Lethal and sublethal effects of fluxametamide on rice-boring pest, rice stem borer Chilo suppressalis

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Abstract

Fluxametamide is a novel isoxazoline insecticide and has been registered in Korea and Japan to control Lepidoptera pests. Rice stem borer, *Chilo suppressalis* (Walker), is a destructive Lepidoptera pest of rice in China, and novel effectively insecticides are required to be developed for controlling it due to its increasing resistance levels. Therefore, the lethal and sublethal effects of fluxametamide on *C. suppressalis* were investigated in the present study. In the lethal assay, the insecticidal activity of fluxametamide with median lethal dose (LD$_{50}$) value of 1.308 mg/kg to the 4th 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 addition, the 3rd instar larvae of *C. suppressalis* were sensitive to fluxametamide than the 4th instar larvae. In the sublethal (LD$_{10}$ and LD$_{30}$) assay, the duration of 3rd to 6th instar larvae was significantly increased, whereas the pupal duration, pupation rate, and life-cycle rate were also significantly increased in F$_0$ generation. Both length and weight of ovarian tube were decreased with the increase of fluxametamide dose, and they in the LD$_{30}$ treatment were significantly lower than those of the control group. In F$_1$ generation, only the duration of eggs was significantly increased with LD$_{30}$ treatment of fluxametamide, other developmental parameters has no significant change. These results suggest that fluxametamide has excellent lethal and sublethal effects on *C. suppressalis* and probably is able to suppress the population growth and progeny of *C. suppressalis*.

Key Message

1. Fluxametamide has better insecticidal activity than chlorantraniliprole against *Chilo suppressalis* larvae;
2. Sublethal dose of fluxametamide could decelerate the growth and development of *C. suppressalis* F$_0$;
3. LD$_{30}$ fluxametamide significantly inhibits the development of *C. suppressalis* ovarian.

1 Introduction

Fluxametamide is a newly registered isoxazoline insecticide in Japan in 2019, which acts on the γ-aminobutyric acid (GABA)-gated chloride channel(Asahi, et al., 2018, Li, et al., 2019). Fluxametamide has high insecticidal activity against agricultural pests, such as Lepidoptera, Thysanoptera, etc., on the vegetable and tea plant, but low toxic to the bee and mammal(Ji, et al., 2021). 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.(Jiang, 2011, Zhang, et al., 2022, Li, Qu, Ye, C.J and L.M, 2014) However the chemical insecticides are still the main tool of its control. Therefore, introduction of new insecticides, which have highly lethal activity against it and no cross-resistance with other insecticides, is very necessary.
Meanwhile, it is worth to noting that the sublethal effect of novel insecticide is required except its lethal effect. To all knowledge, when the lethal dose of the insecticide can directly kill the targeted pest, but with the influence of external factors such as time and the natural environment, etc., the insecticide component generally slowly degrade, and the virulence will gradually decrease and reach to a sublethal dose (Boina, Rogers, Wang and Stelinski, 2010). Therefore, the targeted pest is poisoned by the insecticides without fatal, and still maintains the behavioral ability (Haynes, 1988). Sublethal doses of insecticide can affect the growth, development, and reproduction of insects, and also alter the ecological behavior and resistance of insects (Wang, 2004). For example, after the 3rd instar larvae of *C. suppressalis* treated with sublethal doses (LC$_{10}$ and LC$_{30}$) of chlorantraniliprole, the larval duration was significantly extended, and the larval body weight, longevity of adult, and pupation rate were significantly reduced (Huang, Lu, Han, Du and Wang, 2016). Therefore, the authors speculated that under the treatment of sublethal concentration of chlorantraniliprole, the insects eat 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* (Huang, Lu, Han, Du and Wang, 2016). After 1st instar larvae of *Tryporyza incertulas* 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 (Wang, et al., 2005).

In insect female adults, ovaries are the primary reproductive organs, which regulate the activities of secondary reproductive organs (Bhardwaj, Mittal, Saraf and Kumari, 2020). Therefore, disturbance in ovarian physiology by insecticides affects overall reproductive activities. Insecticides affect the reproductive system either by exerting cytotoxicity and genotoxicity as a result of oxidative stress or through endocrine disruption. Through the study of insect reproductive biology, the analysis of the development process of female reproductive system has certain significance for predicting the development and occurrence regularity of pests.

