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Lethal and Sublethal Effects of Fluxametamide on Rice-Boring Pest, Rice Stem Borer *Chilo suppressalis*

Yao Li [1,†], Yingnan Wang [1,†], Chenglong Qian [1], Tao Tang [2], Ning Shen [1], Wei Wu [3], Jianming Wang [3], Zhaojun Han [1] and Chunqing Zhao [1,*]

[1] Key Laboratory of Integrated Pest Management on Crops in East China, Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, China
[2] Institute of Plant Protection, Hunan Academy of Agricultural Sciences, Changsha 410125, China
[3] Nanjing Dragonfly Smart Agricultural Research Co., Ltd., Nanjing 211225, China
* Correspondence: zcq@njau.edu.cn; Tel.: +86-025-84399025; Fax: +86-025-84399063
† These authors contributed equally to this work.

Abstract: (1) Background: Fluxametamide is a novel isoxazoline insecticide. Rice stem borer *Chilo suppressalis* (Walker) is a destructive Lepidoptera pest of rice in China, and novel effective insecticides are required to be developed for controlling it due to its increasing resistance levels. (2) Results: In the lethal assay, the insecticidal activity of fluxametamide with median lethal dose (LD_{50}) value of 1.308 mg/kg to the fourth-instar larvae of *C. suppressalis* was higher than that of chlorantraniliprole (LD_{50}, 3.112 mg/kg) and lower than that of emamectin benzoate (LD_{50}, 0.006 mg/kg). In the sublethal (LD_{10} and LD_{30}) assay, the duration of third to sixth-instar larvae, the pupal duration, pupation rate, and life cycle rate were significantly increased in F_0 generation. Both the length and weight of the ovarian tube were decreased with the dose increase of fluxametamide, and were significantly smaller in the LD_{30} treatment than those of the control group. In F_1 generation, only the duration of eggs was significantly increased with LD_{30} treatment of fluxametamide, whereas other developmental parameters had no significant change. (3) Conclusion: Fluxametamide had relatively strong lethal and sublethal effects on *C. suppressalis* and probably was able to affect the population growth and progeny of *C. suppressalis*.

Keywords: fluxametamide; lethal effect; sublethal effect; *Chilo suppressalis*; ovary

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1. Introduction

Rice stem borer *Chilo suppressalis* (Walker) is one of the most destructive rice pests in China and greatly reduces the yield and quality of rice. To date, *C. suppressalis* has developed high levels of resistance to several classes of insecticides including fipronil, avermectin, chlorantraniliprole, molosultap, etc. [1–4]. However, chemical insecticides are still the main tool of its control. Therefore, the introduction of new insecticides, e.g., fluxametamide, which have a highly lethal effect against it and no cross-resistance with other insecticides, is very necessary. Fluxametamide is a newly registered isoxazoline insecticide, and was launched in Korea in 2018 and in Japan in 2019 with the registered trade name 'GRACIA'. It acts on the γ-aminobutyric acid (GABA)-gated chloride channel [5,6] leading to signal disorder and loss of physiological function to kill the insect [7,8]. Fluxametamide has high insecticidal activity against agricultural pests, such as Lepidoptera, Thysanoptera, etc., on the vegetable and tea plant, but has low toxicity to the bee and to mammals [9].

Meanwhile, it is worth to noting that a sublethal effect of a novel insecticide is required to be explored in addition of its lethal effect. Instead of the lethal dose of the insecticide directly killing the targeted pest, the insecticide slowly degrades, and the virulence will gradually decrease and reach a sublethal dose from the influence of external factors including time and the natural environment [10]. Therefore, the targeted pest insect is poisoned

by the insecticide without dying, and still maintains the behavioral ability [11]. Sublethal dose(s) of insecticide can affect the growth, development, and reproduction of insects, and also alter the ecological behavior and resistance of insects [12]. For example, after the third-instar larvae of *C. suppressalis* was treated with sublethal doses (LC_{10} and LC_{30}) of chlorantraniliprole, the larval duration was significantly extended, and the larval body weight, longevity of the adult, and pupation rate were significantly reduced [13]. Therefore, the authors speculated that the *C. suppressalis* treated with a sublethal concentration of chlorantraniliprole eats less food, more energy in the body is used for detoxification, and the endocrine system is out of balance, which delays the growth and development of *C. suppressalis* [13]. When the first-instar larvae of *Tryporyza incertulas* was treated with sublethal doses of imidacloprid or buprofezin, their fertility was stimulated, and the fecundity of each female was significantly increased compared with that of the control group (hereinafter referred to as CK) [14].

