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Toxicity and Sublethal Effects of Lambda-Cyhalothrin Insecticide on Parent and Filial Generations of *Henosepilachna vigintioctomaculata* (Coleoptera: Coccinellidae)

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Simple Summary: The ladybug *Henosepilachna vigintioctomaculata* is a widely distributed leaf-eating pest. Lambda-cyhalothrin, a synthetic pyrethroid insecticide, is widely used to control leaf-eating pests. However, the effects of this insecticide on biological activity, cross-generations, and detoxification enzyme activity of this ladybug are imperfectly known. We reared *H. vigintioctomaculata* for at least three generations under experimental conditions to determine the effects of lambda-cyhalothrin on biological activity. We evaluate sublethal effects on F_0 generation adults, and cross-generational effects on F_1 adults, using age–stage bisexual life tables, and examine detoxification enzyme activity of F_0 adults. Sublethal concentrations of lambda-cyhalothrin significantly reduced F_0 adult longevity and average fecundity, and inhibited various life-table parameters in the F_1 population; there was also a cross-generational genetic effect, with population growth being inhibited. Low concentrations of lambda-cyhalothrin significantly inhibit *H. vigintioctomaculata* population growth. Multifunctional oxidase, carboxylesterase, and glutathione S-transferase play important roles in *H. vigintioctomaculata* resistance to lambda-cyhalothrin.

Abstract: Lambda-cyhalothrin is a synthetic pyrethroid insecticide that is widely used to control leaf-eating pests. Because of increased insecticide resistance, an understanding of sublethal cross-generational effects of insecticides is important. We examine the effects of sublethal concentrations (SLC) (LC_{10}, LC_{20}, and LC_{40}) of lambda-cyhalothrin on growth, reproduction, and detoxification enzyme activities of F_0 and F_1 generation *Henosepilachna vigintioctomaculata*. Lambda-cyhalothrin is toxic to adult *H. vigintioctomaculata*, with an LC_{40} at 48 h of 0.355 mg L^{-1}. At SLC, lambda-cyhalothrin significantly reduces the longevity and average fecundity of F_0 and F_1 adults, and prolongs the durations of egg, larval, and pupal stages and adult preoviposition period. Additionally, increased lambda-cyhalothrin concentration significantly decreases net reproductive rates, and both finite and intrinsic rates of increase of the F_1 generation, and significantly increases average generation cycle. Detoxification enzyme activity of F_1 adults treated with SLC of lambda-cyhalothrin for 48 h trends upwards. Results indicate that low concentrations of lambda-cyhalothrin induce glutathione S-transferase and carboxylesterase activities and inhibit multifunctional oxidase activity. The growth, development, and reproduction of the *H. viltioctomaculata* F_1 population remain inhibited by lambda-cyhalothrin treatment to the adult stage, and inhibitory effects increase with increased lambda-cyhalothrin concentration. The control efficacy of lambda-cyhalothrin against *H. viltioctomaculata* shows cross-generational effects.

Keywords: *Henosepilachna vigintioctomaculata*; lambda-cyhalothrin; life table; transgenerational studies; detoxifying enzyme

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1. Introduction

The 28-spotted ladybug *Henosepilachna vigintioctomaculata* (Coleoptera: Coccinellidae) is a crop pest that occurs widely throughout China, and elsewhere in countries such as Korea, Japan, Russia, and Australia. This ladybug is a phytophagous pest that feeds mainly on crops such as Solanaceae, Leguminosae, Cruciferae, and Cucurbitaceae, with adults and larvae eating the tender leaves and stems [1,2]. When leaves are eaten, a semi-transparent parallel-shaped depression is formed, and in severe cases, only leaf veins and the epidermis remain, causing the leaves to wilt, yellow, and die. China is an important producer of potato—a globally important food crop—and *H. vigintioctomaculata* seriously affects potato agriculture [2-4]. Changes in microclimates and promotion of intercropping of corn, vegetables, and potatoes have also led to *H. vigintioctomaculata* becoming both an increasingly serious pest in potato plantations in Yunnan, Guizhou, and Sichuan regions, and a main spreader of potato brown spot disease, which also poses a threat to local potato production. In severe cases, yield can be reduced by 50% [5-7].

The insecticide lambda-cyhalothrin is highly efficient at controlling leaf-eating pests [8]. After spraying, insecticide residue in general gradually decreases over time, and with crop growth [9]. The physiology and behavior of some pests exposed to insecticides at sublethal doses may change [10,11]. Most sublethal insecticide doses have inhibitory or delayed effects on pest populations, but some can also stimulate growth and development, and promote pest proliferation [12]. These sublethal effects on pests can lead to physiological and biochemical changes and insecticide resistance. Therefore, understanding the sublethal effects of insecticides on pests is important to evaluate their efficacy and assess risks associated with their use [13]. Pests can reduce the effects of insecticides by regulating detoxification enzyme activities [14]. Among enzymes, glutathione S-transferase (GST), carboxylesterase (CarE), and multifunctional oxidase (MFO) are the most important. Low concentrations of insecticides or plant secondary metabolites can induce or inhibit the activity of various enzymes, thereby affecting pest metabolic processes, promoting resistance and providing continuous selection pressure, reducing the effectiveness of insecticides for pest control [15,16].

While the median lethal concentration (LC_{50}) or median lethal dose (LD_{50}) have been commonly used to evaluate the effects of insecticides on pests, they only report the response to insecticides at certain developmental stages [17]. Life tables can be used to more comprehensively analyze the effects of pesticides on pests at the population level. Compared with traditional life tables, an age–age bisexual life table increases statistics for males, accurately describes age differentiation of insects, differentiates between pre- and total-oviposition periods of adults, and can comprehensively describe changes in the entire population [18-20]. Beta-cypermethrin, phoxim, and abamectin pesticides are mostly used to control potato ladybird adults [4].While the sublethal effects of insecticides on target species are being increasingly explored, the effects of lambda-cyhalothrin lethal concentration (LC) on growth, development, and fecundity of *H. vigintioctomaculata* are imperfectly known. We first determine the sublethal concentration (SLC) of lambda-cyhalothrin on the F_0 generation, and its effect on the F_1 generation at SLCs. We construct an age–instar bisexual life table for the F_1 population and compare changes in parameters such as developmental duration, survival rate, fecundity, and longevity at different lambda-cyhalothrin concentrations. We also report detoxifying enzyme activities after SLC treatment of F_0 adults. Results provide a theoretical basis for a comprehensive assessment of the potential of lambda-cyhalothrin to control *H. vigintioctomaculata*, and for more informed application of this insecticide.