Fluxametamide is a new insecticide with high insecticidal toxicity, and no report on its sublethal effects on pest is reported to date. Therefore, the physiological parameters including hatchability, developmental duration of larvae, pupation rate, pupal weight and durations, emergence, longevity and fertility of adult, the development of ovaries, were investigated after *C. suppressalis* treated with fluxametamide in this study. These results will provide comprehensive useful information for assessing the potential the lethal effects and sublethal effects of fluxametamide to insect, and for its recommendation in integrated pest management.

### 2 Materials And Methods

#### 2.1 Insect rearing and insecticides

The population of *C. suppressalis* used in this study was reared on an artificial food in the laboratory without exposure to insecticides (Li, Han and Peng, 2015). 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., and, chlorantraniliprole (96%) and
emamectin benzoate (95%) were supplied by our lab.

2.2 Acute toxicity assay

An acute toxicity assay was conducted on 4th instar larvae using the artificial food mixed insecticide
method (Ling, 2019). The lethal activity of insecticide towards C. suppressalis larvae was examined by
the ‘Guideline for Laboratory Bioassay of Pesticides, Part 10: Diet incorporation method (Code:
(chlorantraniliprole, 1, 2, 4, 8, 16, and 32 mg/kg; fluxametamide, 0.5, 1, 2, 3, 4, and 5 mg/kg; emamectin
benzoate, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/kg) of each insecticide were set referring to the
pre-assay. Briefly, 10 g fresh artificial food is 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 plates for treated groups.
Meanwhile, the control group was only treated with a mixture of acetone and 0.1% Tween-80. Ten 4th
instar larvae were used for each replication and triplications were performed for each concentration.
Mortality was recorded at 72 h after treatment.

In addition, the lethal toxicity of fluxametamide to 3rd instar larvae of C. suppressalis was performed as
abovementioned procedures using six experimental doses (0.25, 0.5, 1, 1.5, 2, and 2.5 mg/kg). Both LD_{10}
and LD_{30} of fluxametamide were used for sublethal effect study.

2.3 Sublethal effects of fluxametamide on C. suppressalis
development of F₀ generation

Forty 3rd 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 treatment. A mixture of
acetone and 0.1% Tween-80 was used as the control. After 72 h, all survival larvae were individually
transferred to 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, one pair of male and female adults
emerged on the same day were paired in an oviposition plastic cup (13 cm height; neck diameter: 9.5 cm
upper end and 5.5 cm lower end) containing 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 F₀ generation

For determining the effect of fluxametamide on C. suppressalis ovary, 2-day-old female adult ovaries
were dissected in phosphate buffer saline (PBS) under a stereoscopic microscope (Nikon SMZ25, Nikon
In each treatment (CK, LD<sub>10</sub> and LD<sub>30</sub>), 10 female adults were randomly removed for dissection. First, the female adults on the second day of emergence were prepared under the stereomicroscope, and the head of each adult was cut off with anatomical scissors. The remaining part was placed on a slide dripping with PBS under a stereoscopic microscope. Secondly, the insect body was incised with anatomic tweezers and the outer cuticle was gently torn until it reached the tail. Thirdly, the ovary was slowly separated from the body wall, and transferred to another slide dripping into PBS for observation. Finally, 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<sub>1</sub> generation

To determine whether fluxametamide has carryover activity on the F<sub>1</sub> generation, 120 eggs were taken randomly from each pair of adult moths of each treatment (CK, LD<sub>10</sub> and LD<sub>30</sub>). Newly hatched F<sub>1</sub> larvae were individually transferred to a clean plastic tube containing untreated artificial food. The duration of the F<sub>1</sub> larval period and subsequent stages, and the related survivorship were recorded daily. Pupal weight, pupation rate, emergence rate and female ratio were recorded. Newly emerged F<sub>1</sub> adults were paired in an oviposition plastic cup as described above. The survival rate and the number of laid eggs by F<sub>1</sub> adults were recorded daily.

2.6 Data analysis

The median lethal dose (LD<sub>50</sub>) with corresponding 95% confidential limits (CL) and sublethal doses (LD<sub>30</sub> and LD<sub>10</sub>) of insecticide on 4th instar larvae of C. suppressalis, were calculated using a probit regression analysis by 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 by Tukey's multiple comparison test (P< 0.05).