In insects, ovaries are the primary reproductive organs, which regulate the activities of secondary reproductive organs [15]. Therefore, disturbance in ovarian physiology by an insecticide affects all reproductive activities. As is known, insecticides affect the reproductive system either by exerting cytotoxicity and genotoxicity as a result of oxidative stress or through endocrine disruption. The study of the development process of the female reproductive system is valuable for predicting the development and occurrence regularity of insect populations in insect reproductive biology. To date, there is still no report on the sublethal effect of fluxametamide to any pest as fluxametamide is a newly marketed insecticide. Therefore, the physiological parameters, including hatchability, developmental duration of the larvae, pupation rate, pupal weight and duration, emergence, longevity and fertility of the adult, and the development of ovaries, were investigated in *C. suppressalis*, which were treated with fluxametamide in this study. These results will provide comprehensive useful information for assessing the potential lethal and sublethal effects of fluxametamide to insects, and for its recommendation in integrated pest management.

2. Materials and Methods

2.1. Insect, Chemicals, and Insecticides

The *C. suppressalis* was reared on artificial food in the laboratory without exposure to any insecticide [16]. The rearing conditions were at a temperature of 27 ± 1 °C, relative humidity of 60–70%, and a 16:8 h light:dark photoperiod. Fluxametamide (98%) was supplied by Shenyang Sinochem Agrochemicals R&D Co., Ltd. (Shenyang, China), chlorantraniliprole (96%) was supplied by FMC Corporation (Shanghai, China), and emamectin benzoate (95%) was supplied by Syngenta Nantong Crop Protection Co., Ltd. (Nantong, China). Acetone (chemical purity) was supplied by Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China), and Tween-80 was supplied by Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China).

2.2. Lethal Effect of Fluxametamide on C. suppressalis

The lethal effect of fluxametamide was measured on fourth-instar larvae using the artificial food mixed with insecticide method [17] according to the 'Guideline for Laboratory Bioassay of Pesticides, Part 10: Diet incorporation method' (Code: NY/T1154.10-2008) [18]. Six experimental doses of each insecticide (chlorantraniliprole, 1, 2, 4, 8, 16, and 32 mg/kg; fluxametamide, 0.5, 1, 2, 3, 4, and 5 mg/kg; and emamectin benzoate, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/kg) were set referring to the pre-assay. Briefly, 10 g fresh artificial food was mixed with 100 µL working solution of insecticide, dissolved in a mixture of acetone and 0.1% Tween-80 (1:1, *v*/*v*), and divided into three disposable Petri dishes (ϕ 9 cm) for treated groups. Meanwhile, the CK was only treated with a mixture of acetone (100 µL) and 0.1% Tween-80 (100 µL). Ten fourth-instar larvae were used for each replication, and triplications were performed for each dose. Mortality was recorded at 72 h after treatment.

In addition, the lethal effect of fluxametamide on third-instar larvae of *C. suppressalis* was analyzed as abovementioned procedures using six experimental doses (0.25, 0.5, 1, 1.5, 2, and 2.5 mg/kg).

2.3. Sublethal Effect of Fluxametamide on C. suppressalis Development of F_0 Generation

In the sublethal effect study, the third-instar larvae of *C. suppressalis* were selected, treated with LD_{10} and LD_{30} of fluxametamide, and defined as F_0 generation. In particular, forty third-instar larvae as one replication were treated with artificial food containing a sublethal dose (LD_{10} and LD_{30}) of fluxametamide. Five replications were performed for each sublethal dose. A mixture of acetone (100 µL) and 0.1% Tween-80 (100 µL) was used as the CK. After 72 h, all survival larvae were individually transferred into the same size clean plastic tube (2 cm diameter and 9.5 cm height) containing fresh artificial food, and each larva was defined as one replicate. The physiological parameters including the duration of larva, the pupation rate, the duration and weight of pupa, adult emergence, adult longevity, etc., were recorded every day.

To examine the oviposition period, and the number of laid eggs, the male and female adults which emerged on the same day were paired following 1:1 in an oviposition plastic cup (13 cm height; neck diameter: 9.5 cm upper end and 5.5 cm lower end) containing a small Petri dish of 10% (*w*/*v*) honey solution and A4 paper folded into ridges (10 cm by 10 cm), which was replaced every 2 days.

