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A Study on Toxicity of Several Pesticides on Larval Development of Shrimp *Caridina denticulata denticulata* De Haan

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새뱅이(*Caridina denticulata denticulata* De Haan)의 유생 발생을 통한 수종 살충제 독성에 관한 연구

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ABSTRACT

The effects of insecticide dimilin including the technical grade(TG) and wettable powder (WP-25), inhibiting the synthesis of chitin in the cuticle of insect larvae, and herbicides alachlor and atrazine on the development of shrimp *Caridina denticulata denticulata* were investigated in various concentrations. This species is abundant in the freshwater such as river and pond. The shrimp larvae were reared at dimilin solutions(TG and WP 25), alachlor solutions, and atrazin solutions. The concentrations of dimilin were 0, 1, 5, 10 and 25 ppb dimilin solutions, and those of alachlor and atrazin were 0, 1, 5, 10, and 25 ppm, respectively. Mortality was high at 10 ppb when the larvae were exposed to dimilin and at 25 ppm in the case of alachlor and atrazine solutions. If lethal concentration is defined as a concentration in which less than 10% of larvae reach to the post larval stage from hatching it can be concluded that the lethal concentration of dimilin is 5 ppb, and that of alachlor and atrazin is 25 ppm.

INTRODUCTION

Much research on the pesticides has been conducted throughout the last three decades to produce ingredients which will inhibit only target species without severe influence on the ecosystem(Miura and Takahashi, 1975 ; Post *et al.*, 1974 ; Christiansen and Costlow, 1982 ; Wilson, 1985 ; Kim, 1990). The insect growth regulator dimilin was produced first by Thompson-Hayward Chemical Company of the United States(1974) and was utilized to get rid of the harmful insects, such as gypsy moths, cotton bollweevils and foliar feeders,

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and to control freshwater mosquitoes(Tester and Costlow, 1981 ; Cunningham, 1982). Alachlor and atrazine are active ingredients, widely used as herbicides produced primarily for corn and soybean crops as a preemergent inhibitor of annual grasses, broadleaf weeds and yellow nutsedge(Monsanto Company, 1984). Due to a high rate of application(1.68~4.48 kg/ha), high solubility in water(242 ppm), and high stability, alachlor is persistent in soil and aquatic environment(Weed Sci. Soc. Amer., 1979). Detectable levels of alachlor can persist in soils for up to one year and in farm drainage water for up to four weeks (Skaggs *et al.*, 1980). Because of these characteristics, alachlor can readily leach through soils during heavy rainfall. Wauchope(1978) reported that most concentrations of alachlor were between 0.078~0.184 ppm in drainage streams, While Skaggs *et al.*(1980) found levels in farm ditch-water as high as 2.7 ppm immediately following a runoff event.

Histological examination by Mulder and Gijswijt(1973) revealed the effect of dimilin which intrudes the deposition of endocuticle in insects. Yu and Terriere(1977) showed that dimilin in house fly larvae resulted in the reduction in the ability of the enzyme metabolizing β-ecdysone and distruption of chitin by inhibiting the metabolism of ecdysone. Christiansen *et al.*(1978) also revealed that concentrations of dimilin being toxic to insects are also toxic to the zoeal larvae of crabs.

The present study was conducted to investigate the effects of dimilin, alachlor and atrazine on the larvae of shrimp *Caridina denticulata denticulata*.

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, USA. Dimilin which is white crystalline solid has extremely low solubility of almost 0. 2 mgL^{-1} at 20℃ water. Acetone as a carrier or solvent for TG was put to use in the present experiment because TG is soluble in organic solvents moderately. One ppm(parts per million) dimilin solution was prepared as stock solution by disssolving appropriate amounts in ACS acetone for technical grade or directly in filtered water for wettable powder and stored at 4℃. From this stock solution, working solution of 1 ppm dimilin was made for both ingredients daily in the filtered water. Under the base of this working solution, the filtered water was added to make 4 different dimilin concentrations of 1, 5, 10 and 25 ppb(parts per billion). Dimilin solution of each concentration was supplied as culture solution for shrimp larvae.