3 Results

3.1 Lethal effect of fluxametamide on C. suppressalis larvae
Table 1
Toxicity of three insecticides to 4th instar larvae of *C. suppressalis* after 72 h treatment

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Slope ± SE</th>
<th>LD$_{50}$ (mg/kg)</th>
<th>95% CL</th>
<th>$\chi^2$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluxametamide</td>
<td>2.838 ± 0.357</td>
<td>1.308</td>
<td>0.815–1.839</td>
<td>6.863</td>
<td>0.930</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>1.327 ± 0.219</td>
<td>3.112</td>
<td>1.998–4.420</td>
<td>3.677</td>
<td>0.912</td>
</tr>
<tr>
<td>Emamectin benzoate</td>
<td>2.919 ± 0.430</td>
<td>0.006</td>
<td>0.005–0.008</td>
<td>4.624</td>
<td>0.926</td>
</tr>
</tbody>
</table>

The LD$_{50}$ values of fluxametamide, chlorantraniliprole and emamectin benzoate for 4th 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 3rd instar larvae were 0.09, 0.25 and 0.50 mg/kg at 72 h, respectively (Table A1).

### 3.2 Sublethal effect of fluxametamide on *C. suppressalis* F$_0$ generation

Table 2
Sublethal effects of fluxametamide on F$_0$ larvae duration of *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>3rd instar</th>
<th>4th instar</th>
<th>5th instar</th>
<th>6th instar</th>
<th>3rd to 6th instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>2.41 ± 0.05$^a$</td>
<td>3.42 ± 0.04$^a$</td>
<td>4.33 ± 0.06$^a$</td>
<td>7.94 ± 0.23$^a$</td>
<td>18.01 ± 0.25$^a$</td>
</tr>
<tr>
<td>LD$_{10}$</td>
<td>4.01 ± 0.08$^b$</td>
<td>4.28 ± 0.08$^b$</td>
<td>4.50 ± 0.09$^a$</td>
<td>8.37 ± 0.22$^{ab}$</td>
<td>21.05 ± 0.28$^b$</td>
</tr>
<tr>
<td>LD$_{30}$</td>
<td>4.75 ± 0.09$^c$</td>
<td>4.77 ± 0.11$^c$</td>
<td>4.93 ± 0.09$^b$</td>
<td>9.11 ± 0.26$^b$</td>
<td>23.24 ± 0.32$^c$</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SE$_{s}$). The superscript lower-case letters in the same column indicate a significant difference ($P<0.05$).

The significant difference was observed in the larval duration of *C. suppressalis* treated by different doses of fluxametamide. For the LD$_{10}$ and LD$_{30}$ treatments, the durations from the 3rd to 6th instar were prolonged by 3.04 and 5.23 days ($P<0.0001$; $F=90.067$; $df=2,408$), respectively, compared with control (Table 2).
Table 3
Sublethal effects of fluxametamide on F₀ pupa of *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration (d)</th>
<th>Pupal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female pupa</td>
<td>Male pupa</td>
</tr>
<tr>
<td>CK</td>
<td>7.67 ± 0.08ᵃ</td>
<td>8.21 ± 0.12ᵇ</td>
</tr>
<tr>
<td>LD₁₀</td>
<td>7.43 ± 0.21ᵃᵇ</td>
<td>8.33 ± 0.19ᵇ</td>
</tr>
<tr>
<td>LD₃₀</td>
<td>7.17 ± 0.12ᵇ</td>
<td>7.51 ± 0.10ᵃ</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.036</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>F</em></td>
<td>3.390</td>
<td>8.057</td>
</tr>
<tr>
<td><em>df</em></td>
<td>2, 155</td>
<td>2, 212</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SEs). The superscript lower-case letters in the same column indicate a significant difference (*P* < 0.05).

