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Effects of bifenthrin on *Daphnia magna* during chronic toxicity test and the recovery test

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Abstract: The acute and chronic toxic effects of bifenthrin on *Daphnia magna* were studied. The results showed that 24 h-EC_{50}, 48 h-LC_{50} and 96 h-LC_{50} of bifenthrin on *D. magna* were 3.24, 12.40 and 1.40 μg/L respectively. And the LOEC and NOEC of bifenthrin were 0.02 and 0.004 μg/L respectively. The recovery test of bifenthrin on *Daphnia magna* was presented. *Daphnia magna* (F_0 generation) were exposed during 21 d to different bifenthrin concentrations. Offspring (animals from the first and third brood: F_1 (1st) and F_1 (3rd), respectively) were transferred to a free pesticide medium during a 21 d recovery period. In this recovery study, survival, growth, reproduction (mean total young per female, onset of reproduction and number broods per female) and the intrinsic rate of natural increase (r) were assessed as parameters. Reproduction such as number of young per female as well as length was still reduced in F_1 (1st) generation daphnids from parentals (F_0) exposed to the bifenthrin. However F_1 (3rd) individuals from parentals exposed to pesticide concentrations were able to restore reproduction when a recovery period of 21 d was allowed, but the length of F_1 (3rd) from parentals exposed to the 0.5 and 0.75 μg/L bifenthrin concentration was still significantly effected ($P < 0.05$).

Keywords: bifenthrin; *Daphnia magna*; parental generation (F_0); F_1 (1st) and F_1 (3rd); recovery test; toxic test

Introduction

With the development and application of new pesticide, insecticide has greatly reduced the harm of pest to crops. Bifenthrin is an effective pyrethroid insecticide and acaricide against a wide range of insect pests, and it is very poisonous to mammal, aquatic. This insecticide is widely used to prevent the pest of the cotton. But it also can be enriched in environment when it is used illogical for long-term. As a result, it will seriously affect the growth of aquatic and destroy the balance of ecosystem (Guo, 2001; Institute for the Control of Agrochemicals, the Ministry of Agriculture, 1998).

Cladocerans are important components of many freshwater ecosystems. Despite their importance, little published information is available on the sensitivity of cladocerans to pyrethroid insecticides. The evaluation of the effects of hazardous chemicals on aquatic organisms usually includes chronic tests as well as acute and sublethal studies. But extending chronic toxicity tests to a second generation could increase the cost-effectiveness of the assays. This fact is an important one because, in many cases, the environmental contamination takes place in pulse exposures, not in a continuous manner. So the changing conditions in natural systems make necessary recovery ability after a stress situation.

The toxicity of bifenthrin on *D. magna* has not been reported in China, so the present study aims to evaluate acute and chronic toxicity of bifenthrin to a freshwater cladoceran, *Daphnia magna*. And we also studied the recovery test of bifenthrin on *D. magna*.

1 Materials and methods

1.1 Test organisms

D. magna were obtained from continuous cultures maintained in our laboratory at 20 ± 1 ℃ in OECD M4 culture medium, 250 ± 25 mg/L as $CaCO_3$; pH 7.8 ± 0.2, 16:8-h light: dark photoperiod and a density of below 50 animals/L. The sensitivity of daphnids is accorded with the ISO standard (OECD, 1995).

The medium was renewed thrice a week and daphnids were fed daily with the alga *Scenedesmus obliquus*. These algae were also continuously cultivated in our laboratory using a nutrient medium. Test animals used to start the experiment were ≤24 h juveniles.

1.2 Test chemical

The bifenthrin used in the experiments was 98% pure and is standard sample. Stock solution was prepared by dissolving the toxicant in acetone, and the concentration was 1 g/L. The stock solution was stocked at 4℃.

1.3 Acute toxicity test

Acute toxicity tests were performed in accordance to the standard protocol for *D. magna* acute tests (ISO 6341: 1996). Twenty neonates aged less than 24 h were averagely transferred into 4 glass beakers filled with 20 ml of test solution and incubated at 20 ± 1℃ for 24, 48 and 96 h. 16: 8-h light: dark photoperiod was maintained. The daphnids were fed ($2 - 2.3 \times 10^5$ cells/ml algae) four hours prior to renewal.