2. Materials and Methods

2.1. Insect Rearing and Insecticide

Henosepilachna vigintioctomaculata were collected from Luliang County, Qujing City potato fields, Yunnan Province (103°48′52.02″E, 25°17′20.74″N, altitude 1878 m) in September 2023. Insects were placed in an insect-feeding cage (50 × 50 × 50 cm) at room temperature. Potted potato (Yunshu 108)

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grown at (25 ± 1) °C, relative humidity 70% ± 5%, and a photoperiod 16:8 h L/D were cultivated as a host; fresh leaves were cut to feed ladybugs when plants had grown to 15–20 cm. Before experimentation, adult ladybugs were reared for at least three generations with no exposure to insecticide. Three-day-old adults were used as the initial insect source. Insect feeding conditions were consistent with potato cultivation conditions.

2.2. Insecticide

The stock insecticide solution was 2.5% lambda-cyhalothrin WG (Henan Yongguan Qiaodi Agricultural Science and Technology Co., Ltd.). Multifunctional oxidase (MFO) activity detection kits (A162-1-1), Carboxylesterase (CarE) activity detection kits (A133-1-1) and Glutathione S-transferase (GSH-ST) activity detection kits (A004-1-1) were purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd.

2.3. Determining Biological Activity

The sensitivity of *H. vigintioctomaculata* to lambda-cyhalothrin was determined using a leaf film method [21]. Five lambda-cyhalothrin treatments (1.25, 1.00, 0.75, 0.50, and 0.25 mg L^{-1}) were initially established; water was used as a control. Each treatment contained 30 × 3-d-old similarly sized adult ladybugs, and was replicated three times. Before experimentation, adult ladybugs were starved for 48 h. Potato leaves were immersed in each treatment concentration for 20 s, then removed, placed in an insect box (15 × 12 × 5 cm), dried, and their petiole wrapped with water-soaked cotton wool. Ladybugs were then added. Ladybug mortality was recorded after 48 h of treatment. Feeding conditions were the same as in section 2.1. Ladybugs were pronounced dead if touched by a brush and no reaction occurred. A virulence regression curve, 95% confidence intervals, chi-square values (χ^2), and degrees of freedom (*df*) were calculated for LC_{10}, LC_{20}, and LC_{40} concentrations.

2.4. Effects of Lambda-cyhalothrin Sublethal Treatment on F_0 and F_1 H. vigintioctomaculata

Potato leaves were immersed in LC_{10}, LC_{20}, and LC_{40} concentrations for 20 s, then removed and dried; water was used as a control. Sixty healthy 3-d-old similarly sized male and female adults (1:1, morphological identification) were fed potato leaves and reared in an artificial climate box. After 48 h of exposure to leaves immersed in different concentrations of insecticide (without changing leaves), surviving *H. vigintioctomaculata* were removed, paired male and female, placed in a Petri dish, and fed fresh, untreated potato leaves. The initial stage of the F_0 generation experiment involved approximately 30 individuals, with three biological replicates. At least 15 pairs of ladybug from each insecticide treatment were paired; fresh potato leaves were replaced daily in the insect box. The oviposition date, oviposition amount, and adult longevity of single females were observed regularly (8:00 and 18:00 h). If a male died before a female, it was replaced. We refer to adult contemporary *H. vigintioctomaculata* treated with SLCs of lambda-cyhalothrin as parent generation (F_0), and offspring produced by natural mating of these parents filial generation (F_1) [2].

Eggs (90, oviposition < 5 h) laid on the same day by an F_0 female in each lambda-cyhalothrin treatment and the control group were used as the F_1 generation. These eggs were placed into circular plastic, numbered Petri dishes (diameter 60 mm) with moist filter paper, and then moved to an artificial climate box for hatching, where hatching rate was determined. Feeding conditions were the same as in section 2.1. Individual newly hatched F_1 generation larvae treated with different insecticide concentrations were inoculated separately into Petri dishes for feeding. Fresh potato leaves were placed into Petri dishes, and ladybug development was monitored every 24 h. After 24 h of pupation, pupae were weighed, then transferred into individual centrifuge tubes sealed with absorbent cotton, and returned to the climate chamber. After adult eclosion, pupal duration was determined. For each insecticide concentration treatment, female and male adults that emerged on the same day were randomly selected and paired 1:1 and placed into new culture dishes. A wet cotton ball was placed in the dish to provide moisture, and fresh leaves were replaced the next day. Test insects ate and

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deposited eggs. The number of eggs laid by a single female and the longevity of adult females and males were observed daily (8:00 and 18:00 h) until all adults had died. The number of eggs hatched / the total number of eggs laid by females represents the egg-hatching rate. The time from newly hatched larvae to pupation represents the larval development period, and the time from pupation to adult eclosion represents the pupal development period. The percentage of the number of pupae that could break the shell and become adults divided by the total number of pupae × 100 represents the eclosion rate.

2.5. Determining Detoxifying Enzyme Activities

Detoxifying enzyme activities were determined using the method of Jiang et al [22]. F_0 generation adult ladybugs were fed fresh leaves treated with LC_{10}, LC_{20}, and LC_{40} concentrations of lambda-cyhalothrin, with four replicates per treatment. After 48 h, surviving adults were collected and placed into centrifuge tubes. Each replicate contained 10 individuals, frozen in liquid nitrogen and stored at −80 °C for enzyme activity determination. Ten similarly sized adults were selected from each concentration treatment. PBS (pH 7.4) was added to an ice bath homogenate at a weight (g): volume (mL) ratio of 1:9; the homogenate was centrifuged at 4 °C and 12,000 r/min for 30 min. The supernatant was recovered to determine GST, CarE, and MFO enzyme activities, and protein concentration, following kit instructions.

