Evaluating the Effects of Cu$^{2+}$ on the Development and Reproduction of *Spodoptera litura* (Lepidoptera: Noctuidae) Based on the Age-Stage, Two-Sex Life Table

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Received 12 July 2022; Editorial decision 24 October 2022.

Abstract

Copper (Cu$^{2+}$) is a micronutrient that promotes the development and reproduction of organisms. However, with the rapid expansion of modern industry and agriculture, Cu$^{2+}$ concentrations are increasing, which might have negative impacts on biological and ecological safety. *Spodoptera litura* is not only an intermittent outbreak pest but also can be used as a model organism to assess environmental and ecological risks. Therefore, the effects of the life history and population parameters of *S. litura* fed on artificial diets with different Cu$^{2+}$ concentrations were analyzed using the age-stage, two-sex life table. Our results showed that not only the preadult survival rate but also the intrinsic rate of increase ($\lambda$) and the finite rate of increase ($r$) were significantly increased under exposure to low Cu$^{2+}$ concentrations (2, 4, and 8 mg/kg). In addition, the population growth of *S. litura* was significantly faster, indicating that *S. litura* can adapt well to low concentrations and is likely to undergo outbreaks of damage. Whereas, in addition to a significant reduction in preadult survival rate, population growth rate, pupal weight, pupal length, adult body weight, and oviposition were also significantly reduced under exposure to high Cu$^{2+}$ concentration (32 mg/kg). And when the concentration reached 64 mg/kg, the survival rate of adults was extremely low, suggesting a decrease in the adaptation of *S. litura*. These results can help to understand the population dynamics of *S. litura* and predict potential ecological risks.

Key words: copper, *Spodoptera litura*, age-stage two-sex life table, development, reproduction

Copper (Cu$^{2+}$) is one of the seven micronutrients essential for insect growth, development, and reproduction, which is also a component of many enzymes and proteins in cells (Bost et al. 2016). Appropriate Cu$^{2+}$ concentrations have a catalytic effect on the growth, development, and reproduction of insects (Huang et al. 2012). However, toxic reactions happen when the Cu$^{2+}$ concentration exceeds a certain threshold (Lu et al. 2019). Elevated levels of Cu$^{2+}$ in the environment are attributed to human actions (Liu et al. 2021). For example, the application of the fungicide, Bordeaux mixture to treat fruit trees for downy mildew resulted in a marked increase in Cu$^{2+}$ levels in the soil (Brun et al. 2001, Vogelweith and Thiéry 2018). Additionally, Cu$^{2+}$ is commonly used as a feed additive to boost the immunity and growth performance of livestock and poultry, which leads to Cu$^{2+}$ excretion from feces and deposition in soil (Nicholson et al. 1999, Huang et al. 2010). Cu$^{2+}$ accumulates easily in soil because of its difficulty in degradation (Lu et al. 2019). The Cu$^{2+}$ concentration in 98% of soils fertilized with excrement exceeds the standard values in China (Li et al. 2022). After absorption by plants from the soil, Cu$^{2+}$ is accumulated in plants and transmitted to organisms through the food chain, thereby affecting insects (Lu et al. 2019).

Insects can absorb heavy metals from the environment through respiration, contact, and feeding (Lu et al. 2019, Su et al. 2021), among which, feeding is the primary way to accumulate heavy metals in insects (Wan et al. 2014). When insects feed on diets containing low Cu$^{2+}$ concentrations, it can promote the growth and development of insects, as well as increase body weight and length (Wu et al. 2007). Low Cu$^{2+}$ concentration can also shorten the developmental time of insects (Huang et al. 2012). In addition, insect feeding on leaves applied with Cu$^{2+}$-containing fungicides increased fecundity and even sparked an outbreak (Vogelweith and Thiéry 2018). However, when they feed on food containing higher Cu$^{2+}$ concentrations, insect mortality is increased and population development is adversely affected, such as Boettcherisca peregrina (Wu et al. 2007) and *Apis mellifera* (Di et al. 2016). Proisotoma minuta...
in jars containing soil with the highest Cu²⁺ concentrations showed high mortality, slow growth, and decreased fecundity (Nursita et al. 2005).