2.4. Sublethal Effect of Fluxametamide on C. suppressalis Ovary Development of F_0 Generation

For determining the sublethal effect of fluxametamide on *C. suppressalis* ovaries, 2-day-old female adult ovaries were dissected in phosphate-buffered saline (PBS) under a stereoscopic microscope (Nikon SMZ25, Nikon Instruments Inc., Melville, NY, USA). In each treatment (CK, $LD_{10,}$ and LD_{30}), 10 female adults were randomly selected for dissection. In brief, the head of the adult was cut off with anatomical scissors, and the remaining part was placed in a droplet of PBS on a slide. The insect body was incised with anatomic tweezers and the cuticle was gently torn from the thorax to the tail. The ovary was gently separated from the body and transferred in a droplet of PBS on another slide for observation. At last, the length and weight of the ovarian tube per ovary were measured and counted using Image J analysis software (Version 1.8.0; National Institutes of Health, Rockville Pike, Bethesda, MD, USA).

2.5. Carryover Activity of Fluxametamide at Sublethal Dose on the Progeny of F_1 Generation

To determine whether fluxametamide has carryover activity on the offspring of F_0 (F_1 generation), 120 eggs were randomly taken from each pair of adult moths of each treatment (CK, $LD_{10,}$ and LD_{30}). Newly hatched F_1 larvae were individually transferred into a clean plastic tube containing artificial food. The duration of the F_1 larval period and subsequent stages and the related survivorship were recorded daily, and the pupal weight, pupation rate, emergence rate, and female ratio were calculated. Newly emerged F_1 adults were paired in an oviposition plastic cup as described above. The survival rate and the number of laid eggs by F_1 adults were recorded daily.

2.6. Data Analysis

The median lethal dose (LD_{50}) with corresponding 95% confidential limits (CL) and sublethal doses (LD_{30} and LD_{10}) of insecticide of *C. suppressalis* larvae were calculated using a probit regression analysis with a chi-square test with SPSS v 22 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for significance analysis in SPSS using Tukey's multiple comparison test ($p < 0.05$).

3. Results

3.1. Lethal Effect of Fluxametamide on C. suppressalis Larvae

The LD_{50} values of fluxametamide, chlorantraniliprole, and emamectin benzoate for the fourth-instar larvae of *C. suppressalis* were 1.308, 3.112, and 0.006 mg/kg at 72 h, respectively (Table 1). In addition, the LD_{10}, LD_{30}, and LD_{50} values of fluxametamide for the third-instar larvae at 72 h were 0.09, 0.25, and 0.50 mg/kg, respectively (Table A1).

Table 1. Lethal effect of insecticide on fourth-instar larvae of *C. suppressalis* after 72 h treatment.

Insecticide	Slope ± SE	LD_{50} (mg/kg)	95% CL	χ^2	R^2
Fluxametamide	2.838 ± 0.357	1.308	0.815–1.839	6.863	0.930
Chlorantraniliprole	1.327 ± 0.219	3.112	1.998–4.420	3.677	0.912
Emamectin benzoate	2.919 ± 0.430	0.006	0.005–0.008	4.624	0.926

3.2. Sublethal Effect of Fluxametamide on C. suppressalis F_0 Generation

A significant difference was observed in the larval duration of *C. suppressalis* treated with different doses of fluxametamide. For the LD_{10} and LD_{30} treatments, the durations from the third to sixth instar were prolonged by 3.04 and 5.23 days ($p < 0.0001$; $F = 90.067$; $df = 2, 406$), respectively, compared with CK (Table 2).

Table 2. Sublethal effect of fluxametamide on F_0 larvae duration of *C. suppressalis*.

Parameter	Duration (Days)				
	Third Instar	Fourth Instar	Fifth Instar	Sixth Instar	Third to Sixth Instar
CK	2.41 ± 0.05 ^{c}	3.42 ± 0.04 ^{c}	4.33 ± 0.06 ^{b}	7.94 ± 0.23 ^{b}	18.01 ± 0.25 ^{c}
LD_{10}	4.01 ± 0.08 ^{b}	4.28 ± 0.08 ^{b}	4.50 ± 0.09 ^{b}	8.37 ± 0.22 ^{ab}	21.05 ± 0.28 ^{b}
LD_{30}	4.75 ± 0.09 ^{a}	4.77 ± 0.11 ^{a}	4.93 ± 0.09 ^{a}	9.11 ± 0.26 ^{a}	23.24 ± 0.32 ^{a}
p	<0.0001	<0.0001	<0.0001	0.003	<0.0001
F	253.812	78.619	13.570	6.010	90.067
df	2, 535	2, 525	2, 505	2, 406	2, 406

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

In the LD_{30} treatment, the pupal durations of the female and male were shortened by 0.50 days ($p = 0.036$; $F = 3.390$; $df = 2, 155$) and 0.70 days ($p < 0.0001$; $F = 8.057$; $df = 2, 212$), respectively, compared with CK. No significant effect was found in the LD_{10} treatment. In addition, no significant effect was found in the female and male weight at any treated concentration of fluxametamide (Table 3).