2.6. Data Analysis

Probability unit analysis was used to analyze the toxicity regression equation of lambda-cyhalothrin using SPSS 26.0 (IBM Co., Ltd., Armonk, NY, USA); SLCs were determined. One way-ANOVA was used on data related to detoxification enzymes. A Tukey's multiple range test was used for multiple comparisons, and Student's t tests were used to identify significant differences between treatments in pairwise comparisons. Original growth and development, survival rate, and fecundity data for the F_1 generation were collated and imported into Twosex-MSChart software (v 5/7/2024) to calculate developmental duration, adult longevity, adult preoviposition period (APOP), total preoviposition period (TPOP), fecundity, net reproductive rate (R_0), finite rate of increase (λ), intrinsic rate of increase (r), and average generation cycle (T) for each stage [23-26]. All variances and standard errors were obtained by using 100,000 random sampling bootstraps, and differences for each parameter in insecticide treatments were evaluated by paired bootstrap tests [27,28]. Plots were generated using SigmaPlot 14.0. Specific calculation formulas are:

$$l_x = \sum_{j=1}^{\beta} s_{xj} \tag{1}$$

$$M_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}} \tag{2}$$

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \tag{3}$$

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \tag{4}$$

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$$\lambda = e^{r} \tag{5}$$

$$T = \frac{\ln(R_0)}{r} \tag{6}$$

3. Results

3.1. Toxicity of Lambda-cyhalothrin to Henosepilachna vigintioctomaculata Adults

After 48 h treatment, LC_{10}, LC_{20}, and LC_{40} values for lambda-cyhalothrin exposure to *H. vigintioctomaculata* adults were 0.193, 0.251 and 0.355 mg L^{-1}, respectively. Three concentration values overlapped with other 95% CL values for subsequent experiments (Table 1).

Table 1. Sublethal concentrations of lambda-cyhalothrin to newly hatched adult *H. vigintioctomaculata* (F_0) 48 h after treatment.

Insecticide	Number of samples	LC_{10} (95% CL) (mg L^{-1})	LC_{20} (95% CL) (mg L^{-1})	LC_{40} (95% CL) (mg L^{-1})	χ^2 (*df*)	Slope ± SE	*p*-value
Lambda-cyhalothrin	450	0.193 (0.112–0.265)	0.251 (0.184–0.352)	0.355 (0.258–0.432)	9.53	3.91 ± 0.59	0.023

3.2. Effect of Sublethal Concentration of Lambda-cyhalothrin on Longevity and Fecundity of F_0 H. vigintioctomaculata

LC_{10}, LC_{20}, and LC_{40} concentrations of lambda-cyhalothrin significantly affected the longevity and average fecundity of F_0 adults (Table 2). Compared with the control group, the higher the concentration of lambda-cyhalothrin, the shorter the adult life span (female and male), with the life span of females being slightly longer. After exposure to lambda-cyhalothrin concentrations (LC_{10}, LC_{20}, and LC_{40}), individual egg production and F_1 egg hatching rates also significantly decreased compared with the control group.

Table 2. Impact of sublethal concentrations of lambda-cyhalothrin on F_0 generation adult *H. vigintioctomaculata* longevity and fecundity.

Parameter	Control	Lambda-cyhalothrin (LC_{10})	Lambda-cyhalothrin (LC_{20})	Lambda-cyhalothrin (LC_{40})
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Male longevity (d)	24.39 ± 0.30 a	24.00 ± 0.23 a	21.45 ± 0.29 b	20.09 ± 0.33 c
Female longevity (d)	26.27 ± 0.34 a	26.00 ± 0.21 a	24.29 ± 0.39 b	21.79 ± 0.29 c
Fecundity (eggs laid/female)	65.66 ± 9.21 a	59.11 ± 7.09 b	42.12 ± 5.56 c	31.09 ± 4.76 d
F_1 egg Hatching rate	84.29 ± 11.55 a	68.73 ± 9.79 b	58.26 ± 9.60 c	49.46 ± 7.88 d

Note: Different letters within a row indicate significant differences ($P < 0.05$).

3.3. Effect of Sublethal Concentration of Lambda-cyhalothrin on Growth, Development, Fecundity, and Pupal Weight of F_1 H. vigintioctomaculata

LC_{10}, LC_{20}, and LC_{40} concentrations of lambda-cyhalothrin affected each F_1 generation developmental stage (Table 3). Compared with the control, the duration of egg, larval, and pupal stages in the LC_{10} and LC_{20} treatment groups differed significantly ($P < 0.05$); the lifespan of male and female adults in the LC_{40} concentration treatment group was 16.57% and 19.76% significantly shorter than control group adults. APOP and TPOP were prolonged with increase lambda-cyhalothrin

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concentration (control group APOP and TPOP durations were the shortest (6.20 d and 23.53 d), and those in the LC_{40} treatment were the longest (7.10 d and 30.45 d, respectively). Compared with other treatments, the numbers of eggs laid by females exposed to the LC_{40} concentration was lowest (13.40 eggs/female); the number of eggs laid by females in the control group (55.59 eggs/female) was 1.13×, 1.70×, and 4.15× that of LC_{10}, LC_{20}, and LC_{40} treatments, respectively. Additionally, compared with controls, the pupal weight and adult emergence rate of the F_1 generation were also significantly lower in the LC_{10}, LC_{20}, and LC_{40} treatments ($P < 0.05$), with the effect stronger with increased insecticide concentration (Fig. 1, Table 3).

Table 3. Fecundity and duration of various life-history parameters of progeny of lambda-cyhalothrin-treated *H. vigintioctomaculata.*

Parameter (duration, days)	Treatment			
	Control	Lambda-cyhalothrin (LC_{10})	Lambda-cyhalothrin (LC_{20})	Lambda-cyhalothrin (LC_{40})
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Egg (d)	5.66 ± 0.07 c	6.48 ± 0.07 b	6.70 ± 0.07 b	7.12 ± 0.05 a
Larva (d)	15.05 ± 0.17 c	16.32 ± 0.23 b	16.60 ± 0.21 b	18.69 ± 0.30 a
Pupa (d)	4.59 ± 0.08 c	5.40 ± 0.13 b	5.71 ±0.15 b	6.17 ± 0.19 a
Male longevity (d)	23.53 ± 0.25 a	22.52 ± 0.31 b	20.12 ± 0.17 c	18.88 ± 0.21 d
Female longevity (d)	25.40 ± 0.27 a	24.86 ± 0.18 b	23.21 ± 0.46 c	21.19 ± 0.26 d
APOP (d)	6.20 ± 0.47 b	7.00 ± 1.36 a	7.00 ± 0.59 a	7.10 ± 0.82 a
TPOP (d)	23.53 ± 0.52 d	26.75 ± 1.44 c	29.79 ± 1.24 b	30.45 ± 1.70 a
Eclosion rate %	85.98 ± 0.13 a	80.54 ± 0.76 b	71.02 ± 0.54 c	60.66 ± 0.73 d
Fecundity (eggs laid/female)	55.59 ± 8.72 a	48.79 ± 7.08 b	32.71 ± 3.23 c	13.40 ± 3.06 d

Note: APOP, adult pre-oviposition period; TPOP, total pre-oviposition period. Data are presented as means ± SE. Different letters within a row indicate significant differences based on paired bootstrap tests ($P < 0.05$). Standard errors are estimated from 100,000 bootstrap resamples.