Spodoptera litura (Lepidoptera: Noctuidae), is an ecologically adaptable phytophagous insect (Chen et al. 2021a), which serves as a model organism to evaluate environmental risks (Jin et al. 2020a). It has become a popular research subject in the fields of nutrition (Chen et al. 2021b), ecotoxicology (Shu et al. 2009), and insect toxicology (Qin et al. 2020). It is critical to explore the possible reasons for its outbreaks because S. litura is an agricultural pest with intermittent outbreaks. The growth, development, and reproduction performances of the insects are affected by the surrounding environment (Green et al. 2019). These performances are typically linked to the composition and content of consumed nutrients (da Silva et al. 2021). The concentration of various components in the diet, such as minerals and proteins, has an impact on how insects fed with artificial diets perform (Jin et al. 2020b). Therefore, it is worth evaluating the effects of artificial diets containing various Cu²⁺ concentrations on the performance of S. litura.

The life table method is a versatile and effective tool for analyzing insect growth, development, reproduction, and population dynamics (Wang et al. 2017). By establishing the age-stage, two-sex life table, the development, survival, and reproduction of S. litura on artificial diets supplemented with various Cu²⁺ can be accurately determined. This study aimed to assess the adaptation of S. litura to different Cu²⁺ concentrations in diets, as well as the potential consequences of high Cu²⁺ levels. Our results revealed the trends of changes in the population growth of S. litura with various Cu²⁺ concentrations, which may help to understand the potential ecological risks of Cu²⁺ contamination.

Materials and Methods

Source of Test Insects

Eggs of S. litura were provided by the School of Life Sciences, Sun Yat-sen University. All larvae were consecutively fed on an artificial diet in a sensitive light incubator (HP400G-C, Wuhan Ruihua Instrument Equipment Co., Ltd., China) at 27 ± 1°C, 70 ± 5% relative humidity, and a photoperiod of 12:12 (L:D) h before pupating in sterile sand with 15% water content. The adults were fed with 10% honey water as food.

Artificial Diet Preparation

The artificial diets for S. litura larvae were prepared according to the formula and method provided by Zhang et al. (2016). Briefly, 16 g of agar and 50 g of sucrose were put into a 2-L beaker filled with 1 liter of deionized water and boiled. Thereafter, 100 g of each maize flour, soybean flour, and bran flour, along with 12 g of yeast powder, were added, and the mixture was boiled for 30 min. After lowering the temperature of the mixture to approximately 60°C, 2 g of sorbic acid, 6 g of ascorbic acid, 2 g of methyl p-hydroxybenzoate, and 2 g of multivitamin particles were added and mixed well to obtain the artificial diet.

Cu²⁺ used for this experiment was derived from CuCl₂·2H₂O (CAS: 10125-13-0, AR, Macklin Biochemical Co., Ltd., Shanghai, China), which was previously dissolved into deionized water to achieve the final target concentrations of the diets. The initial Cu²⁺ concentration was set as 2 mg/kg based on the Cu²⁺ enrichment potential of leafy vegetables, as reported by Niu (2019) and Kachenko and Singh (2006). According to a series of pre-experiments to determine the concentration of S. litura, the final Cu²⁺ concentrations in the diets were 2, 4, 8, 16, 32, and 64 mg/kg, and the formula and preparation methods mentioned above were used for each treatment. Diet without Cu²⁺ was used as the blank control. All prepared artificial diets were poured into seven transparent plastic boxes (15.5 cm in length, 11 cm in width, and 6 cm in height) and refrigerated at 4°C for further use.

Test Insect Rearing

Newly hatched larvae were randomly picked from egg masses and individually fed on artificial diets with different Cu²⁺ concentrations in a plastic Petri dish (5.5 cm diameter and 1 cm height). Forty newly hatched larvae were tested in each treatment. Artificial diets were replaced, and feces and diet residues were cleaned up daily. The larvae were separately pupated in 32-compartment plastic trays with sterile sand. After pupation, the pupae were weighed using an electronic balance (AX224ZH/E, Ohaus Instrument Co., Ltd., Changzhou, China), and the length of the pupae was measured with a vernier caliper. After emergence, the adults were kept in sealed plastic boxes (3 cm bottom diameter, 3.8 cm top diameter, and 3.2 cm height) to be weighed (net weight without the plastic box). Females and males that emerged at the same time were paired and reared in disposable plastic cups (4.3 cm bottom diameter, 6.7 cm top diameter, and 7.5 cm height) with pieces of paper for spawning. When emerged females outnumbered males or when males died first after mating, males were supplemented for pairing from mass-rearing populations under the same conditions, and vice versa. No analysis used data from the replacement moths. A cotton ball with 10% honey water was placed in the bottom of the cup and replaced daily. Eggs were collected from the spawning paper and cup walls (in rare cases) and counted daily using a stereo microscope (SMZ745, Nikon, Tokyo, Japan). The spawning paper was changed daily.