Table 3. Sublethal effect of fluxametamide on duration and weight of F_0 *C. suppressalis* pupa.

Parameter	Duration (Days)		Pupal Weight (mg)	
	Female Pupa	Male Pupa	Female	Male
CK	7.67 ± 0.08 ^{a}	8.21 ± 0.12 ^{a}	51.87 ± 1.32 ^{a}	41.98 ± 0.82 ^{a}
LD_{10}	7.43 ± 0.21 ^{ab}	8.33 ± 0.19 ^{a}	51.09 ± 1.51 ^{a}	42.33 ± 0.88 ^{a}
LD_{30}	7.17 ± 0.12 ^{b}	7.51 ± 0.10 ^{b}	48.64 ± 1.39 ^{a}	40.79 ± 0.93 ^{a}
p	0.036	<0.0001	0.269	0.465
F	3.390	8.057	1.325	0.768
df	2, 155	2, 212	2, 155	2, 212

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

No difference was found in female and male adult durations, the number of eggs per female, or the hatching rate of eggs when F_0 was exposed to different doses of fluxam-

etamide (Table 4). Both mean fecundity and hatchability were decreased while its dose increased, whereas there was no significant difference among these treatments.

Table 4. Sublethal effect of fluxametamide on duration, mean fecundity and hatchability of F_0 *C. suppressalis* adults.

Parameter	Duration (Days)		Mean Fecundity (Egg/Female)	Hatchability (%)
	Female Adult	Male Adult		
CK	4.22 ± 0.14 [a]	2.86 ± 0.15 [a]	90.03 ± 8.42 [a]	76.00 ± 3.23 [a]
LD_{10}	4.11 ± 0.24 [a]	3.13 ± 0.17 [a]	79.06 ± 9.83 [a]	73.28 ± 2.50 [a]
LD_{30}	3.68 ± 0.25 [a]	3.23 ± 0.19 [a]	80.13 ± 6.73 [a]	62.84 ± 8.93 [a]
p	0.164	0.280	0.625	0.144
F	1.831	1.283	0.473	2.013
df	2, 149	2, 192	2, 75	2, 50

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

In this study, the normal pupa of *C. suppressalis* was brown and shiny (Figure 1A) and the wings of the normal adult were fully developed and formed a symmetric ridge-like shape (Figure 1B, C). However, after being treated with fluxametamide at a sublethal dose, some pupae could not be generated, and the pupal tail was crumpled and darker in color (Figure 1D); some adults failed to be eclosed from pupae, manifested as pupal shells that cannot be detached, or the wings were curled (Figure 1E–G).

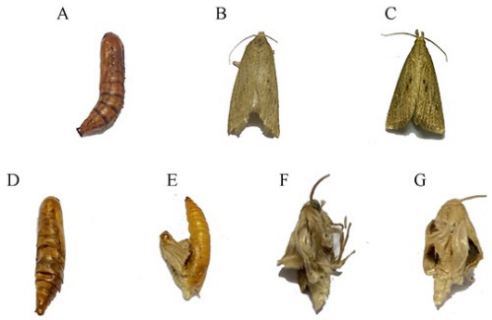

Figure 1. Various phenotypes of the *C. suppressalis* from pupa to adult. (**A**) normal pupa; (**B**) normal female; (**C**) normal male; (**D**) unable to eclose; (**E**–**G**) failure of eclosion.

Compared with the CK, the pupation rate in the LD_{10} and LD_{30} treatments was significantly decreased by 13.0% and 27.0% ($p < 0.0001$; $F = 16.029$; $df = 2, 12$), respectively, and their life cycle rate was significantly decreased by 15.00% and 27.00% ($p < 0.0001$; $F = 23.238$; $df = 2, 14$), respectively (Table 5). Other biological parameters including female rate and emergence rate were not significantly different among three treatments.

Table 5. Sublethal effect of fluxametamide on biological parameters of F_0 *C. suppressalis* pupae and adults.