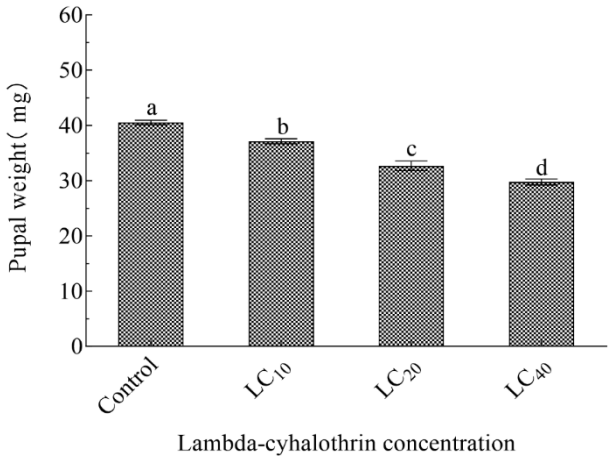

Figure 1. Pupal weight of progeny of lambda-cyhalothrin-treated *H. vigintioctomaculata.* Note: Data are means ± SE; different lowercase letters above whiskers indicate significant differences ($P < 0.05$, Tukey's multiple range test).

3.4. Population Parameters

Compared with the control, LC_{10}, LC_{20}, and LC_{40} treatments significantly affected F_1 generation life table parameters. Values for r, λ, and R_0 of the F_1 population decreased with increased lambda-cyhalothrin concentration, and T increased (Table 4).

Table 4. Population parameters of F_1 *H. vigintioctomaculata* descended from the F_0 generation exposed to lambda-cyhalothrin.

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Parameters	Concentration treatments			
	Control	Lambda-cyhalothrin (LC_{10})	Lambda-cyhalothrin (LC_{20})	Lambda-cyhalothrin (LC_{40})
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Intrinsic rate of increase, r d^{-1}	0.087 ± 0.006 a	0.062 ± 0.007 b	0.041 ± 0.005 c	0.017 ± 0.007 d
Finite rate of increase, λ d^{-1}	1.081 ± 0.006 a	1.064 ± 0.007 b	1.041 ± 0.005 c	1.017 ± 0.009 d
Net reproductive rate, R_0 (offspring/individual)	25.98 ± 6.75 a	13.66 ± 3.64 b	9.16 ± 1.25 c	3.68 ± 0.96 d
Mean generation time, T (d)	38.97 ± 0.46 c	41.69 ± 0.74 b	53.98 ± 1.80 a	54.52 ± 1.91 a

3.5. Age–Stage Specific Maternity

At each concentration the l_x curves follow a similar trend: there is a period of stability, then gradual, then steep decline (Fig. 2), indicating that death of individuals in the *H. vigintioctomaculata* F_1 population mainly occurred in later stages. The f_x curves are more variable, indicating greater variation in the emergence and oviposition of female adults, resulting in the curve appearing high and low. For LC_{10}, LC_{20} and LC_{40} lambda-cyhalothrin concentrations, peak adult female f_x values were 13.77 (37 d), 14.11 (55 d), and 8.46 (58 d) (Fig. 2). The peak number of eggs of f_x in the control group was greatest (15.62 eggs d^{-1}). Over time, the age-specific survival rate l_x of the control group trended down. The m_x curve indicated that the control group began reproducing at 33 d, whereas in other concentrations reproduction began 2–11 d later. Multiple peaks appear in the mx curve, indicating changes in individual spawning periods. The age-specific oviposition rate l_xm_x in LC_{40}-treated individuals decreased sharply, from 5.00 individuals d^{-1} in the control group to 1.70 individuals d^{-1} (Fig. 2).

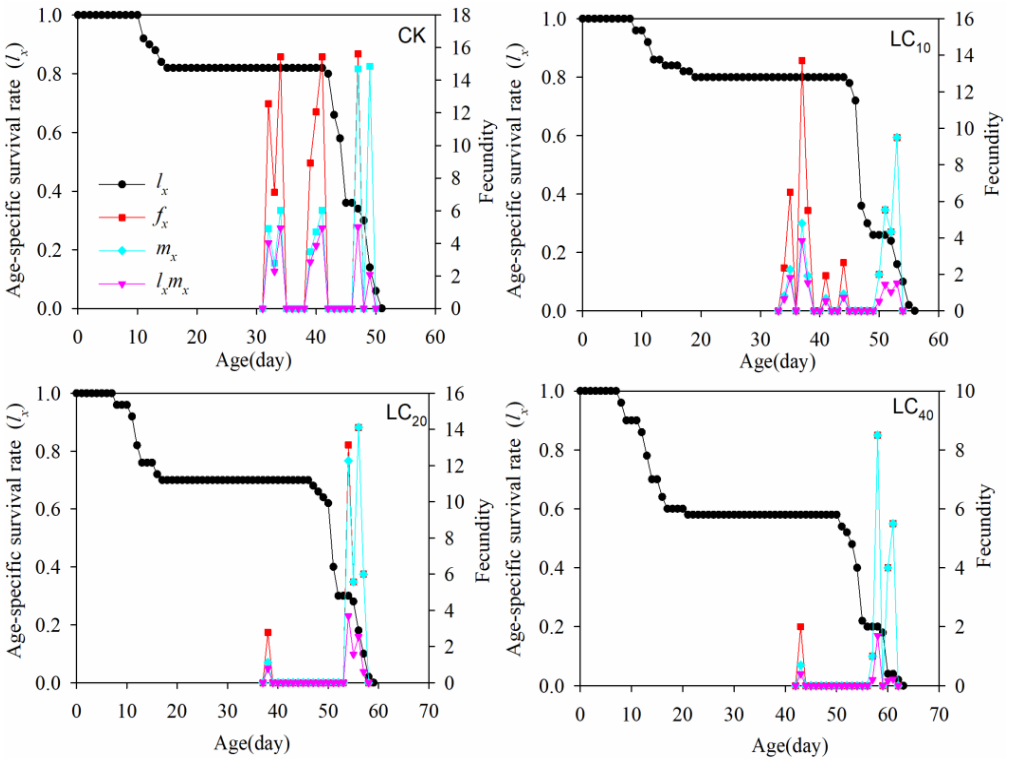

Figure 2. l_x, f_x, m_x and l_xm_x values of F_1 *H. vigintioctomaculata* descended from F_0 ladybugs treated with lambda-cyhalothrin and control values.