Life Table Study

Individual larvae of S. litura were checked for molting daily, using their head capsules and exuviae as evidence of molting to the next instar. Developmental and survival data for each larval to the adult stage, as well as female fecundity, were recorded daily until all tested individuals died. All original life history data of S. litura were analyzed using the computer program TWOSEX-MSChart (Chi 2020b), which was based on the age-stage, two-sex life table (Chi and Liu 1985, Chi 1988). Life history parameters, namely age-stage specific survival rates (s_x), finite rate of increase (r), age-specific survival rate (l_x), age-stage specific fecundity (f_x), age-specific fecundity (m_x), and age-specific maturity (f_m) were calculated using the aforementioned program. The variances and standard errors of the population parameters, including the intrinsic rate of increase (r), finite rate of increase (r), net reproductive rate (R_0), and mean generation time (T), were calculated by the bootstrap technique with 100,000 random replications. At the 5% significance level, the differences between the six treatments in developmental time for each stage, preadult survival, adult longevity, adult preoviposition period (APO), total preoviposition period (TOP), oviposition period, and fecundity were compared using the pairing bootstrap test (Efron and Tibshirani 1993, Huang and Chi 2012). The age-specific survival rate (l_x) , which indicates the survival rate of new eggs to age x regardless of the stage, was calculated using the following formula:

\[ l_x = \frac{1}{T} \sum_{j=1}^{T} s_{xj} \] (1)

The age-specific fecundity (m_x) was calculated as:
Table 1. Survival rates for different stages and female ratio of *S. litura* exposed to different Cu\(^{2+}\) concentrations

| Parameters               | Control | 2        | 4        | 8        | 16       | 32       | 64       |
|--------------------------|---------|----------|----------|----------|----------|----------|----------|
| 1st instar (%)           | 100.00  | 100.00   | 95.00    | 100.00   | 85.00    | 90.00    | 36       |
| 2nd instar (%)           | 82.50   | 33       | 95.00    | 100.00   | 81.58    | 79.41    | 25       |
| 3rd instar (%)           | 90.91   | 30       | 100.00   | 100.00   | 85.19    | 80.00    | 20       |
| 4th instar (%)           | 100.00  | 30       | 100.00   | 100.00   | 95.65    | 90.00    | 18       |
| 5th instar (%)           | 100.00  | 30       | 100.00   | 100.00   | 95.65    | 90.00    | 18       |
| 6th instar (%)           | 96.67   | 29       | 100.00   | 100.00   | 94.44    | 92.35    | 14       |
| 7th instar (%)           | 100.00  | 29       | 100.00   | 100.00   | 94.44    | 92.35    | 14       |
| 8th instar (%)           | 100.00  | 29       | 100.00   | 100.00   | 94.44    | 92.35    | 14       |
| Prepupa (%)              | 93.10   | 27       | 100.00   | 100.00   | 94.44    | 92.35    | 14       |
| Pupa (%)                 | 70.37   | 19       | 100.00   | 100.00   | 94.44    | 92.35    | 14       |
| Larva-adult (%)          | 45.00   | 18       | 100.00   | 100.00   | 94.44    | 92.35    | 14       |
| Sex ratio                | 0.73    | 8/11     | 0.83     | 15/18    | 0.70     | 14/20    | 0.67     |

The female ratio was not calculated because of the presence of only one female adult (Note: *n*, the total number of insects for different stages; sex ratio, females to males).

\[
m_x = \sum_{x=0}^{\infty} e^{-r(x+1)} R_x m_x = \frac{R_0 e^r}{\lambda}
\]

According to the Euler–Lotka formula, the intrinsic rate of increase \(r\) was calculated by the iterative bisection method (Goodman 1982):

\[
\lambda = e^r
\]

The net reproductive rate \(R_0\) was calculated as:

\[
R_0 = \sum_{x=0}^{\infty} I_x m_x
\]

The mean generation time \(T\) was calculated as:

\[
T = \frac{\ln R_0}{\lambda}
\]

The computer program TIMING-MSChart (Chi 2020a) was used to evaluate the data from the age-stage, two-sex life table. 10 eggs were used as the initial population in each treatment to simulate the population growth and predict the population dynamics of the descendants of *S. litura* fed with different Cu\(^{2+}\) concentrations.