Parameter	Pupation Rate (%)	Female Rate (%)	Emergence Rate (%)	Complete Full Life Cycle Rate (%)
CK	81.50 ± 3.59 [a]	48.13 ± 3.48 [a]	88.47 ± 1.09 [a]	72.00 ± 2.67 [a]
LD_{10}	68.50 ± 2.69 [b]	39.62 ± 4.53 [a]	83.55 ± 3.45 [a]	57.00 ± 2.15 [b]
LD_{30}	54.50 ± 3.74 [c]	41.36 ± 8.47 [a]	83.82 ± 2.12 [a]	45.00 ± 3.45 [c]
p	<0.0001	0.575	0.307	<0.0001
F	16.029	0.579	1.304	23.238
df	2, 12	2, 12	2, 12	2, 12

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

3.3. Sublethal Effect of Fluxametamide on C. suppressalis F_1 Generation

The sublethal effect of fluxametamide on the duration of egg and larvae of F_1 generation is presented in Table 6. Compared with the CK, the egg duration in the LD_{30} treatment was significantly prolonged by 0.17 days ($p < 0.0001$; $F = 14.105$; $df = 2, 357$), and the third-instar duration was significantly prolonged by 0.18 days in the LD_{10} treatment and shortened by 0.07 days in the LD_{30} treatment ($p = 0.045$; $F = 3.138$; $df = 2,349$), respectively.

Table 6. Sublethal effect of fluxametamide on duration of F_1 egg and larvae of *C. suppressalis*.

Developmental Stage	Duration (Days)			*p*	*F*	*df*
	CK	LD_{10}	LD_{30}			
Egg	5.99 ± 0.01 ^{b}	6.03 ± 0.02 ^{b}	6.16 ± 0.03 ^{a}	<0.0001	14.105	2, 357
First instar	3.76 ± 0.04 ^{a}	3.71 ± 0.04 ^{a}	3.81 ± 0.04 ^{a}	0.205	1.590	2, 357
Second instar	3.56 ± 0.05 ^{a}	3.46 ± 0.05 ^{a}	3.48 ± 0.06 ^{a}	0.396	0.929	2, 356
Third instar	3.53 ± 0.06 ^{ab}	3.64 ± 0.05 ^{a}	3.46 ± 0.05 ^{b}	0.045	3.138	2, 349
Fourth instar	4.05 ± 0.04 ^{a}	3.97 ± 0.05 ^{a}	4.05 ± 0.05 ^{a}	0.295	1.224	2, 345
Fifth instar	5.10 ± 0.07 ^{a}	5.07 ± 0.08 ^{a}	5.11 ± 0.07 ^{a}	0.934	0.068	2, 337
Sixth instar	8.76 ± 0.30 ^{a}	8.76 ± 0.29 ^{a}	8.94 ± 0.29 ^{a}	0.890	0.117	2, 289
Larval stage	28.81 ± 0.38 ^{a}	28.43 ± 0.36 ^{a}	28.78 ± 0.35 ^{a}	0.712	0.340	2, 289

Note: Values are shown as mean ± standard error (SE); superscript lowercase letters in the same row indicate the significant difference ($p < 0.05$).

No significant difference was observed in F_0 for other biological parameters, including the pupal duration, pupal weight, adult duration, the mean fecundity, hatchability, the pupation and emergence rates, and female ratio in the three treatments (Tables 7–9).

Table 7. Sublethal effect of fluxametamide on duration and weight of F_1 *C. suppressalis* pupa.

Parameter	Duration (Days)		Pupal Weight (mg)	
	Female Pupa	Male Pupa	Female	Male
CK	7.02 ± 0.08 ^{a}	7.66 ± 0.11 ^{a}	64.76 ± 1.73 ^{a}	49.61 ± 1.28 ^{a}
LD_{10}	7.00 ± 0.12 ^{a}	7.50 ± 0.07 ^{a}	64.50 ± 2.17 ^{a}	48.68 ± 1.18 ^{a}
LD_{30}	7.11 ± 0.08 ^{a}	7.55 ± 0.08 ^{a}	59.72 ± 1.71 ^{a}	46.26 ± 1.16 ^{a}
p	0.681	0.438	0.105	0.146
F	0.385	0.829	2.302	1.948
df	2, 112	2, 147	2, 112	2, 147

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

Table 8. Sublethal effect of fluxametamide on duration, mean fecundity, and hatchability of F_1 *C. suppressalis* adult.