Alachlor, 2-chloro-2'-6'-diethyl-N-(methoxymethyl) acetanilide, and atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine, are the active ingredients used as a preemergent inhibitor of annual grasses, broadleaf weeds and yellow nutsedge(Monsanto, 1984). Ten ml of alachlor and atrazine, emulsifiable concentrate(EC), were pipetted into a 500 ml glass bottle in order to evaporate the odor of these herbicides, respectively. Certified ACS acetone was then added to make a final volume of 480 ml stock solution of alachlor, which produced a nominal concentration of 1 ppt(parts per thousand). ACS acetone was added to make 1 ppt of atrazine solution. These stock solutions were stored at 4℃. To these stock solutions, the filtered water was added to give final concentrations of 1, 5, 10, and 25 ppm of alachlor and atrazine, respectively.

Ovigerous shrimps *C. denticulata denticulata* were collected from the ponds located in Chinyong, Kyongsangnam-do from May to September in 1990 and 1991. The shrimps with eggs were placed in the circular aquarium and fed with the flesh of clams *Tapes philippinarum*. The examination for the maturity of egg mass was undertaken by a stereomicroscope. After the microscopic inspection, shrimps with eggs which are ready to hatch were transferred to the 1,000 ml beaker filled with the filtered water and antibiotic for inhibiting bacteria.

Adult shrimps with eggs were put in the room temperature and the larvae were cultured in a cabinet at a photoperiod of 14 h light : 10 h darkness and a constant temperature of 25℃ shortly after hatching. Algae and pellet were supplied as a food for the shrimp larvae. The larvae were reared in the Carolina Culture bowls with the inside diameter of 8 and 2.5 cm in depth. Ten larvae were reared in each bowl containing almost 60 ml solution and the number of total larvae in each concentration was 200 from the same hatching. This experiment has been carried out in 3 replicates in order to give the accuracy of analysis. Living larvae, and numbers of dead larvae and exuviae were recorded daily. Living larvae were transferred to the pesticidal solutions at each concentration containing a few drops of algae shortly after stereomicroscopic inspection.

RESULTS AND DISCUSSION

The percent survival of shrimp larvae was considerably reduced with dimilin concentrations of both the technical grade(TG) and the wettable powder(WP-25). At 1 ppb dimilin concentration, survival was similar to that in the control group whereas almost no shrimp larvae have survived at 10 ppb and 25 ppb dimilin treatment. Mulder and Gijswijt(1973) observed that the toxicity of diflubenzuron with different particle size increased as the particle size becomes smaller. Mulla and Darwazeh(1975) found different effect to mosquito larvae in the same dimilin formulation with different carriers and particle sizes. Jarvinen and Tanner(1982) reported that the technical grade and encapsulated ingredients of parathion have similar solubilities, but the encapsulated ingredient was shown to be 45~60% and 19~35% less toxic than the technical grade in static and chronic tests, respectively.

However, Wilson and Costlow(1986) demonstrated that the two formulations of dimilin (TG and WP-25) were similar in their efficacy as determined by both acute and chronic toxicity tests with grass shrimp larvae. For example, at 1 ppb 53% and 57% of the larvae to day 15 from hatching successfully exuviated to the postlarval stage for WP-25 and TG formulations, respectively. The results according to the present study are similar from those of Wilson and Costlow(1986). The results have shown that there are no differences in the percent survival to post larvae between TG and WP-25 dimilin at each concentration(60 and 63% for TG and WP-25 at 5 ppb). Significant differences in the survival have not been occurred between 1 ppb and 5 ppb dimilin concentration even though these experiments were repeated three times for all the dimilin concentrations. Among the shrimp larvae exposed to 10 ppb dimilin to day 8, about 3%(for TG) and 7%(for WP-25) of the larvae molted to the succeeding stage whereas approximately 96% of the larvae exuviated in the control(Figs. 1 and 3).