1.4 Chronic toxicity test

Daphnids (≤24 h old) were exposed during 21 d to five bifenthrin concentrations: 0 (blank control), 0.001, 0.004, 0.02, 0.1 and 0.5 μg/L, plus the acetone control following the OECD guideline (OECD, 1995). Daphnids were raised individually in 50-ml glass beakers containing 20 ml of test solution (OECD M4 culture medium, the appropriate pesticide concentration and food). The algae *Scenedesmus obliquus* (at a density of 5×10^5 cells/ml) was used as food. A total of 10 replicates for each treatment were carried out. The test temperature was 20 ± 1℃, and a 16: 8-h light: dark photoperiod was maintained.

1.5 Recovery test

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Daphnids(≤24 h old) were exposed during 21 d to six bifenthrin concentrations: 0 (blank control), 0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 μg/L, plus the acetone control (0.0002%) following the OECD guideline(OECD, 1995). Daphnids(parental generation, F_0) were raised individually in 50-ml glass beakers containing 20 ml of test solution (OECD M4 culture medium, the appropriate pesticide concentration and food). The test temperature was 20 ± 1 ℃, and the other condition was similar to the chronic toxicity test.

From the first brood of each pesticide treatment, 10 neonates (≤ 24 h old) were selected and individually transferred to 50-ml glass beakers in a toxicant free medium (recovery period) to start the tests of first generation(F_1 1st brood) which was not exposed to bifenthrin, plus the controls (acetone and blank controls).

To start another experiment, 10 neonates(≤24 h old) from the third brood of the parental generation (F_0) were collected from each exposure pesticide concentration and individually transferred to 50-ml beakers containing 20 ml of toxicant free medium, plus the controls. Subsequently, these new-born daphnids (F_1 3rd brood) were not exposed to bifenthrin.

Size (body length), fecundity and survival of *D. magna* generations were monitored during 21 d. Longevity, time to the first reproduction, total number of neonates per female and number of broods, were the criteria used. Neonates were counted daily and discarded.

The intrinsic rate of natural increase(r) was calculated using the formula: $R_0 = \sum l_x m_x$; $T = \sum x l_x m_x / \sum l_x m_x$; $r = \ln R_0 / T$; where l_x is the proportion of individuals surviving to age x, m_x is the age-specific fecundity (number of neonates produced per surviving female at age x), and x is days. The r integrates the measures of age-specific survival and fecundity to estimate the effect of toxicant exposures on population growth. As r calculated in *D. magna* organisms after 21 d is indistinguishable from r estimated for the entire life-span, due to the great importance of early reproduction (Van Leeuwen, 1985; Villarroel, 1999), all calculations were based on 21 d experiments.

1.6 Statistical analysis

Data from the acute test were dealt with Excel computer program. Data from the generations of *D. magna* studied were analyzed using analysis of variance(ANOVA) to detect significant differences($P < 0.05$) between treated group and control values with an SPSS computer program.

2 Results and discussion

2.1 Acute test

Bifenthrin 24 h-EC_{50}, 48 h-LC_{50} and 96 h-LC_{50} value (Fig.1) for *D. magna* in the experimental conditions were 3.24 μg/L(2.85—3.68 μg/L), 12.40 μg/L(11.87—12.95 μg/L) and 1.40 μg/L(0.94—2.07 μg/L) respectively. It was high toxicant on the basis of classification standard of toxicity(Xiong, 2001). Bifenthrin is a neurotoxin, so it will stimulate the nerve system of *D. magna* in some concentration(less than LC_{50} concentration), and *D. magna* will be in a condition of being excited for a long-term. So when we observe the 24 h-EC_{50}, the behavior of *D. magna* was very rapid compared with blank control if it was inhibited. Therefore we can conclude that the bifenthrin causes *D. magna* to a state of being excited by means of stimulating the nerve system, and *D. magna* acted so rapidly that it would die because of too tired.

It was too difficult to measure the 24 h-LC_{50} of bifenthrin on *D. magna*. When the maximal concentration reached to 0.5 mg/L(the solubility of bifenthrin in water is 0.1 mg/L), the heart still did not stop, but its behavior was inhibited early(more than 5.5 μg/L), this also was owing to that bifenthrin is a neurotoxin. When the concentration was 5.5 μg/L(the maximal concentration in EC test), the behavior of *D. magna* has been inhibited completely, but by reason of the stimulation of bifenthrin, the heart still beat continuously, thus the 24 h-LC_{50} value was out and away higher than the 24 h-EC_{50} value.