Parameter	Duration (Days)		Mean Fecundity (Egg/Female)	Hatchability (%)
	Female Adult	Male Adult		
CK	5.34 ± 0.24 ^{a}	4.88 ± 0.26 ^{a}	132.38 ± 10.92 ^{a}	72.63 ± 3.35 ^{a}
LD_{10}	4.82 ± 0.26 ^{a}	4.42 ± 0.28 ^{a}	123.54 ± 11.68 ^{a}	69.41 ± 2.48 ^{a}
LD_{30}	5.60 ± 0.22 ^{a}	4.56 ± 0.35 ^{a}	133.33 ± 15.78 ^{a}	63.94 ± 4.26 ^{a}
p	0.082	0.570	0.850	0.201
F	2.562	0.565	0.163	1.636
df	2, 106	2, 134	2, 77	2, 77

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

Table 9. Sublethal effect of fluxametamide on biological parameters of F_1 *C. suppressalis* pupae and adults.

Parameters	Pupation Rate (%)	Female Ratio (%)	Emergence Rate (%)	Complete Full Life Cycle Rate (%)
CK	80.83 ± 0.83 [a]	42.23 ± 1.61 [a]	84.53 ± 1.81 [a]	68.33 ± 1.67 [a]
LD_{10}	83.33 ± 2.20 [a]	32.96 ± 4.36 [a]	85.19 ± 4.31 [a]	70.83 ± 2.20 [a]
LD_{30}	79.17 ± 3.00 [a]	37.74 ± 2.32 [a]	84.17 ± 0.58 [a]	66.67 ± 3.00 [a]
p	0.454	0.172	0.965	0.495
F	0.905	2.392	0.036	0.792
df	2, 6	2, 6	2, 6	2, 6

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

3.4. Sublethal Effect of Fluxametamide on C. suppressalis Ovary

After the third-instar larvae of *C. suppressalis* were treated with a sublethal dose of fluxametamide, their female adults were dissected and the length and weight of the ovarian tube of *C. suppressalis* were obtained (Table 10 and Figure 2). The length and weight of the ovarian tube were decreased with the increased dose of fluxametamide. In the CK, LD_{10}, and LD_{30} treatments, the length of the ovarian tube was 8.74 ± 0.93, 7.77 ± 2.07, and 5.47 ± 1.33 mm/ larva, respectively, and the weight of the ovarian tube was 15.50 ± 4.00, 12.79 ± 3.26, and 8.81 ± 3.16 mg/larva, respectively. Significant differences in the length and weight of the ovarian tube were observed between LD_{30} and CK, which indicated that the LD_{30} fluxametamide significantly inhibited the ovarian development of *C. suppressalis.*

Table 10. Sublethal effect of fluxametamide on the length and weight of the ovarian tube of *C. suppressalis.*

Parameters	Length of Ovarian Tube (mm/larva)	Weight of Ovarian Tube (mg/larva)
CK	8.74 ± 0.93 [a]	15.50 ± 4.00 [a]
LD_{10}	7.77 ± 2.07 [ab]	12.79 ± 3.26 [ab]
LD_{30}	5.47 ± 1.33 [b]	8.81 ± 3.16 [b]
p	0.016	0.029
F	5.790	4.704
df	2, 13	2, 13

Note: Values are shown as mean ± standard error (SE). The superscript lowercase letters in the same column indicate the significant difference ($p < 0.05$).

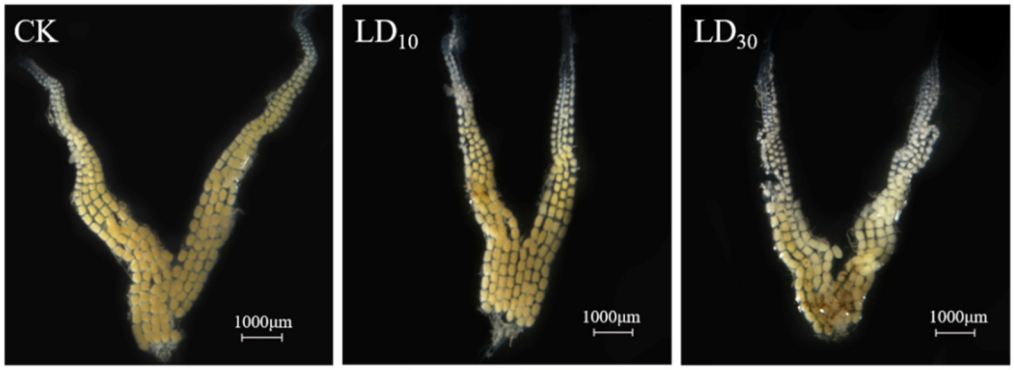

Figure 2. Sublethal effect of fluxametamide on the size of *C. suppressalis* ovary. Significant differences were shown as different lowercase letters above the bars if $p < 0.05$ (Tukey's test).

