	－

diflubenzuron formulations was similar to that in the control group (Figs. 1 and 2). Also for each concentration, there is no difference in the effect of diflubenzuron on the duration of larval development when using either of the two formulations (Figs. 2 and 4). Dimilin had different effects on the different stages in *L. corrugatus*, and early larval stage of *L. corrugatus* were more sensitive to the TG than WP-25 (Tables 1 and 2). As a whole, authors demonstrated TG and WP-25 are similar in their efficacy in acute and chronic toxicity tests with *L. corrugatus* larvae. The results of the present investigation are different from those of Mulder and Gijswijt (1973). They observed that the toxicity of diflubenzuron with different particle sizes increased as the particle size of the active ingredient decreased. For example at 0.3 ppb, the mortality of *Pieris brassicae* was 6, 43, and 100 when the mean particle size of diflubenzuron used was 15, 7 and 4 μ, respectively. Since the particle size of diflubenzuron in WP-25 is much smaller than that obtained for TG. By the Mulder and Gijswijt (1973), it is expected that WP-25 is more toxic than TG at the same concentration. But from the present study, there is no significant difference between the two formulation used. One possible explanation for these differences is that the highest test concentration used in the present investigation (25.0 ppb) is far below the water solubility (0.2 ppm) of diflubenzuron at 20℃. Thus, there was probably no crystallization when acetonic solution of the TG formulation was added to seawater as suggested by Cunningham (1982). Probably at concentrations greater than the water solubility, some differences in the biological activity between TG and WP-25 diflubenzuron may occur. At 0.01 ppm, according to Mulder and Gijwijt (1973), there was no significant increase in effectiveness of diflubenzuron with decreasing particle size. The toxicity of both formulations is similar at concentrations of less than 10 ppm (which is the general concentration used in laboratory with Dimilin).

In conclusion, the effects of formulation and carriers on the toxicity of pesticides are complex and there is no single explanation for the differences observed when they do occur. 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 곤충 성장억제제인 Dimilin (TG and WP-25) 의 영향에 대해 주름꽃게 유생을 대상으로 실험하였다.

주름꽃게 유생을 Dimilin, TG와 WP-25 대조군과 농도 0.1, 1.0, 5.0, 10.0 그리고 25.0 ppb에서 각각 사육하여 유생의 생존율과 사망률을 매일 조사하였다.

TG와 WP-25는 주름꽃게의 유생기에 따라 차이를 보이지만, 초기 유생기에는 TG가 WP-25보다 더 강한 효과를 보인다. 치사농도를 10% 이하의 유생이 부화후 마지막 zoea 유생기에 도달하는 것으로 정의한다면 곤충성장억제제인 Dimilin 은 5.0 ppb 이상이 치사 농도이다. 두 성분 TG와 WP-25는 주름꽃게의 발생 기간에는 중요한 차이를 보이지 않고 있다.

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