For the LD₃₀ 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 control. No significant effect was found in the LD₁₀ treatment. In addition, no significant effect was found in the female and male weight at any concentration of fluxametamide (Table 3).
### Table 4

**Sublethal effects of fluxametamide on F₀ adult of *C. suppressalis***

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration (d)</th>
<th>Mean fecundity (egg/female)</th>
<th>Hatchability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female adult</td>
<td>Male adult</td>
<td></td>
</tr>
<tr>
<td><strong>CK</strong></td>
<td>4.22 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.86 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.03 ± 8.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;10&lt;/sub&gt;</strong></td>
<td>4.11 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.13 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.06 ± 9.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;30&lt;/sub&gt;</strong></td>
<td>3.68 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.23 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.13 ± 6.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.164</td>
<td>0.280</td>
<td>0.625</td>
</tr>
<tr>
<td><em>F</em></td>
<td>1.831</td>
<td>1.283</td>
<td>0.473</td>
</tr>
<tr>
<td><em>df</em></td>
<td>2, 149</td>
<td>2, 192</td>
<td>2, 75</td>
</tr>
</tbody>
</table>

**Note:** Values are shown as means ± standard errors (SE<sub>s</sub>). The superscript lower-case letters in the same column indicate a 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 exposed to different doses of fluxametamide (Table 4). Both mean fecundity and hatchability were decreased while its dose increased, whereas there was no significant difference among these treatments.

In this study, the normal pupa of *C. suppressalis* is brown and shiny (Fig. 1-A) and the wings of the normal adult are fully developed and form a roof ridge (Fig. 1-B, C). However, after fluxametamide treatment at sublethal doses, some pupa could not be able to emerge, and the pupal tail was crumpled, darker in color, and the surface lost luster (Fig. 1-D), while some adults failed to emerge from pupae, manifested as pupal shells that cannot be detached, or the wings were curled (Fig. 1-E, F, G).
Table 5
Sublethal effects of fluxametamide on F₀ *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pupation rate (%)</th>
<th>Female rate (%)</th>
<th>Emergence rate (%)</th>
<th>Complete full life cycle rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>81.50 ± 3.59a</td>
<td>48.13 ± 3.48a</td>
<td>88.47 ± 1.09a</td>
<td>72.00 ± 2.67a</td>
</tr>
<tr>
<td>LD₁₀</td>
<td>68.50 ± 2.69b</td>
<td>39.62 ± 4.53a</td>
<td>83.55 ± 3.45a</td>
<td>57.00 ± 2.15b</td>
</tr>
<tr>
<td>LD₃₀</td>
<td>54.50 ± 3.74c</td>
<td>41.36 ± 8.47a</td>
<td>83.82 ± 2.12a</td>
<td>45.00 ± 3.45c</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.0001</td>
<td>0.575</td>
<td>0.307</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>F</em></td>
<td>16.029</td>
<td>0.579</td>
<td>1.304</td>
<td>23.238</td>
</tr>
<tr>
<td><em>df</em></td>
<td>2, 12</td>
<td>2, 12</td>
<td>2, 12</td>
<td>2, 12</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SEs). The superscript lower-case letters in the same column indicate a significant difference (*P* < 0.05).

Compared with the control group, the pupation rate in the LD₁₀ and LD₃₀ 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.

### 3.3 Sublethal effect of fluxametamide on *C. suppressalis* F₁ generation
Sublethal effects of fluxametamide on the duration of egg and larvae of F₁ generation from 3rd instar larvae of F₀ generation are presented in Table 6. Compared with the control, the egg duration in the LD₃₀ treatment was significant prolonged by 0.17 days \((P < 0.0001; F = 14.105; df = 2, 357)\), and the 3rd instar duration was significant prolonged by 0.18 days in the LD₁₀ treatment and shortened by 0.07 days in the LD₃₀ treatment \((P = 0.045; F = 3.138; df = 2,349)\), respectively.

No significant difference was observed in other biological parameters, including the pupal duration and weight of female and male, the adult duration of female and male, the mean fecundity of reproductive females, hatchability, the pupation and emergence rates, and female ratio in three treatments (Tables 7–9).
Table 7
Sublethal effects of fluxametamide on F$_1$ pupa of *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration (d)</th>
<th>Pupal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female pupa</td>
<td>Male pupa</td>
</tr>
<tr>
<td>CK</td>
<td>7.02 ± 0.08\textsuperscript{a}</td>
<td>7.66 ± 0.11\textsuperscript{a}</td>
</tr>
<tr>
<td>LD$_{10}$</td>
<td>7.00 ± 0.12\textsuperscript{a}</td>
<td>7.50 ± 0.07\textsuperscript{a}</td>
</tr>
<tr>
<td>LD$_{30}$</td>
<td>7.11 ± 0.08\textsuperscript{a}</td>
<td>7.55 ± 0.08\textsuperscript{a}</td>
</tr>
<tr>
<td>$P$</td>
<td>0.681</td>
<td>0.438</td>
</tr>
<tr>
<td>$F$</td>
<td>0.385</td>
<td>0.829</td>
</tr>
<tr>
<td>$df$</td>
<td>2, 112</td>
<td>2, 147</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SE$_s$). The superscript lower-case letters in the same column indicate a significant difference ($P<0.05$).