Data Analysis

Statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago, IL). The data (pupal weight, pupal length, and adult body weight) satisfied normal distribution and homoscedasticity by Shapiro–Wilk and Levene’s tests, respectively. Differences in data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s HSD test for multiple comparisons \((p < 0.05, \text{statistically significant})\). The Pearson correlation coefficient analysis was performed to determine the association among different parameters (such as pupal weight and pupal length, and pupal weight and adult body weight).

Results

Survival Rates and Sex Ratio of *S. litura* at Different Cu\(^{2+}\) Concentrations

Most larvae had seven instars, and approximately half of the larvae passed through eight instars under all treatments (including control), except under 4 mg/kg Cu\(^{2+}\) concentration (Table 1). The survival rates of larvae exposed to 2–8 mg/kg Cu\(^{2+}\) concentrations were higher than 90% for each stage, whereas the survival rates of 2nd instar larvae were lower in the control group and 16 mg/kg treatment. When larvae were exposed to 4 mg/kg Cu\(^{2+}\) concentration, the highest survival rate was obtained (85%). Mortality was found for each stage after exposure to the Cu\(^{2+}\) concentration of 32 mg/kg. By contrast, there was only one adult present and no fecundity under Cu\(^{2+}\) exposure at 64 mg/kg. Therefore, its raw data could not be calculated using the bootstrap technique based on the age-stage, two-sex life table, which was absent from other figures and tables (except Fig. 1). The female-to-male ratio of *S. litura* was <1 for all treatments (including control).

Developmental Time, Preadult Survival, and Adult Longevity of *S. litura* at Different Cu\(^{2+}\) Concentrations

The developmental time of 1st and 2nd instar larvae exposed to 32 mg/kg Cu\(^{2+}\) concentration (2.57 d and 1.71 d, respectively) was significantly shorter than that in the untreated control (2.80 d) and 2 mg/kg Cu\(^{2+}\) treatment (2.24 d), respectively (Table 2). The developmental durations of 3rd instar larvae and preadult stage (including larval stage) were significantly shorter under 4 mg/kg and 8 mg/kg Cu\(^{2+}\) treatments than those under 32 mg/kg treatment but not significantly different from the untreated control. The developmental time of the 6th instar larvae at the Cu\(^{2+}\) concentration of 16 mg/kg (3.94 d) was significantly longer than that of the control (3.07 d). After exposure to the Cu\(^{2+}\) concentration of 32 mg/kg, the developmental time of 8th instar larvae was 9.50 d, which was significantly longer than that under other treatments and in the control. The variation in adult longevity was dependent mainly on males. The adult longevity of *S. litura* in 4 mg/kg Cu\(^{2+}\) treatment (11.20 d) was significantly longer than that in 16 mg/kg (8.45 d) and 32 mg/kg (7.70 d) Cu\(^{2+}\) treatments. In addition, the preadult survival rates of *S. litura* exposed to 2 mg/kg (82.54%) and 4 mg/kg (85.01%) Cu\(^{2+}\) concentrations were significantly higher than those in the untreated control (47.51%) and 16 mg/kg (55.00%) and 32 mg/kg (26.18%) treatments.

Pupal Weight, Pupal Length, and Adult Body Weight of *S. litura* at Different Cu\(^{2+}\) Concentrations

The pupal weight \((F_{1,45} = 15.322, \ P < 0.001)\), pupal length \((F_{1,45} = 11.034, \ P < 0.001)\), and adult body weight \((F_{1,45} = 9.399, \ P = 0.003)\).
P < 0.001) of *S. litura* were significantly affected by Cu²⁺ concentrations (Table 3). With the elevation of Cu²⁺ concentrations in diets, pupal weight, pupal length, and body weight gradually increased, peaking under 4 mg/kg Cu²⁺ treatment (0.5474 g, 2.2183 cm, and 0.3452 g, respectively). Subsequently, a downward trend was observed, reaching minimums in the 32 mg/kg Cu²⁺ treatment (0.3824 g, 2.0075 cm, and 0.2158 g, respectively), which were significantly lower than those in the untreated control and other Cu²⁺ treatments except 16 mg/kg. In addition, there was a significant positive correlation between pupal weight and pupal length under exposure to different Cu²⁺ concentrations (Table 3). Pupal weight was also positively correlated with body weight.