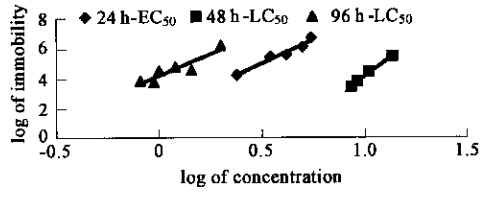

Fig.1 24 h-EC_{50}, 48 h-LC_{50} and 96 h-LC_{50} of bifenthrin on *Daphnia magna*

2.2 Chronic test

The effect of chronic bifenthrin concentrations on the survival and reproduction of *D. magna* is shown in Table 1. Reproduction was significantly reduced ($P < 0.05$) at pesticide concentrations higher than 0.02 μg/L.

Parameters such as days to first brood and No. broods per adult decreased obviously at the concentration of 0.5 μg/L. The outcome of these two parameters was consistent, and these parameters were no more sensitive than reproduction.

Exposure to the selected bifenthrin concentrations affected the length of *D. magna* at 0.004, 0.1 and 0.5 μg/L.

2.3 Recovery test

The effect of sublethal bifenthrin concentration on the survival, growth and reproduction of *D. magna* parental generation(F_0) is shown in Table 2. All parameters studied during the chronic experiments were affected by the sublethal levels of the pesticide used.

Survival of *D. magna* parental generation(F_0) did not decrease below 1.0 μg/L(Table 2). Daphnids had survived only for five days, and had no offspring. The length and days to first brood was significantly reduced($P < 0.05$) from 0.5 μg/L to 0.75 μg/L, particularly days to first brood increased from five days (blank control) to ten days (0.5 μg/L). Number of broods per female decreased above 0.25 μg/L.

The results of the reproduction tests with the first generation of daphnids (F_1 1st) are shown in Table 3. Reproductive parameters such as length and number of young per female were still affected by the concentration of pesticide at which their mothers were exposed. And the days to first brood and number of broods per female had recovered just like the blank control. F_1 generation (1st) (21 d recovery period) from mothers exposed 0.75 μg/L only produced 115 young per female(167 in controls) and the length(3.01 cm) was smaller than in the controls(3.27 cm). However, F_1 (1st) daphnids transferred to clean water showed higher reproduction than their mothers exposed to the pesticide

(Table 1). For example, F_0 daphnids exposed to 0.75 μg/L produced only 61 young per female and the length was 2.65 cm. We do not have any data from reproduction parameters in F_1 generation from F_0 daphnids exposed to the highest bifenthrin tested because those animals did not reproduce.

Table 1 Results of chronic test of bifenthrin

Bifenthrin, μg/L	Length, cm	Longevity, d	Time to first brood, d	Number of broods per female	Number of young per female
Blank control	3.06 ± 0.09	21.0 ± 0.0	7.1 ± 0.4	6 ± 0.0	139 ± 14
Acetone control	3.06 ± 0.08	21.0 ± 0.0	7.0 ± 0.0	6 ± 0.0	140 ± 13
0.001	3.00 ± 0.05	21.0 ± 0.0	7.0 ± 0.0	6 ± 0.0	132 ± 17
0.004	2.96 ± 0.09*	21.0 ± 0.0	7.0 ± 0.0	6 ± 0.0	130 ± 13
0.02	2.99 ± 0.08	21.0 ± 0.0	7.1 ± 0.3	6 ± 0.0	124 ± 15*
0.1	2.95 ± 0.09*	21.0 ± 0.0	7.0 ± 0.0	6 ± 0.0	111 ± 12*
0.5	2.76 ± 0.07*	21.0 ± 0.0	14 ± 1.2*	3.8 ± 0.5*	57 ± 10*

Notes: $P < 0.05$; the data from chronic test indicated that LOEC of bifenthrin is 0.02 μg/L, and NOEC is 0.004 μg/L

Table 2 Size, survival and fecundity of F_0 generation of *D. magna* exposed to several concentrations of bifenthrin in a 21 d life study

Bifenthrin, μg/L	Length, cm	Longevity, d	Time to first brood, d	Number of broods per female	Number of young per female
Blank control	3.14 ± 0.10	21.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	199 ± 16
Acetone control	3.11 ± 0.07	21.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	191 ± 7
0.05	3.07 ± 0.13	21.0 ± 0.0	7.0 ± 0.0	6.9 ± 0.4	179 ± 9*
0.1	3.10 ± 0.08	21.0 ± 0.0	7.0 ± 0.0	6.6 ± 0.5	176 ± 20*
0.25	3.10 ± 0.10	20.5 ± 0.9	7.8 ± 1.0	6.2 ± 0.4*	153 ± 17*
0.5	2.90 ± 0.06*	21.0 ± 0.0	12.0 ± 2.1*	4.4 ± 0.8*	95 ± 22*
0.75	2.65 ± 0.13*	21.0 ± 0.0	15.8 ± 1.4*	3.1 ± 0.4*	61 ± 10*
1.0	–	4.6 ± 0.2*	–	0*	0*