4. Discussion

As one of the most serious harmful insect pests in the paddy, *C. suppressalis* has developed resistance to many kinds of insecticides including fipronil, chlorantraniliprole,

avermectin, etc. Compared with 2019, the resistance ratio of the *C. suppressalis* population to chlorantraniliprole in Hubei in 2020 was up to 11–28 times and reached moderate resistance. The resistance of *C. suppressalis* populations in southern areas of China such as Zhejiang, Anhui, Jiangxi, and Hunan to chlorantraniliprole was high-level: as high as 2060 times higher. The resistance of *C. suppressalis* to abamectin in Jiangxi and Hunan was up to moderate-to-high levels [3]. Therefore, it is necessary to introduce new insecticides, e.g., isoxazoline insecticides, to control *C. suppressalis*. As the first isoxazoline insecticide used for the control of agricultural pests, fluxametamide acts on insect ionotropic GABA receptors and has high insecticidal activity to several types of agricultural pests [9]. In this study, we aimed to clarify its insecticidal activity and its sublethal effect on the contemporary and progeny biological characteristics against *C. suppressalis*.

According to our results, fluxametamide exhibited high insecticidal activity against the fourth and third-instar larvae. The LD_{50} of fluxametamide against the fourth-instar larvae of *C. suppressalis* was 1.308 mg/kg at 72 h, which demonstrated that the lethal activity of fluxametamide was higher than that of chlorantraniliprole (3.112 mg/kg) and lower than that of emamectin benzoate (0.006 mg/kg) (Table 1), which is consistent with the toxic trend of emamectin benzoate and chlorantraniliprole in *C. suppressalis* as previously reported [19]. The LD_{50} of fluxametamide against the third-instar larvae of *C. suppressalis* at 72 h was lower than that against the fourth-instar larvae, suggesting that the sensitivity of *C. suppressalis* to fluxametamide decreased with the increase in developmental stage. Similar results were observed in the sensitivity of *C. suppressalis* larvae to flubendiamide and chlorantraniliprole [20]. Therefore, fluxametamide can be applied to control *C. suppressalis* and is better to be used at the earlier developmental stage.

Sublethal doses of insecticide(s) could affect insect population dynamics through impairment of developmental and reproductive traits. In this study, the biological parameters of third-instar *C. suppressalis* larvae were determined using artificial food containing fluxametamide; the results showed that the duration of larvae (female and male), the pupation rate, and the life cycle rate were significantly affected. Similar results have also been reported for the sublethal effect of insecticide to Lepidoptera. For example, after the second-instar larvae of rice leaf folders *Cnaphalocrocis medinalis* (Guenée) treated with a sublethal dose (LD_{10} or LD_{25}) of chlorpyrifos, the larval duration was significantly prolonged, and the pupal duration was significantly shortened [21]. After larvae of the cutworm *Spodoptera litura* Fabricius were treated with LC_{10} or LC_{25} of metaflumizone, F_0 pre-pupal and pupal durations, pupation rate, and the probability of test worms completing the entire life cycle were significantly decreased compared with the CK [22]. After diamondback moth *Plutella xylostella* L. larvae were treated with LC_{10} or LC_{25} of spinetoram, their pupation rate and the probability of completing the entire life cycle (81.61% or 75.72%) were significantly lower than those of the CK [23]. In summary, Lepidoptera pests treated with a sublethal dose of insecticide mostly manifest as prolonged larval duration, shortened pupal duration, decreased pupation rate, and a decline in the number of those completing the entire life cycle, which could reduce the proliferation rate of the population. We speculated that the reason for this phenomenon is that the sublethal dose of insecticide can inhibit the feeding of a test insect, which results in insufficient nutrition and eventually prolongs the developmental periods and affects the quality of pupae. However, some sublethal doses of insecticide cause significant prolongation of the larval and pupal durations of the F_0 generation [24,25]. For example, the larval and pupal stage of *P. xylostella* and *S. litura* were significantly prolonged by a sublethal dose of spinosad (0.04 and 0.16 mg/kg) [24] and fluralaner (LD_5 and LD_{15}) [25]. Therefore, the reasons for this discrepancy still require further study.