### Biological Parameters of *S. litura* at Different Cu²⁺ concentrations

As for biological parameters of *S. litura*, only one female adult exhibited fecundity under 32 mg/kg Cu²⁺ treatment. By contrast, many females laid eggs in other treatments and control (Table 4). Although APOP was not significantly different among the treatments, TPOP in the 16 mg/kg Cu²⁺ treatment was significantly longer (48.33 d) than in the untreated control (43.00 d). No significant differences were observed in the oviposition period among the treatments; however, the oviposition period was the longest at the Cu²⁺ concentration of 4 mg/kg (4.23 d) and the shortest at 16 mg/kg (3.00 d). In the case of low Cu²⁺ concentrations (2–8 mg/kg), the mean fecundity followed an increasing trend, with 2244.77 eggs under 4 mg/kg Cu²⁺ treatment, which was significantly higher than that under 16 mg/kg treatment (947.46 eggs).

#### Age-stage Specific Survival Rate (sxj)

The age-stage specific survival rate (sxj) of *S. litura* at the larval stage decreased linearly under 32 and 64 mg/kg Cu²⁺ treatments, leading to a significant reduction in the survival rates of both the pupal and adult stages (Fig. 1). The survival rates of larvae exposed to 2 and 4 mg/kg Cu²⁺ concentrations in the immature stage were as high as 90%, and the survival rates in the pupal stage were more than 80%.

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**Table 2. Developmental time for different stages, adult longevity, and preadult survival of *S. litura* exposed to different Cu²⁺ concentrations**

| Parameters                      | Control 2  | 4  | 8  | 16 | 32 |
|---------------------------------|-----------|----|----|----|----|
| 1st instar (days)               | 2.80 ± 0.7a | 2.72 ± 0.9ab | 2.66 ± 0.08ab | 2.73 ± 0.08ab | 2.68 ± 0.08ab | 2.57 ± 0.08b |
| 2nd instar (days)               | 2.06 ± 0.29ab | 2.24 ± 0.22a | 2.05 ± 0.19ab | 1.76 ± 0.18ab | 2.00 ± 0.23ab | 1.71 ± 0.14b |
| 3rd instar (days)               | 3.87 ± 0.29ab | 3.92 ± 0.34bc | 3.63 ± 0.23c | 3.67 ± 0.19c | 3.58 ± 0.19c | 5.58 ± 0.36a |
| 4th instar (days)               | 3.90 ± 0.36a | 3.92 ± 0.34a | 3.90 ± 0.24a | 3.67 ± 0.25a | 3.42 ± 0.26a | 4.02 ± 0.48a |
| 5th instar (days)               | 3.23 ± 0.21a | 3.29 ± 0.29a | 2.84 ± 0.20a | 3.06 ± 0.23a | 3.64 ± 0.44a | 4.04 ± 0.74a |
| 6th instar (days)               | 3.07 ± 0.12b | 3.17 ± 0.24ab | 3.72 ± 0.40ab | 3.12 ± 0.25ab | 3.94 ± 0.43a | 3.37 ± 0.25ab |
| 7th instar (days)               | 4.64 ± 0.44a | 4.24 ± 0.37a | 4.41 ± 0.32a | 4.44 ± 0.57a | 4.36 ± 0.36a | 6.88 ± 2.09a |
| 8th instar (days)               | 5.17 ± 0.44a | 5.00 ± 0.35b | 5.84 ± 0.69b | 5.40 ± 0.35b | 4.71 ± 0.39b | 9.50 ± 1.60a |
| Preadult (days)                 | 1.93 ± 0.09a | 1.51 ± 0.08b | 1.76 ± 0.10ab | 2.03 ± 0.11a | 1.79 ± 0.12ab | 1.76 ± 0.12b |
| Larva (days)                    | 28.00 ± 1.12abc | 27.35 ± 0.80bc | 26.19 ± 0.69c | 27.16 ± 0.90c | 29.46 ± 0.64a | 31.12 ± 1.75a |
| Pupa (days)                     | 11.74 ± 0.18ab | 11.79 ± 0.13a | 11.88 ± 0.13a | 11.36 ± 0.16bc | 11.27 ± 0.15c | 11.84 ± 0.21ab |
| Preadult (days)                 | 42.26 ± 1.32abc | 42.12 ± 0.83bc | 40.64 ± 0.62c | 40.50 ± 0.69c | 43.18 ± 0.72ab | 45.99 ± 1.72a |
| Female longevity (days)         | 8.89 ± 1.66a | 10.61 ± 1.01a | 9.79 ± 0.78a | 11.14 ± 1.10a | 9.45 ± 1.52a | 10.11 ± 2.38a |
| Male longevity (days)           | 11.45 ± 1.35abc | 10.33 ± 0.97abc | 12.20 ± 0.86a | 9.86 ± 1.44abc | 7.45 ± 1.14b | 6.17 ± 2.33bc |
| Adult longevity (days)          | 10.37 ± 1.06ab | 10.46 ± 0.69ab | 11.20 ± 0.62a | 10.50 ± 0.90ab | 8.45 ± 0.95b | 7.79 ± 1.61b |
| Preadult survival (%)           | 47.51 ± 0.08c | 82.54 ± 0.66a | 85.01 ± 0.07ab | 70.00 ± 0.07ab | 55.00 ± 0.08bc | 26.18 ± 0.07d |