Many shrimp larvae died in the course of molting process and possessed deformed swimming setae and rostral spine. Therefore, many larvae could not reach to the post larval stage and showed abnormality, especially at 10 ppb and 25 ppb dimilin treatment(TG and WP-25). According to the result of the present study, there was a considerable delay in the larval development of shrimp *C. denticulata denticulata* when the shrimp larvae were

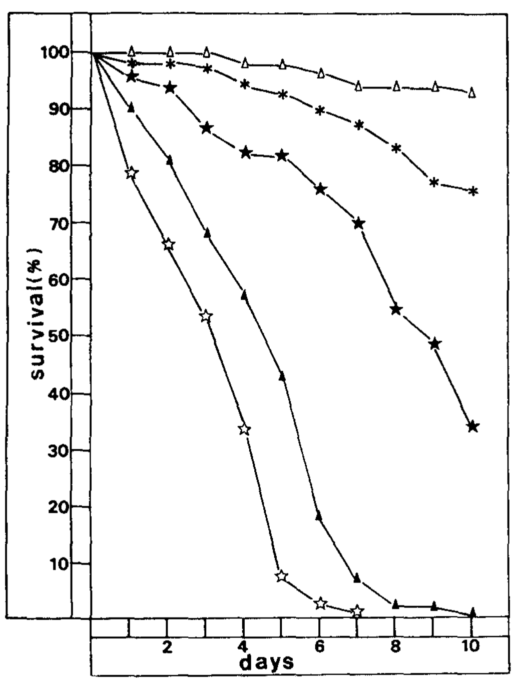

Fig. 1. Survival of *Caridina denticulata denticulata* larvae in control and 4 different concentrations of technical grade dimilin.
Symbols : △, control ; ✳, 1 ppb ; ★, 5 ppb ; ▲, 10 ppb ; ☆, 25 ppb.

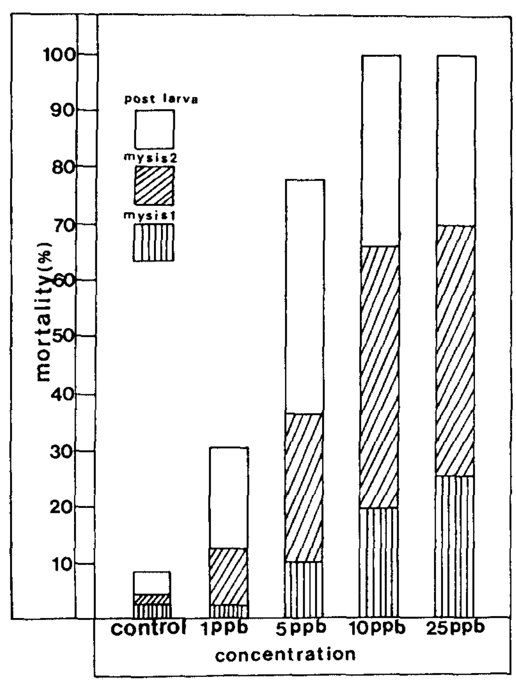

Fig. 2. Mortality of *Caridina denticulata denticulata* larvae at each stage in control and 4 different concentrations of technical grade dimilin.