3.6. Effect of Lambda-cyhalothrin Exposure on Detoxifying Enzyme Activity

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Detoxifying enzyme activities in adult *H. vigintioctomaculata* treated with SLCs of lambda-cyhalothrin trended upwards (Fig. 3). After 48 h exposure at LC_{20} and LC_{40} concentrations, female GST activities decreased significantly by 28.88% and 44.88% compared with control values, and male activities decreased significantly by 39.39% and 44.95%, respectively ($P < 0.05$); at LC_{10} there was an increase in GST activity. CarE activity first increased, then decreased with increased lambda-cyhalothrin concentration; enzyme activities in the LC_{40} treatment were significantly lower than those in either other treatment ($P < 0.05$). With increased treatment concentration, MFO activity was inhibited. Additionally, the GST activity of adults in the LC_{20} treatment and CarE activity in the LC_{10} and LC_{40} treatments differed significantly ($P < 0.05$).

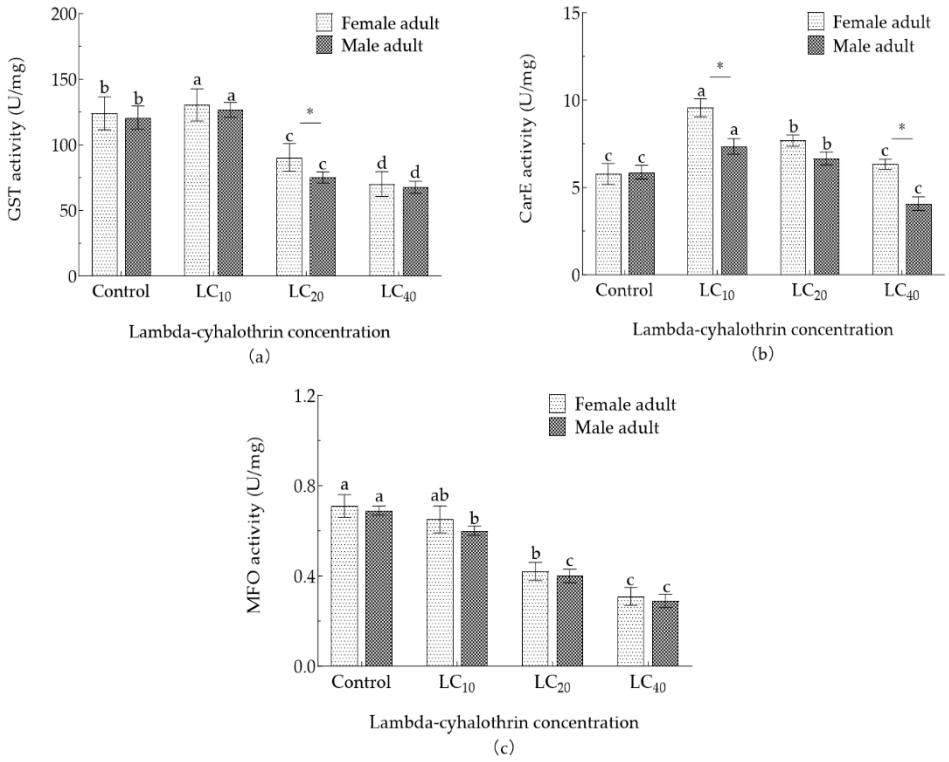

Figure 3. (**a**) GST, (**b**) CarE, and (**c**) MFO activities in *H. vigintioctomaculata* adults treated with lambda-cyhalothrin. Note: Asterisks indicate significant differences in detoxifying enzyme activities between females and males (* $P < 0.05$, independent samples t-test).

4. Discussion

Insecticides are used extensively in agriculture, and they contribute to the development of pest resistance [17]. Integrated pest-management programs underscore the importance and necessity of moderate insecticide use [29]. Lambda-cyhalothrin is an efficient, broad-spectrum and quick-acting pyrethroid insecticide developed by ICI, UK. It has contact and stomach poisoning effects, and has no internal absorption effect. It is mainly used to control pests with chewing or piercing and sucking mouthparts [8,9,14]. Using a leaf-dip method we evaluate the bioactivity of lambda-cyhalothrin against adult *H. vigintioctomaculata* [4]. Our LC_{40} value of 0.355 mg L^{-1} indicates that this insecticide exhibits potent toxicity toward *H. vigintioctomaculata*. Chemical control is the main strategy to manage *H. vigintioctomaculata*. However, post-application in the field, insecticide toxicity generally decreases because of various abiotic environmental factors, producing SLCs [30]. This reduced toxicity can induce sublethal effects in certain pest individuals that may be passed on to subsequent generations. Sublethal effects may influence the growth, development, behavior, and reproductive capacity of target pests and their offspring, and change population dynamics [30-32].

Many studies have reported sublethal insecticide concentrations to exert significant inhibitory effects. For instance, treatment with chlorantraniliprole at LC_{10} concentrations significantly reduces

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the survival rate and longevity of F_1 generations of *Sogatella furcifera* [33]. Studies on development in *Cydia pomonella* under lambda-cyhalothrin stress have yielded similar results [34]. We report SLCs of lambda-cyhalothrin (LC_{10}, LC_{20}, and LC_{40}) to significantly reduce the lifespan and average oviposition of the F_0 generation *H. vigintioctomaculata* compared with control group ladybugs (Table 2). Furthermore, exposure to LC_{40} concentrations significantly reduced the F_1 population size, with delays observed in egg, larval, and pupal developmental duration, further indicative of sublethal effects (Table 3). These findings are consistent with those for exposure of *Paracoccus marginatus* to SLCs of spirotetramat, with extended nymphal periods from F_0 to F_2 generations and pre-adult delays [35]. We also report pupal weight—an important indicator of insect stress resistance and environmental adaptability—of F_1 generation *H. vigintioctomaculata* to decrease with increased SLC of lambda-cyhalothrin [36]. Leaves treated with LC_{40} concentrations also showed negligible feeding damage, likely attributable to the antifeedant properties of lambda-cyhalothrin [37]. Reduced feeding reduced growth and development of *H. vigintioctomaculata*, and reduced body weight.