Values represent the mean ± SE. The standard errors were estimated through 100,000 bootstrap resampling. Different lowercase alphabets within the same row mean a significant difference (paired bootstrap test, *p* < 0.05).
When Cu²⁺ concentrations grew from 0 to 32 mg/kg, the larval duration (survival duration) of S. litura extended from approximately 40 days to 65 days.

**Age-specific Survivability and Age-stage Specific Fecundity**

The $l_{x}$ curve is a simplified version of the $s_{x}$ curve, showing a decreasing trend in the survival rate of S. litura with age (Fig. 2). Among the 11th and 38th days, the survival rate was significantly lower at 16 mg/kg Cu²⁺ treatment (approximately 75%) than that in the treatment with Cu²⁺ concentration ranging from 2 to 8 mg/kg (approximately 90%). After the 38th day, the survival rates decreased sharply under each Cu²⁺ treatment except 32 mg/kg. After the 38th day, the survival rates were significantly higher relative to the control group and 16 mg/kg Cu²⁺ treatment. Moreover, when the Cu²⁺ concentration grew from 0 to 4 mg/kg, r, $\lambda$, and $R_{e}$ gradually climbed to the peak and began decreasing as the concentrations increased further.

**Total Population Projection of S. litura at Different Cu²⁺ concentrations**

The total population projection means of S. litura were started with 10 eggs using the TIMING-MSChart program (Fig. 3). The total population of S. litura increased the fastest in the 4 mg/kg Cu²⁺ treatment, followed by 2 and 8 mg/kg treatments, while the slowest in the 32 mg/kg Cu²⁺ treatment. The total population under 16 mg/kg Cu²⁺ treatment was more consistent with the control. After 70 d, the predicted total population sizes were 5,412, 7,464, and 6,385, respectively, at Cu²⁺ concentrations (2, 4, and 8 mg/kg), which were significantly higher relative to the control group and 16 mg/kg Cu²⁺ treatment (2,183 and 2,024, respectively), whereas the predicted total population sizes were the lowest under 32 mg/kg Cu²⁺ treatment (204).