Table 8
Sublethal effects of fluxametamide on F$_1$ adult of *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration (d)</th>
<th>Mean fecundity (egg/female)</th>
<th>Hatchability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female adult</td>
<td>Male adult</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>5.34 ± 0.24\textsuperscript{a}</td>
<td>4.88 ± 0.26\textsuperscript{a}</td>
<td>132.38 ± 10.92\textsuperscript{a}</td>
</tr>
<tr>
<td>LD$_{10}$</td>
<td>4.82 ± 0.26\textsuperscript{a}</td>
<td>4.42 ± 0.28\textsuperscript{a}</td>
<td>123.54 ± 11.68\textsuperscript{a}</td>
</tr>
<tr>
<td>LD$_{30}$</td>
<td>5.60 ± 0.22\textsuperscript{a}</td>
<td>4.56 ± 0.35\textsuperscript{a}</td>
<td>133.33 ± 15.78\textsuperscript{a}</td>
</tr>
<tr>
<td>$P$</td>
<td>0.082</td>
<td>0.570</td>
<td>0.850</td>
</tr>
<tr>
<td>$F$</td>
<td>2.562</td>
<td>0.565</td>
<td>0.163</td>
</tr>
<tr>
<td>$df$</td>
<td>2, 106</td>
<td>2, 134</td>
<td>2, 77</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SE$_s$). The superscript lower-case letters in the same column indicate a significant difference ($P<0.05$).
### Table 9
Sublethal effects of fluxametamide on F₁ of *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pupation rate (%)</th>
<th>Female ratio (%)</th>
<th>Emergence rate (%)</th>
<th>Complete full life cycle rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>80.83 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.23 ± 1.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.53 ± 1.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.33 ± 1.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD&lt;sub&gt;10&lt;/sub&gt;</td>
<td>83.33 ± 2.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.97 ± 4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.19 ± 4.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.83 ± 2.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD&lt;sub&gt;30&lt;/sub&gt;</td>
<td>79.17 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.74 ± 2.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.17 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.67 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.454</td>
<td>0.172</td>
<td>0.965</td>
<td>0.495</td>
</tr>
<tr>
<td><em>F</em></td>
<td>0.905</td>
<td>2.392</td>
<td>0.036</td>
<td>0.792</td>
</tr>
<tr>
<td><em>df</em></td>
<td>2, 6</td>
<td>2, 6</td>
<td>2, 6</td>
<td>2, 6</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SEs). The superscript lower-case letters in the same column indicate a significant difference (*P* < 0.05).

### 3.4 Sublethal effect of fluxametamide on *C. suppressalis* ovary

After the 3rd instar larvae of *C. suppressalis* were treated with sublethal doses of fluxametamide and their female adults were dissected, the length and weight of the ovarian tube of *C. suppressalis* were obtained (Table 10 and Fig. 2). The length and weight of ovarian tube were decreased with the increased dose of fluxametamide. The ovarian tube length in the CK, LD<sub>10</sub> and LD<sub>30</sub> treatments was 8.74 ± 0.93, 7.77 ± 2.07 and 5.47 ± 1.33 mm / larva, respectively. Their weight of ovarian tube was 15.50 ± 4.00, 12.79 ± 3.26 and 8.81 ± 3.16 mg / larva, respectively. By variance test, significant differences in the length and weight of ovarian tube were observed between LD<sub>30</sub> and CK, which indicated that the LD<sub>30</sub> fluxametamide significantly inhibited the ovarian development of *C. suppressalis*. 
Table 10
Sublethal effect of fluxametamide on the length and weight of ovarian tube of *C. suppressalis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Length of ovarian tube (mm/larva)</th>
<th>Weight of ovarian tube (mg/larva)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>8.74 ± 0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.50 ± 4.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD&lt;sub&gt;10&lt;/sub&gt;</td>
<td>7.77 ± 2.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.79 ± 3.26&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD&lt;sub&gt;30&lt;/sub&gt;</td>
<td>5.47 ± 1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.81 ± 3.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.016</td>
<td>0.029</td>
</tr>
<tr>
<td><em>F</em></td>
<td>5.790</td>
<td>4.704</td>
</tr>
<tr>
<td>df</td>
<td>2, 8</td>
<td>2, 8</td>
</tr>
</tbody>
</table>

Note: Values are shown as means ± standard errors (SEs). The superscript lower-case letters in the same column indicate a significant difference (*P* < 0.05).