The impact of insecticides on pest fecundity manifests their sublethal effects [31]. The toxic excitatory response is influenced by various factors, with exposure duration (or generational exposure) being a key determinant [38]. Low doses of imidacloprid stimulate peach aphids, leading to increased methylation levels in the F_2 generation compared with the F_1 generation. This phenomenon may be linked to genetic adaptability induced by low-dose insecticide stress [39]. We report SLCs of lambda-cyhalothrin to also induce transgenerational effects in *H. vigintioctomaculata*. Specifically, the average oviposition, r, λ, and R_0 values of both F_0 and F_1 generations decreased with increased lambda-cyhalothrin concentration, limiting F_1 population growth (Tables 2 and 3). A similar phenomenon is reported for pests such as *Paracoccus marginatus*, *Aphis gossypii*, and *Rhizoglyphus robini* [9,14,40]. These findings suggest that insecticide treatments can effectively slow transgenerational population growth of a variety of species. This inhibitory effect may be attributed to downregulation of gene expression associated with vitellogenin (Vg) and its receptor synthesis in females exposed to sublethal doses of insecticide. For example, the reduced expression of Vg and vitellogenin receptor (VgR) genes leads to decreases in respective contents [40-42]. Furthermore, age–stage specific maternity curves (Figure 2) indicate that lambda-cyhalothrin stress significantly suppresses the F_1 generation reproductive capacity, with the effect increasing at higher concentrations. Interestingly, many insecticides (e.g., triazophos, thiamethoxam) regulate pest fecundity by modulating expression of Vg and VgR through the TOR protein kinase and juvenile hormone signaling pathways [43,44]. Whether lambda-cyhalothrin similarly regulates the reproductive capacity of *H. vigintioctomaculata* adults via these pathways is unknown.

The reduced sensitivity of insects to insecticides typically involves multiple mechanisms, with the most prominent being the regulation of insecticide metabolism through detoxifying enzymes [45]. These enzymes play important roles in the metabolic processing of chemicals in insects. Insecticides can induce or inhibit their activity, and by exerting continuous selective pressure, drive the evolution of insecticide resistance [46]. Insecticides can affect various population parameters in insects such as developmental periods, reproductive capacity, and adult longevity. Additionally, they can influence the activity of detoxifying enzymes [47]. Different insecticides or plant secondary metabolites have distinct inhibitory or inducing effects on detoxifying enzyme activities in the same pest species [31]. For example, after 24 h of spirotetramat exposure, low concentrations induced a significant increase in GST and CarE activity in *Bradysia odoriphaga* [48]. Similarly, treatment with lambda-cyhalothrin significantly increased GST activity in *Cydia pomonella*, but suppressed CarE activity [49]. We report that after 48 h of exposure to lambda-cyhalothrin, LC_{10} concentrations induced an increase in both GST and CarE activities in adult *H. vigintioctomaculata*. At LC_{20}, the CarE activity in adult males and females was significantly higher than in the control group, but lower than in the LC_{10} treatment group, possibly because of the lower insecticide dose (where CarE was activated to participate in insecticide metabolism). Additionally, the GST activity of adults in the LC_{20} treatment and CarE activity in the LC_{10} and LC_{40} treatments differed significantly ($P < 0.05$). Similar GST and CarE activity results were reported by Kinareikina [50]. At increased dosage, enzyme activity was progressively

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suppressed. The degree of MFO activity correlated positively with insecticide dosage. At low lambda-cyhalothrin concentrations the activities of CarE and GST in *H. vigintioctomaculata* were induced, but MFO activity was significantly reduced. Further molecular-level studies on higher resistance strains are required to elucidate the underlying mechanisms of resistance to lambda-cyhalothrin.

After exposure of adult *H. vigintioctomaculata* to SLCs of lambda-cyhalothrin in the F_0 generation, F_1 population growth was significantly reduced, and the degree of reduction increased with increased insecticide concentration. This suggests that field application of lambda-cyhalothrin exerts transgenerational effects on *H. vigintioctomaculata*. In agricultural practice, the rational use of lambda-cyhalothrin to mitigate damage caused by *H. vigintioctomaculata* to crops is viable. However, the dosage and frequency of application must be managed to avoid enhancing pest fitness or promoting resistance through prolonged use of a single class of insecticide. Furthermore, the implementation of insecticide rotation—using two or more insecticides with different modes of action—could delay development of resistance and support sustainable pest management strategies.

5. Conclusions

As a new insecticide with high efficiency, low toxicity, and low residue, lambda-cyhalothrin has high insecticidal activity against Coleoptera pests. We report the effects of SLCs of this insecticide on the growth and development, longevity and fecundity of *H. vigintioctomaculata* using an age–age bisexual life table. Compared with the control, male and female longevity and fecundity all decreased, and other life table parameters deceased with increased SLC. At low concentrations this insecticide also increases the activity of detoxifying metabolic enzymes. Field application of lambda-cyhalothrin has a cross-generational effect on *H. vigintioctomaculata* that may affect F_1 generations. This provides a reference for how to improve evaluation of the effects of insecticides.

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References

1. Matsishina, N.V.; Ermak, M.V.; Fisenko, P.V.; Sobko, O.A. *Henosepilachna vigintioctomaculata* Motschulsky (Coleoptera: Coccinellidae): morphotypes in an East Asian population. *J. Insect Biodivers.* **2023**, *38*, 15-23, doi:10.12976/jib/2023.38.1.2.
2. Li, W.; Bashir, N.H.; Chen, H.; Li, X.; Wang, Z.; Du, L.; Tian, R. Effect of different host plants on the growth, development, and reproduction of *Henosepilachna vigintioctomaculata* (Coleoptera: Coccinellidae). *Chin. J. Appl. Entomol.* **2024**, *61*, 287-294, doi:10.7679/j.issn.2095-1353.2024.032.
3. Ze, L.J.; Jin, L.; Li, G.Q. The compatible effects of RNA interference targeting laccase2 with biocontrol in *Henosepilachna vigintioctopunctata*. *Entomol. Gen.* **2023**, *43*, 117-126, doi:10.1127/entomologia/2023/1644.