**Discussion**

Due to rapid industrialization and extensive human activities, heavy metal pollution has recently become a serious threat to ecosystems, endangering insect diversity (Lefcort et al. 2010), animal health (Verma et al. 2018), and ecological balance (Huang et al. 2012). S. litura has been extensively studied as a bioindicator of heavy metal pollution (Huang et al. 2012, Ali et al. 2019, Jin et al. 2020a). Ali

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**Table 3. Pupal weight, pupal length, adult weight, and correlations between different parameters (pupal weight and pupal length, and pupal weight and adult body weight) of S. litura exposed to different Cu²⁺ concentrations**

| Treatment (mg/kg) | Pupal weight (g) | Pupal length (cm) | Body weight (g) | Pupal weight and pupal length | Pupal weight and body weight |
|------------------|------------------|-------------------|-----------------|-----------------------------|------------------------------|
| Control          | 0.5286 ± 0.0125a | 2.2042 ± 0.0156a  | 0.3122 ± 0.0167ab| 0.6890                      | 0.0002                      | 0.6679 ± 0.0034             |
| 2                | 0.5448 ± 0.0093a | 2.2111 ± 0.0166a  | 0.3238 ± 0.0099a | 0.7782                      | <0.0001                     | 0.5951 ± 0.0003             |
| 4                | 0.5474 ± 0.0109a | 2.2183 ± 0.0164a  | 0.3452 ± 0.0112a | 0.8589                      | <0.0001                     | 0.6297 ± 0.0001             |
| 8                | 0.5046 ± 0.0150ab| 2.1600 ± 0.0204ab | 0.3156 ± 0.0122ab| 0.9161                      | <0.0001                     | 0.6266 ± 0.0008             |
| 16               | 0.4492 ± 0.0167b | 2.0804 ± 0.0297bc | 0.2667 ± 0.0092bc| 0.9376                      | <0.0001                     | 0.6201 ± 0.0027             |
| 32               | 0.3824 ± 0.0262c | 2.0075 ± 0.0435c  | 0.2158 ± 0.0168c | 0.9599                      | <0.0001                     | 0.9851 ± 0.0001             |

The parameter values are the mean ± SE, with different lowercase alphabets in the same column indicating significant differences (p < 0.05, Tukey’s HSD test). The correlation values are the correlation coefficient ($r$) and $P$ (Pearson correlation coefficient).

**Table 4. Biological parameters of S. litura exposed to different Cu²⁺ concentrations**

| Biological parameters | Control | Treatment (mg/kg) |
|-----------------------|---------|-------------------|
|                       |         | 2                 | 4                 | 8                 | 16                | 32                |
| APOP (days)           | 4.50 ± 1.21a | 4.21 ± 0.58a    | 5.39 ± 0.77a      | 5.38 ± 0.72a      | 6.00 ± 0.92a      | 7.00               |
| TPOP (days)           | 43.00 ± 1.73b | 46.07 ± 1.15ab  | 45.46 ± 1.54ab    | 45.84 ± 1.29ab    | 48.33 ± 1.39a     | 56.00              |
| Oviposition period (days) | 4.17 ± 0.91a | 4.22 ± 0.53a    | 4.23 ± 0.69a      | 3.93 ± 0.82a      | 3.00 ± 0.74a      | 5.00               |
| Fecundity (eggs/female) | 1497.89 ± 514.42ab | 1521.25 ± 233.64ab | 2244.77 ± 444.17a | 1998.63 ± 504.17ab | 947.46 ± 321.34bc | 366.82 ± 231.38c   |

Values are the mean ± SE. The standard error estimates were resampled using 100,000 bootstraps within the same row followed by different lowercase alphabets indicating significant differences (paired bootstrap test, p < 0.05) (Note: APOP, adult preoviposition period; TPOP, total preoviposition period; APOP, TPOP, and oviposition period were not involved in the paired bootstrap test because only one female adult laid eggs at 32 mg/kg.)
et al. (2019) discovered that even low concentrations of the heavy metals lead (Pb) and zinc (Zn) significantly reduced the weight, length, survival, and growth indices of *S. litura* larvae in Pakistan industrial regions. This suggests that it is important to consider the impacts of heavy metal contamination on insects. In this study, we found that different Cu²⁺ concentrations in the diets influenced larval developmental time, pupal weight, adult longevity, survival rate, and fecundity, indicating that heavy metal Cu²⁺ can affect the population development of *S. litura*.