### 4 Discussion

As the first isoxazoline insecticide, fluxametamide acts on insect ionotropic GABA receptors and has highly insecticidal activity to agricultural pests (Ji, et al., 2021). To clarify the insecticidal activity of fluxametamide against *C. suppressalis* and its sublethal effects on the contemporary and progeny biological characteristics of this pest, the lethal and sublethal doses of fluxametamide on *C. suppressalis* were investigated in this study. We found that fluxametamide exhibits excellent insecticidal activity towards the 4th and 3rd instar larvae. The LD<sub>50</sub> of fluxametamide for 4th instar larvae of *C. suppressalis* was 1.308 mg/kg at 72 h, which demonstrated that the insecticidal activity of fluxametamide was lower than that of chlorantraniliprole (3.112 mg/kg) and higher than that of emamectin benzoate (0.006 mg/kg) (Table 1), which is consistent with the trend of emamectin benzoate and chlorantraniliprole toxicity to *C. suppressalis* as previously reported (Ling, 2019). And the LD<sub>50</sub> of fluxametamide for 3rd instar larvae of *C. suppressalis* was 0.50 mg/kg at 72 h, which was lower than that of 4th instar larvae, suggesting that the susceptibility of *C. suppressalis* to fluxametamide decreased with the increase of ages. The similar results were observed in the sensitivity of *C. suppressalis* larvae to flubendiamide and chlorantraniliprole (Liang, Wang, Chen and Xian, 2014). Therefore, according to the perspective of activity and combined with its non-interaction resistance, fluxametamide can be applied to the prevention and control of *C. suppressalis* in the future.

Sublethal doses of insecticides could affect insect population dynamics through impairment of developmental and reproductive traits. In this study, the biological parameters of *C. suppressalis* of 3rd instar larvae were determined with an artificial food containing fluxametamide, the results showed the larvae duration, the female and male duration, the pupation rate and the life-cycle rate were significantly effect. Similar results with our study have also been reported on the sublethal effects of insecticide to Lepidoptera. For example, after the 2nd instar larvae of *Cnaphalocrocis medinalis* treated with the
sublethal doses (LD_{10} and LD_{25}) of chlorpyrifos, the larval duration was significantly prolonged, and the pupal duration was significantly shortened (Yang, Wang, Xu, Lu and Lv, 2018). The Spodoptera litura larvae were treated with LC_{10} and LC_{25} of metaflumizone, F_{0} pre-pupal and pupal durations were significantly shortened, the pupation rate was significantly decreased, and the probability of test worms completing the entire life cycle was decreased significantly compared with the control group (Ma, 2019). After the spinetoram of LC_{10} and LC_{25} treated the larvae of Plutella xylostella, the pupation rate was significantly lower than that of the control group, and the probability of completing the entire life cycle was 81.61% and 75.72%, respectively, which was significantly lower than that of the control group of 92.76% (Tamilselvan, Kennedy and Suganthi, 2021). In summary, Lepidoptera pests treated with sublethal dose of insecticides will mostly manifest as prolonging the larval duration, shortening pupal duration, decreasing pupation rate, declining in the number of test worms that complete the entire life cycle. They will directly lead to a prolonged generation cycle, and the number of adult insects will decrease, thereby reducing the proliferation rate of the population. These may be due to the sublethal dose of insecticide will inhibit the feeding of test worms, resulting in insufficient nutrition, which eventually leads to prolonged developmental periods and affects the quality of pupae. Interestingly, some sublethal doses of insecticides cause significant prolongation of the larval and pupal durations of F_{0} generation. For example, the larval and pupal stages of Plutella xylostella and Spodoptera litura were significantly prolonged by sublethal doses of spinosad (0.04 and 0.16 mg/kg) (Wang, Gong, Li, Qiu and Wang, 2009) and fluralaner (LD_{5} and LD_{15}) (Liu, et al., 2018). However, the reasons for this discrepancy will need to be further explored.