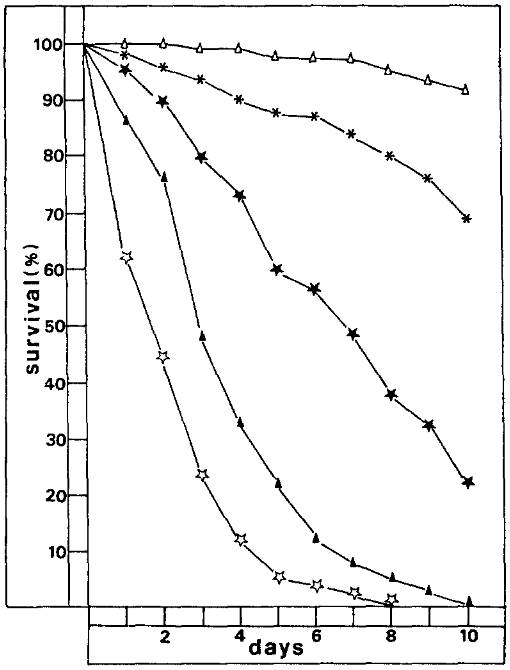

Fig. 3. Survival of *Caridina denticulata denticulata* larvae in control and 4 different concentrations of wettable powder dimilin.
Symbols : △, control ; ✳, 1 ppb ; ★, 5 ppb ; ▲, 10 ppb ; ☆, 25 ppb.

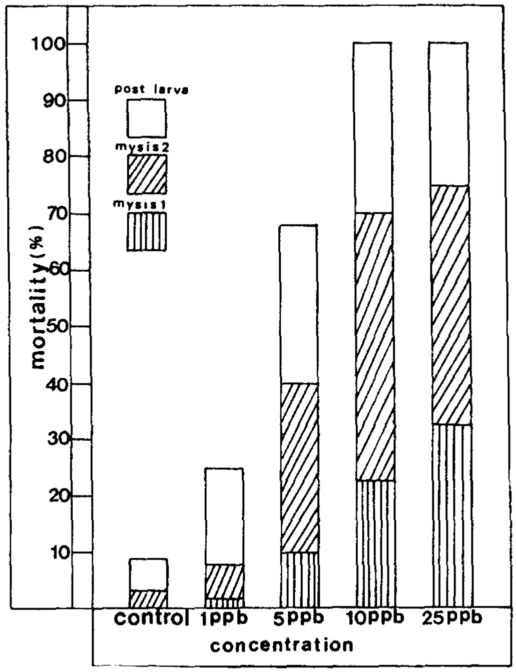

Fig. 4. Mortality of *Caridina denticulata denticulata* larvae at each stage in control and 4 different concentrations of wettable powder dimilin.

treated with dimilin of 1 ppb and 5 ppb concentration.

Thompson-Hayward Chemical Company(1974) reported that the synthesis of chitin in many 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% larvae died at 1 ppb to 3 ppb dimilin, whereas 95% of mosquito *Culex pipiens* died at 6 ppb and 20 ppb dimilin treatment for the second and the fourth instars, respectively. 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 ppb dimilin on various days during intermolt period showed a mortality greater than 95% and older larvae were more sensitive to this chemical agent.

Cunningham(1976) indicated that brine shrimp *Artemia* nauplii exposed to dimilin greater than 10 ppb would die over 3 days. According to the result of the present study, mortality of *C. denticulata denticulata* larvae was found to be increased with increasing dosage. All the larvae died in the postlarval stage by exposing to 10 ppb and 25 ppb dimilin solutions. In comparison, the mortality in 1 ppb and the control groups were between 20 and 25%, and between 5 and 7%, respectively. Mortality of the shrimp larvae exposed to dimilin

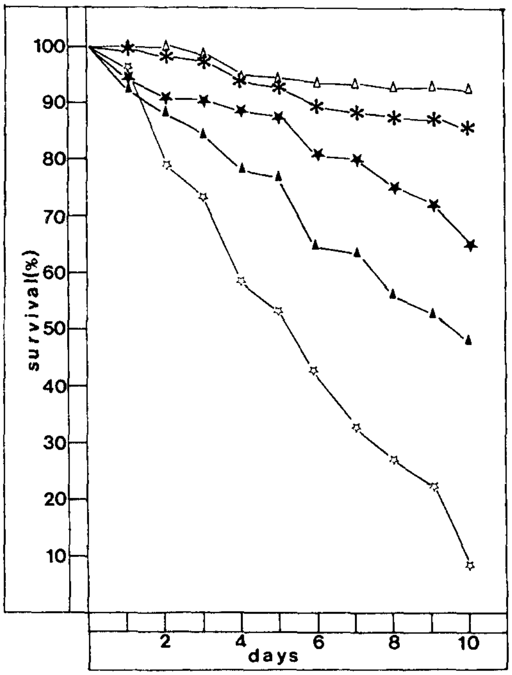

Fig. 5. Survival of *Caridina denticulata denticulata* larvae in control and 4 different concentrations of alachlor.
Symbols :△, control ; ✳, 1 ppm ; ★, 5 ppm ; ▲, 10 ppm ; ☆, 25 ppm.