Note: * $P < 0.05$

Table 3 Size, survival and fecundity of F_1 (1st) offspring-generation of *D. magna* transferred to medium without toxicant during 21 d (recovery period) from a parental generation (F_0) pre-exposed to different bifenthrin concentrations

Bifenthrin, μg/L	Length, cm	Longevity, d	Time to first brood, d	Number of broods per female	Number of young per female
Blank control	3.27 ± 0.07	21.0 ± 0.0	7.3 ± 0.5	7.0 ± 0.0	167 ± 11
Acetone control	3.23 ± 0.10	20.7 ± 0.5	7.1 ± 0.3	7.0 ± 0.0	154 ± 16
0.05	3.14 ± 0.11*	21.0 ± 0.0	7.2 ± 0.4	6.9 ± 0.4	138 ± 18*
0.1	3.14 ± 0.10*	21.0 ± 0.0	7.2 ± 0.4	6.9 ± 0.3	145 ± 23*
0.25	3.22 ± 0.11	21.0 ± 0.0	7.1 ± 0.3	7.0 ± 0.0	140 ± 19*
0.5	3.07 ± 0.08*	21.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	125 ± 18*
0.75	3.01 ± 0.10*	21.0 ± 0.0	6.8 ± 0.4*	7.0 ± 0.0	115 ± 19*
1.0	No offspring				

Note: * $P < 0.05$

Table 4 shows the results of F_1 (3rd brood) daphnids from mother (F_0) exposed to the pesticide concentrations. As we can see in the table, these daphnids transferred to clean water showed no significant differences ($P < 0.05$) with control values except the length of daphnids in 0.5 and 0.75 μg/L concentrations.

Table 4 Size, survival and fecundity of F_1 (3rd) offspring-generation of *D. magna* transferred to medium without toxicant during 21 d (recovery period) from a parental generation (F_0) pre-exposed to different bifenthrin concentrations

Bifenthrin, μg/L	Length, mm	Longevity, d	Time to first brood, d	Number of broods per female	Number of young per female
Blank control	3.34 ± 0.13	20.1 ± 1.7	7.0 ± 0.0	7.0 ± 0.0	182 ± 21
Acetone control	3.27 ± 0.05	21.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	180 ± 26
0.05	3.31 ± 0.04	21.0 ± 0.0	6.8 ± 0.5	7.0 ± 0.0	175 ± 23
0.1	3.30 ± 0.05	21.0 ± 0.0	6.9 ± 0.5	6.9 ± 0.4	182 ± 24
0.25	3.33 ± 0.14	21.0 ± 0.0	6.8 ± 0.4	7.0 ± 0.0	181 ± 35
0.5	3.10 ± 0.11*	19.7 ± 2.4	7.0 ± 0.0	7.0 ± 0.0	181 ± 20
0.75	3.05 ± 0.11*	20.6 ± 0.5	7.0 ± 0.0	7.0 ± 0.0	174 ± 23
1.0	No offspring				

Note: * $P < 0.05$

Time of first reproduction and number of broods per female of F_1 (1st and 3rd broods) transferred to clean water were not significantly different ($P > 0.05$) to the controls (Tables 3 and 4). Compared these results with those from F_0 generation exposed to bifenthrin (Table 2), it can be concluded that "time of first reproduction" and "number of broods per female" were not good parameter to estimate the effect of this pesticide on *D. magna* population, because the

effect of bifenthrin was not very clear.

Number of young produced per female was the most sensitive parameter. Number of young per female of parental generation(F_0) was all significantly affected in the range of 0.05 μg/L to 0.75 μg/L, and decreased with the increase of concentration. In the recovery test of first generation, F_1 (1st) daphnids showed higher reproduction than their mothers, but, compared to the controls, they are still significantly affected. While after 21 d recovery, the F_1(3rd) had no significant differences with controls. This result indicated that, number of young per female was a relatively sensitive parameter, however, when the poor environmental conditions had passed, this parameter would recover soon.