Fecundity is an important indicator of insect population dynamics. Most studies have shown that sublethal doses of insecticide can significantly change the mean fecundity of reproductive females (MFRF) and hatchability [26,27]. However, in this study, the adult longevity, female ratio, the MFRF, hatchability, and emergence rate of *C. suppressalis* F_0 generation were nonsignificantly reduced after third-instar larvae were treated with

the sublethal dose of fluxametamide. Similarly, there was no significant difference in the fecundity after the fifth-instar larvae of fall armyworm *S. frugiperda* (J. E. Smith) were treated with LC_{10} or LC_{25} methoxyfenozide, and the MFRF was 264 and 356 eggs, respectively, which was reduced compared with the CK (393 eggs/female), and there was no significant change in the female ratio and hatchability [28]. However, after third-instar *S. frugiperda* larvae were treated with a sublethal dose of spinetoram, no significant difference was observed in the MFRF and hatchability [29]. Therefore, we speculated that the type and tested dose of insecticide, the age of insect, etc., might affect the fertility of the insect based on the abovementioned results.

In this study, the F_1 generation of *C. suppressalis* was not significantly affected by the sublethal dose of fluxametamide, and its larval development duration, pupal weight, adult longevity, the MFRF, hatchability, pupation rate, and other parameters were negligible compared with the CK. Only the egg duration in the LD_{30} treatment was significantly longer than the CK, and the third-instar larval duration became shorter. Similarly, after the third-instar larvae of *C medinalis* were treated with chlorantraniliprole or emamectin benzoate at LC_{10}, LC_{25}, or LC_{50}, respectively, the duration of F_1 eggs was prolonged with the increase in the sublethal dose, and little effect was observed on the pupal weight and pupal duration of the offspring when the contemporary first to third instar larvae were treated [30]. Therefore, we speculated that residual insecticides in the body are probably excreted or metabolized, and do not accumulate in the insect body during the growth and development of offspring.

The reproductive system is critical for the development of an insect population [31]; therefore, the gonad development of females has been extensively studied [32–34]. In general, the most direct effect of an insecticide on insect fecundity is on its reproductive organs. Due to the different development characteristics of male and female gonads, only female insects are still developing in the adult stage [35]. Therefore, the development of ovaries was selected for study of the sublethal effect of fluxametamide on *C. suppressalis*. In this study, both the length and weight of the ovarian tube were significantly reduced in the LD_{30} of fluxametamide. Similarly, multiple nuclear polyhedrosis virus of beet armyworm *S. exigua* (Hübner) (*Se*MNPV) at a sublethal dose (10^2 PIB/larva and 10^3 PIB/larva) effectively reduced the fecundity of *S. exigua*, and the length and weight of the ovary tubes were smaller than those of the CK [35]. The most direct response of the ovarian development of *C. suppressalis* to fluxametamide is the changes in length and weight of the ovarian tubes. However, the MFRF of *C. suppressalis* nonsignificantly decreased compared with the CK. A similar result was observed in the third-instar larvae of *Plutella xylostella* treated with a sublethal dose of emamectin benzoate [36]. When the third instar larvae of *Plutella xylostella* were treated with sublethal doses of emamectin benzoate, the length of the ovarian tube in the LC_{20} treatment group significantly decreased by 15.6 % compared with the CK, whereas the MFRF significantly increased [36]. We speculated that when subjected to some adverse external stimuli, including insecticide, insects initially undergo a compensatory response after the initial inhibitory response, and the compensatory response may exceed the performance of CK. This is the stimulatory phenomenon of overcompensation in Calabrese's study [37]. To our knowledge, this is the first report related to fluxametamide's effects on insect reproduction.

5. Conclusions

In conclusion, fluxametamide has not only high lethal activity but also a significantly sublethal effect, e.g., a significant delay of the growth and development of the F_0 generation, and significant inhibition of the ovarian development in LD_{30} treatment in the third instar larvae of *C. suppressalis*. Our results will provide scientific guidance for the use of fluxametamide in the field to effectively control agricultural pests including *C. suppressalis*.

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Appendix A

Table A1. Toxicity of fluxametamide to third-instar larvae of *C. suppressalis*.

Time (h)	LD_{50} (mg/kg) (95% CL)	LD_{30} (mg/kg) (95% CL)	LD_{10} (mg/kg) (95% CL)	Slope ± SE	R^2
48	1.11 (0.80–1.54)	0.52 (0.27–0.73)	0.17 (0.04–0.31)	1.576 ± 0.332	0.934
72	0.50 (0.31–0.68)	0.25 (0.11–0.39)	0.09 (0.03–0.18)	1.764 ± 0.328	0.938

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