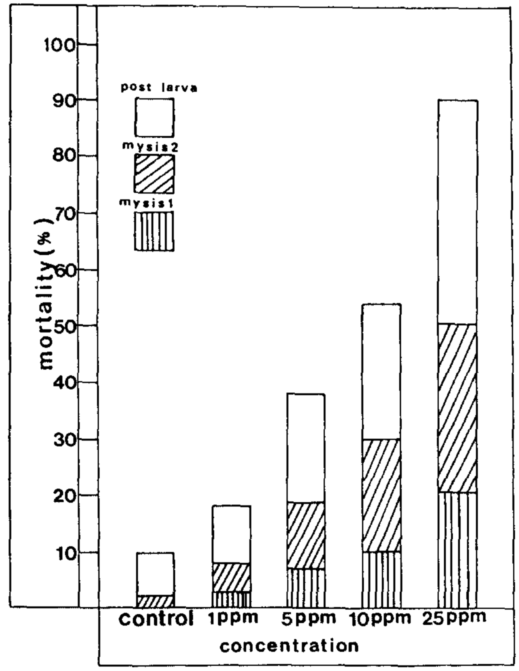

Fig. 6. Mortality of *Caridina denticulata denticulata* larvae at each stage in control and 4 different concentrations of alachlor.

formulations including TG and WP-25 was always the highest during molting to the postlarval stage(Figs. 2 and 4). If lethal concentration is defined as concentration in which larvae less than 10 percent of shrimp larvae were reached to the postlarval stage from hatching, it can be concluded that the lethal concentration of dimilin(TG and WP-25) is 10 ppb.

The larvae of the control group looked completely healthy during the intermolt period until the shedding of exuviae started whereas most of the shrimp larvae exposed to dimilin solutions were unable to carry out their molts completely and died in the course of molting. The new cuticle of larvae treated with dimilin also looked weaker than that of the control group.

When exposed to 25 ppm of alachlor and atrazine, most of shrimp larvae could not reach to the young adult(Figs. 6 and 8). At 25 ppm of alachlor and atrazine, survival was significantly lower than at 10 ppm, whereas survival at 1 ppm and at control was similar to each other. In field studies Skaggs *et al.*(1980) reported that alachlor concentration in water of ditch draining a coastal plains farm peaked at times of peak flow and usually ranged up to approximately 0.07 ppm. Takacs *et al.*(1988) observed that the sublethal concentrations for

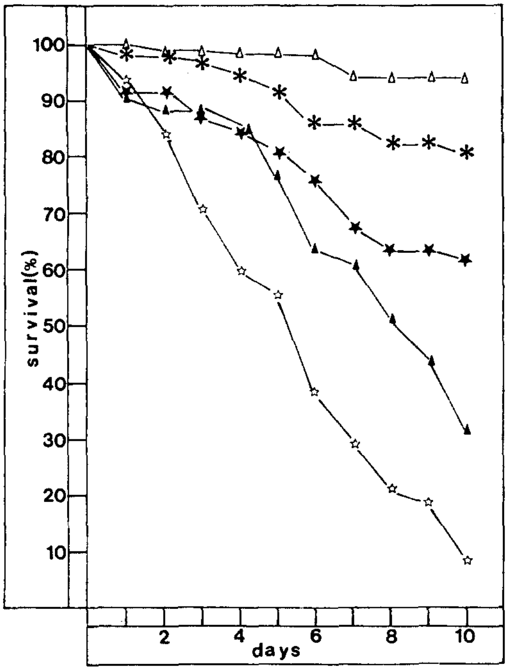

Fig. 7. Survival of *Caridina denticulata denticulata* larvae in control and 4 different concentrations of atrazin.
Symbols : △, control ; ✳, 1 ppm ; ★, 5 ppm ; ▲, 10 ppm ; ☆, 25 ppm.