Length of 21 d old daphnids from F_0 generations was significantly reduced($P < 0.05$) at bifenthrin concentration from 0.5 μg/L to 0.75 μg/L, however females of F_1 (1st brood) from mothers previously exposed to pesticide showed a mean length significantly reduced($P < 0.05$) except 0.25 μg/L after 21 d in a pesticide free medium, and females of F_1 (3rd) was still significantly affected($P < 0.05$) at 0.5 μg/L to 0.75 μg/L similar to F_0 generations. From this result it can be concluded that bifenthrin above 0.5 μg/L had clear effect on length of *D. magna*, and the recovery of length is difficult. This fact would be due to a bifenthrin transfer from the exposed mothers to their offspring, and this effect seemed to be longer.

The results of the intrinsic rate of natural increase(r) of *D. magna*(F_0, F_1 1st and F_1 3rd) are shown in Figs.2—4. Bifenthrin concentrations above 0.25 μg/L significantly reduced r values of F_0 generation, and the effect seemed to be deeper as the concentrations of bifenthrin increased. Daphnids of F_1(1st) generation from females treated with 0.5 and 0.75 μg/L pesticide, still showed significant differences ($P < 0.05$) compared to blank controls even after a recovery period in clean water. But the effect of bifenthrin on daphnids of F_1 (1st) generation was reduced compared to their parentals(F_0). After a 21 d recovery period, F_1(3rd) generation had no significant compared to controls.

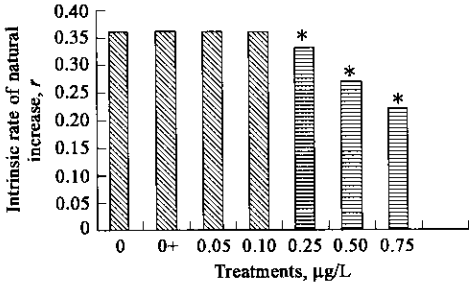

Fig.2 The effect of bifenthrin on the intrinsic rate of natural increase(r) in F_0-generation of *Daphnia magna* ($P < 0.05$)

In the recovery test, the intrinsic rate of natural increase (r) in F_0, F_1 (1st and 3rd) was no more sensitive than number of young and length. We also had observed the intrinsic rate of natural increase(r) in F_1 (3rd) daphnids from parentals(F_0) exposed to 0.05 and 0.1 μg/L was higher than controls. This result showed that when environment conditions are returned to no pollution, offspring F_1 (3rd) from mothers which were exposed to low pesticide would develop faster. Cleuvers *et al*.(Cleuvers, 1997) observed that the later onset of reproduction increases the probability that neonates are born after the poor environmental conditions have passed. Munzinger(Munzinger, 1992) studied the effect of nickel on seven generations of *D. magna* and found an adaptation towards nickel as the intrinsic rate of population growth(r) increased in the exposed generations.

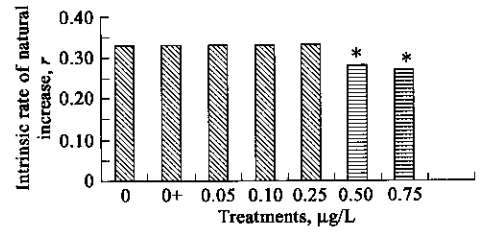

Fig.3 The effect of bifenthrin on the intrinsic rate of natural increase(r) in F_1(1st) of *Daphnia magna* ($P < 0.05$)

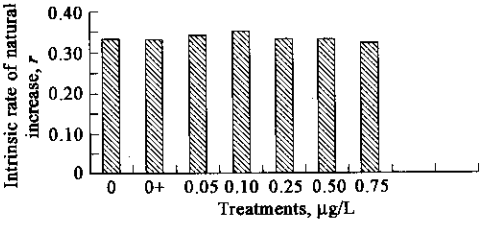

Fig.4 The effect of bifenthrin on the intrinsic rate of natural increase(r) in F_1(3rd) of *Daphnia magna* ($P < 0.05$)

Acetone controls did not differ significantly($P > 0.05$) in any of the studied reproductive parameters of *D. magna* F_0 and F_1 (1st and 3rd) generations. And mortality in the acetone and blank controls of generations F_0, F_1-1st and F_1-3rd never exceeded 10% at the end of the experiment.

It will be interesting to continue with this investigation of the pesticide transfer from a parental generation to the offspring, and found the causation. It will be important also to study the role of intergenerational toxicant transfer in the physiology of the organisms exposed to contaminants and the ability of the offspring whose mothers were pre-exposed to the stress conditions to recover.

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