4. Ding, X.; Aerziguli, R.; Ji, L.; Liu, S.; Fu, K.; Tursun, A.; Guo, W. The occurrence and harm of *Henosepilachna vigintioctomaculata*. *Xinjiang Agric. Sci.* **2022**, *59*, 983-989, doi:10.6048/j.issn.1001-4330.2022.04.024.
5. Tamang, S.; Alam, T.; Rana, L. Biointensive integrated pest management of potato. In *Biointensive integrated pest management for horticultural crops*, 1 ed.; Kumar, A., Saha, S., Choudhary, J.S., Eds.; CRC Press: London, 2021; pp. 207-240.
6. Nithish, A. Study on management of damage caused by *Henosepilachna vigintioctopunctata* in brinjal. *J. Pharmacog. Phytochem.* **2020**, *9*, 2874-2881.
7. Zhou, J.; Xu, R.; Chen, Z.; Jia, Y.; Xu, K. Phototactic behavior of *Henosepilachna vigintioctomaculat*a motschulsky (Coleoptera: Coccinellidae). *Coleopts. Bull.* **2015**, *69*, 806-812, doi:10.1649/0010-065X-69.4.806.
8. Keyhanian, A.A.; Barari, H.; Mobasheri, M.T. Comparison of the efficacy of insecticides, alphacypermethrin and lambda-cyhalothrin, against canola flea beetles. *Appl. Entomol. Phytopathol.* **2021**, *44*, 113-122.
9. Qiu, Y.; Chen, Z. Intergenerational effects of sublethal lambda-cyhalothrin exposure on *Aphis gossypii* glover (Hemiptera: Aphididae) reproduction and development. *Insects* **2024**, *15*, 173, doi:10.3390/insects15030173.
10. Wang, D.; Wang, Y.M.; Liu, H.Y.; Xin, Z.; Xue, M. Lethal and sublethal effects of spinosad on *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **2013**, *106*, 1825-1831, doi:10.1603/EC12220.
11. Boina, D.R.; Onagbola, E.O.; Salyani, M.; Stelinski, L.L. Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid, *Diaphorina citri*. *Pest Manag. Sci.* **2009**, *65*, 870-877, doi:10.1002/ps.1767.
12. Cutler, G.C.; Amichot, M.; Benelli, G.; Guedes, R.N.C.; Qu, Y.; Rix, R.R.; Ullah, F.; Desneux, N. Hormesis and insects: Effects and interactions in agroecosystems. *Sci. Total Environ.* **2022**, *825*, 153899, doi:10.1016/j.scitotenv.2022.153899.
13. Gul, H.; Güncan, A.; Ullah, F.; Desneux, N.; Liu, X. Intergenerational sublethal effects of flonicamid on cotton aphid, *Aphis gossypii*: An age-stage, two-sex life table study. *Insects* **2024**, *15*, 529, doi:10.3390/insects15070529.
14. Li, J.Y.; Chen, Y.T.; Chen, Y.; Zhao, J.W.; Fu, J.W.; Shi, M.Z. Sublethal effects of lambda-cyhalothrin on the biological characteristics, detoxification enzymes, and genes of the papaya mealybug, *Paracoccus marginatus*. *J. Pest Sci.* **2024**, 1-15, doi:10.1007/s10340-024-01837-3.
15. van Leeuwen, T.; Dermauw, W. The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. *Ann. Rev. Entomol.* **2016**, *61*, 475-498, doi:10.1146/annurev-ento-010715-023907.
16. Guo, W.; Yang, H.; Duan, M.; Xu, H.; Zhao, W.; Wang, C. Sublethal effects of tolfenpyrad and its impact on detoxifying enzymes to *Spodoptera exigua*. *Plant Protect.* **2024**, *50*, 168-175, doi:10.16688/j.zwbh.2023282.
17. Batool, N.; Abubakar, M.; Noureldeen, A.; Naqqash, M.N.; Alghamdi, A.; Al Dhafar, Z.M.; Baakdah, F.; Mozūratis, R. Toxicity and sublethal effect of chlorantraniliprole on multiple generations of *Aedes aegypti* L. (Diptera: Culicidae). *Insects* **2024**, *15*, 851, doi:10.3390/insects15110851.
18. Li, W.; Bashir, N.H.; Naeem, M.; Tian, R.; Tian, X.; Chen, H. Age-stage, two-sex life table of *Atractomorpha lata* (Orthoptera: Pyrgomorphidae) at different temperatures. *Insects* **2024**, *15*, 493, doi:10.3390/insects15070493.
19. Borges, I.; Dury, G.J.; Soares, A.O. Population growth parameters of *Scymnus nubilus* fed single-aphid diets of *Aphis fabae* or *Myzus persicae*. *Insects* **2024**, *15*, 486, doi:10.3390/insects15070486.
20. Ahn, J.J.; Choi, K.S. Population parameters and growth of *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) under fluctuating temperature. *Insects* **2022**, *13*, 113, doi:10.3390/insects13020113.
21. Guo, L.; Bian, Q.; Zhang, H.; Gao, X.; Liang, P. Bioassay technique for *Plutella xylostella*: Leaf-dip method. *Chin. J. Appl. Entomol.* **2013**, *50*, 556-560.
22. Jiang, M.; Qian, X.; Zhou, Z.; Liu, Y.; Zhang, M.; Yang, Y. Impacts of sublethal doses of spinetoram on the biological traits and detoxifying enzymes of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae). *Insects* **2024**, *15*, 990, doi:10.3390/insects15120990.
23. Chi, H.; Liu, H.S.I. Two new methods for study of insect population ecology. *Bull. Inst. Zool Acad. Sin.* **1985**, *24*, 225-240.
24. Chi, H. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* **1988**, *17*, 26-34, doi:10.1093/ee/17.1.26.