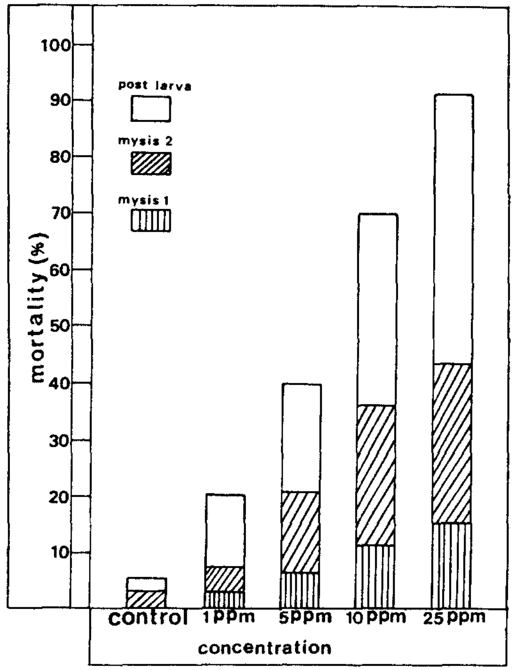

Fig. 8. Mortality of *Caridina denticulata denticulata* larvae at each stage in control and 4 different concentrations of atrazine.

the cuab *R. harrisii* larvae range from 10 to 27ppm alachlor. The results of the present investigation was similar to those of Takacs *et al.*(1988). If lethal concentration is defined as concentration at which shrimp larvae less than 10 percent were reached to the postlarval stage from hatching, it can be concluded that the lethal concentration of alachlor and atrazine is 25 ppm. Even though the present study indicates that shrimp larvae are considerably sensitive to alachlor and atrazine, it is possible that even greater sensitivity of the larvae exists in the atrazine treatment than in the alachlor treatment(Figs. 5 and 7). Helling *et al.*(1988) also supports the results of the present investigation. In the experiment on the average residues of atrazine, alachlor and cyanazine, they reported mobility and the amount of remaining herbicide are in the order of atrazine, alachlor and cyanazine. Therefore in the furture experiments, the effect of alachlor and atrazine on the survival of shrimps with eggs will be considered.

적 요

곤충 유충에 있어 각피의 키틴합성을 저해하는 살충제인 dimilin의 두가지 제제 TG와 WP-25

그리고 제초제 alachlor와 atrazine이 생물에 미치는 영향을 알아보기 위해 새뱅이(*Caridina denticulata denticulata*)의 유생을 실험종으로 하여 실험하였다.

새뱅이 새우의 유생을 dimilin(TG와 WP-25), alachlor, atrazine의 용액에서 사육하였으며, dimilin의 사육 농도는 0, 1, 5, 10, 25 ppb이었으며 alachlor와 atrazine의 경우 0, 1, 5, 10, 25 ppm이었다. 새뱅이 새우의 유생이 dimilin을 포함한 사육수에서 자란 경우 TG와 WP-25 모두 10 ppb 이상에서 치사율이 가장 높았고 alachlor와 atrazine의 경우 모두 25ppm에서 치사율이 가장 높았다. 치사 농도를 10% 이하의 새우유생이 부화후 마지막 후기 유생기에 도달하는 것으로 정의한다면 dimilin(TG와 WP-25)은 10 ppb이상의 농도에서, 제초제 alachlor와 atrazine은 공히 25 ppm이상의 농도에서 새뱅이 새우 유생에 치사영향을 준다고 할 수 있다.

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