Fecundity is an important indicator of insect population dynamics. Most studies have shown that sublethal doses of insecticides can significantly change the mean fecundity of reproductive females and hatchability. However, in this study, the adult longevity, female ratio, the mean fecundity of reproductive females, hatchability and emergence rate of C. suppressalis F_{0} generation were reduced after 3rd instar larvae treated with a sublethal dose of fluxametamide, however there was no significant difference compared with the control group. Similarly, after the 5th instar larvae of S. frugiperda treated with LC_{10} and LC_{25} methoxyfenozide, the mean fecundity of reproductive females was 264 and 356 grains, respectively, compared with the control group (393 grains/female), and there was no significant change in the female ratio and hatchability (Zarate, et al., 2011). After 3rd instar larvae of S. frugiperda treated with sublethal doses of spinetoram, no significant impact was observed on the mean fecundity of reproductive females and hatchability (Gao and Chen, 2021). Based on above results, we speculated that the type of insecticide and its tested dose, the age of the test worm and so on, may all be factors affecting the fertility of the test worm.

In this study, after the treatment with the sublethal dose of fluxametamide, C. suppressalis F_{1} generation was not significantly affected, and its larval development duration, pupal weight, adult longevity, the mean fecundity of reproductive females, hatchability, pupation rate and other indicators were negligible compared with the control group. Only the egg duration in the LD_{30} treatment was significantly longer than the control, and the 3rd instar larval duration was shortened. Studies have shown that after the 3rd
instar larvae of *C. medinalis* treated with chlorantraniliprole and emamectin benzoate at LC$_{10}$, LC$_{25}$, LC$_{50}$, respectively, the duration of F$_1$ eggs was prolonged with the increase of the sublethal dose, and when the contemporary 1st to 3rd instar larvae were treated with these insecticides at sublethal dose, little effect was observed on the pupal weight and pupal duration of the offspring (Liang, 2014), probably residual insecticides in the body are excreted or metabolized, and do not form accumulation in the insect body during the growth and development of offspring.

The reproductive system is critical for development of insect population. Therefore, the gonad development of females has been extensively studied. In general, the most direct effect of an insecticide on insect fecundity is its reproductive organs. Due to the different development characteristics of male and female gonad, only female insects are still developing in the adult stage (Huang, 2012). Therefore, the development of ovaries was selected for study of sublethal effect of fluxametamide to *C. suppressalis*. In this study, both length and weight of ovarian tubes was significantly reduced in the LD$_{30}$ fluxametamide. Similarly, multiple nuclear polyhedrosis virus (SeMNPV) at sublethal dose (10$^2$ PIB/larva and 10$^3$ PIB/larva) effectively reduced the fecundity of *Spodoptera exigua*, and the length of ovary tubes and the weight of ovaries were smaller than those of the control (Huang, 2012). To our knowledge, this is the first report related to the fluxametamide to insect reproduction. The most direct response of the ovarian development of *C. suppressalis* to fluxametamide is the changed length and weight of ovarian tubes.

## 5 Conclusion

In conclusion, it can be obtained that fluxametamide not only has outstanding acute toxicity to *C. suppressalis*, but also has a significantly sublethal effects, e.g. significantly delay the growth and development of the F$_0$ generation, and the LD$_{30}$ fluxametamide significantly inhibit the ovarian development of *C. suppressalis*. Our results will provide the scientific guidance for the dosage of fluxametamide in the field to effectively control agricultural pests.

## Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Chunqing Zhao and Zhaojun Han contributed to the study conception and design. Material preparation was performed by Chenglong Qian and Yao Li. Data collection and analysis were performed by Yao Li and Yingnan Wang. The first draft of the manuscript was written by Ning Shen and Tao Tang. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This research does not involve the use of animal or humans in the experiment action.

References


26. Appendices

**Figures**
Figure 1

Various forms of the *C. suppressalis* from pupa to adult

A, normal pupa; B, normal female; C, normal male; D, unable eclosion pupa; E-G, failure of eclosion
Figure 2

Sublethal effect of fluxametamide on *C. suppressalis* ovary

Note, sublethal effects of fluxametamide on the size (A), length (B) and weight (C) of *C. suppressalis* ovary were shown. The statistically significant differences were shown as different lowercase letters above the bars, when $P < 0.05$ (Turkey’ s test).

**Supplementary Files**

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- Appendices.docx