 doi:10.20944/preprints202502.0822.v1

25. Chi, H.; Su, H.Y. Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. *Environ. Entomol.* **2006**, *35*, 10-21, doi:10.1603/0046-225X-35.1.10.
26. Tuan, S.J.; Lee, C.C.; Chi, H. Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age - stage, two - sex life table. *Pest Manag. Sci.* **2014**, *70*, 805-813, doi:10.1002/ps.3618.
27. Chi, H. *TWOSEX-MSChart: A computer program for the age-stage, two-sex life table analysis*; National Chung Hsing University in Taiwan: Taichung City, Taiwan, China, 2023.
28. Efron, B.; Tibshirani, R.J. *An introduction to the bootstrap*; Chapman and Hall: New York, NY, USA, 1993.
29. Dean, A.N.; Niemi, J.B.; Tyndall, J.C.; Hodgson, E.W.; O'Neal, M.E. Developing a decision - making framework for insect pest management: A case study using *Aphis glycines* (H emiptera: A phididae). *Pest Manag. Sci.* **2021**, *77*, 886-894, doi:10.1002/ps.6093.
30. Havasi, M.; Zahedi Golpayegani, A.; Bandani, A. The sublethal concentration of *Cyflumetofen adversely* affect demographic parameters of *Tetranychus urticae* (Acari: Tetranychidae): Using age-stage, two-sex life tables. *Int. J. Acarol.* **2022**, *48*, 331-337, doi:10.1080/01647954.2022.2050807.
31. Fan, R.; Fan, Z.; Sun, Z.; Chen, Y.; Gui, F. Insecticide susceptibility and detoxification enzyme activity of frankliniella occidentalis under three habitat conditions. *Insects* **2023**, *14*, 643, doi:10.3390/insects14070643.
32. Wang, D.; Gong, P.; Li, M.; Qiu, X.; Wang, K. Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pest Manag. Sci.* **2009**, *65*, 223-227, doi:10.1002/ps.1672.
33. Yang, H.; Wang, Z.; Jin, D. Sublethal effects of chlorantraniliprole on the experimental populations of the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). *Acta Entomol. Sin.* **2012**, *55*, 1161-1167, doi:10.16380/j.kcxb.2012.10.008.
34. Ju, D.; Liu, Y.X.; Liu, X.; Dewer, Y.; Mota-Sanchez, D.; Yang, X.Q. Exposure to lambda-cyhalothrin and abamectin drives sublethal and transgenerational effects on the development and reproduction of Cydia pomonella. *Ecotoxicol. Environ. Saf.* **2023**, *252*, 114581, doi:10.1016/j.ecoenv.2023.114581.
35. Li, J.Y.; Chen, Y.T.; Wang, Q.Y.; Zheng, L.Z.; Fu, J.W.; Shi, M.Z. Sublethal and transgenerational toxicities of chlorfenapyr on biological traits and enzyme activities of *Paracoccus marginatus* (Hemiptera: Pseudococcidae). *Insects* **2022**, *13*, 874, doi:10.3390/insects13100874.
36. Li, D.; Peng, C.; Ma, R.; Chen, Y.; Gui, F.; Sun, Z. Effects of sublethal concentrations chlorfenapyr on growth, development and enzyme activity of *Tuta absoluta*. *J. Environ. Entomol.* **2024**, *46*, 1349-1357, doi:10.3969/j.issn.1674-0858.2024.06.11.
37. Bian, D.; Ren, Y.; Ye, W.; Dai, M.; Li, F.; Wei, J.; Sun, H.; Li, B. Evaluation of tolerance to λ-cyhalothrin and response of detoxification enzymes in silkworms reared on artificial diet. *Ecotoxicol. Environ. Saf.* **2022**, *232*, 113232, doi:10.1016/j.ecoenv.2022.113232.
38. Agathokleous, E.; Brown, P.H.; Calabrese, E.J. A gift from parent to offspring: Transgenerational hormesis. *Trends Plant Sci.* **2021**, *26*, 1098-1100, doi:10.1016/j.tplants.2021.08.006.
39. Ayyanath, M.-M.; Cutler, G.C.; Scott-Dupree, C.D.; Prithiviraj, B.; Kandasamy, S.; Prithiviraj, K. Gene expression during imidacloprid-induced hormesis in green peach aphid. *Dose Response* **2014**, *12*, 480-497, doi:10.2203/dose-response.13-057.Cutler.
40. Alimirzaee, S.; Khajehali, J.; Van Leeuwen, T. Hormetic effects of neonicotinoid insecticides on *Rhizoglyphus robini* (Acari: Acaridae). *Pestic. Biochem. Physiol.* **2023**, *192*, 105396, doi:10.1016/j.pestbp.2023.105396.
41. Ullah, F.; Gul, H.; Tariq, K.; Desneux, N.; Gao, X.; Song, D. Thiamethoxam induces transgenerational hormesis effects and alteration of genes expression in *Aphis gossypii*. *Pestic. Biochem. Physiol.* **2020**, *165*, 104557, doi:10.1016/j.pestbp.2020.104557.
42. Wang, L.; Zhu, J.; Wang, Q.; Ji, X.; Wang, W.; Huang, W.; Rui, C.; Cui, L. Hormesis effects of sulfoxaflor on Aphis gossypii feeding, growth, reproduction behaviour and the related mechanisms. *Sci. Total Environ.* **2023**, *872*, 162240, doi:10.1016/j.scitotenv.2023.162240.

 doi:10.20944/preprints202502.0822.v1

43. Zhou, C.; Yang, X.; Yang, H.; Gong, M.; Long, G.; Jin, D. Role of SfJHAMT and SfFAMeT in the reproductive regulation of *Sogatella furcifera* and its expression under insecticide stress. *Pestic. Biochem. Physiol.* **2021**, *173*, 104779, doi:10.1016/j.pestbp.2021.104779.
44. Zhou, C.; Yang, X.; Yang, H.; Gong, M.; Long, G.; Jin, D. Role of insecticide-mediated transcription of the TOR and JH signaling pathway-related genesin the regulation ofreproduction in *Sogatella furcifera*. *Entomol. Gen.* **2022**, *42*, 771-779, doi:10.1127/entomologia/2022/1496.
45. Van Leeuwen, T.; Dermauw, W. The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. *Annu. Rev. Entomol.* **2016**, *61*, 475-498, doi:10.1146/annurev-ento-010715-023907.
46. Hafeez, M.; Liu, S.; Jan, S.; Ali, B.; Shahid, M.; Fernández‐Grandon, G.M.; Nawaz, M.; Ahmad, A.; Wang, M. Gossypol‐induced fitness gain and increased resistance to deltamethrin in beet armyworm, *Spodoptera exigua* (Hübner). *Pest Manag. Sci.* **2019**, *75*, 683-693, doi:10.1002/ps.5165.
47. van Leeuwen, T.; van Pottelberge, S.; Tirry, L. Biochemical analysis of a chlorfenapyr‐selected resistant strain of *Tetranychus urticae* Koch. *Pest Manag. Sci.* **2006**, *62*, 425-433, doi:10.1002/ps.1183.
48. Zhao, Y.; Wang, Q.; Ding, J.; Wang, Y.; Zhang, Z.; Liu, F.; Mu, W. Sublethal effects of chlorfenapyr on the life table parameters, nutritional physiology and enzymatic properties of *Bradysia odoriphaga* (Diptera: Sciaridae). *Pestic. Biochem. Physiol.* **2018**, *148*, 93-102, doi:10.1016/j.pestbp.2018.04.003.
49. Li, P.R.; Shi, Y.; Ju, D.; Liu, Y.X.; Wang, W.; He, Y.S.; Zhang, Y.Y.; Yang, X.Q. Metabolic functional redundancy of the *CYP9A* subfamily members leads to P450‐mediated *lambda*‐cyhalothrin resistance in *Cydia pomonella*. *Pest Manag. Sci.* **2023**, *79*, 1452-1466, doi:10.1002/ps.7317.
50. Kinareikina, A.; Silivanova, E. Impact of insecticides at sublethal concentrations on the enzyme activities in adult *Musca domestica* L. *Toxics*, 2023, 11, 47, doi.org/10.3390/ps.11010047.