The survival rate is the guarantee for population development in insects. Low Cu²⁺ concentrations (2, 4, and 8 mg/kg) improved the survival rate of *S. litura* since Cu²⁺ is an essential trace element in the organism (Bost et al. 2016). However, high Cu²⁺ concentrations can negatively affect the organism’s survival (Rehman et al. 2019). The survival rates of *S. litura* larvae exposed to high Cu²⁺ concentrations (>16 mg/kg) were decreased rapidly. Our findings are similar to those of Shu et al. (2015), who found that high Pb concentration (200 mg/kg) significantly reduced the survival rate of *S. litura* larvae. While the binding of Cu²⁺ to metallothionein has a detoxifying effect (Andreani et al. 2020), excess Cu²⁺ still damages the structure of the insect midgut (Miranda et al. 2022). Similar results have been observed for other heavy metals such as Cadmium (Cd) (Baghban et al. 2014), Zn (Ali et al. 2019), and Pb (Shu et al. 2015). Intestinal microbiota imbalance by the disruption of midgut structure affects nutrient and energy absorption (Chai et al. 2022), which reduces the survival, growth, and development of insects. The survival rate of the younger larvae (2nd and 3rd instars) of *S. litura* was lower than that of older larvae with increasing Cu²⁺ concentrations, suggesting that younger larvae may be less tolerant to heavy metals (Jiang}

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**Table 5. Population parameters of *S. litura* exposed to different Cu²⁺ concentrations**

| Population Parameters | Control  | 2 mg/kg | 4 mg/kg | 8 mg/kg | 16 mg/kg | 32 mg/kg |
|-----------------------|---------|---------|---------|---------|---------|---------|
| \( r (\text{day}^{-1}) \) | 0.1254 ± 0.0134ab | 0.1349 ± 0.0083a | 0.1483 ± 0.0091a | 0.1402 ± 0.0094a | 0.1091 ± 0.0100b | 0.0627 ± 0.0074c |
| \( \lambda (\text{day}^{-1}) \) | 1.1337 ± 0.0151ab | 1.1445 ± 0.0095a | 1.1599 ± 0.0105a | 1.1506 ± 0.0108a | 1.1153 ± 0.0111b | 1.0647 ± 0.0079c |
| \( R_0 (\text{offspring/individual}) \) | 299.98 ± 135.45ab | 570.91 ± 144.20ab | 785.28 ± 227.13a | 699.39 ± 228.27ab | 260.30 ± 107.88b | 45.67 ± 23.30c |
| \( T (\text{days}) \) | 44.34 ± 1.06c | 46.89 ± 1.81bc | 44.70 ± 1.12c | 46.36 ± 1.37c | 50.10 ± 1.01b | 59.27 ± 0.01a |

The standard error estimates were resampled using 100,000 bootstraps. Differences between treatments were determined using the paired bootstrap test. The means within the same row followed by different lowercase alphabets indicate significant differences \((p < 0.05)\) (Note: \( r \), intrinsic rate of increase; \( \lambda \), finite rate of increase; \( R_0 \), net reproductive rate; and \( T \), mean generation time).
et al. 2018). Kafel et al. (2012) also noted that the later stages of Spodoptera exigua larvae might be more resistant to heavy metals than during earlier stages.

High Cu²⁺ concentrations (16 and 32 mg/kg) significantly lengthened the developmental time of S. litura larvae compared with low Cu²⁺ concentrations. Similar results were also found for Boettcherisca peregrine (Wu et al. 2007) and Aedes aegypti (Miranda et al. 2022). Shu et al. (2012) reported that the larval stage of S. litura was significantly prolonged with an increase in Pb concentration. Chen et al. (2021a) found a decrease in the levels of development-related proteins when S. litura was exposed to high Pb concentrations. Protein is one of the most important nutrients in insect development and reproduction (Barragan-Fonseca et al. 2019). According to Hemati et al. (2012) and Di et al. (2021), protein deficiency significantly slowed the developmental rate of Helicoverpa armigera and S. litura larvae. Ding and Wang (2006) demonstrated that the larval developmental time of Drosophila melanogaster was significantly increased and that most of the protein bands gradually weakened at high Cu doses. Therefore, we hypothesize that the prolonged developmental time of S. litura larvae exposed to high Cu²⁺ concentrations might be due to decreased protein content by Cu²⁺, resulting in a longer developmental period.

The nutritional and physical conditions of the larvae may influence their traits during the pupal stage (He et al. 2021). Low Cu²⁺ concentrations promoted the survival and development of S. litura larvae, and increased pupal weight, while high Cu²⁺ concentrations did the opposite. Similarly, Di et al. (2016) observed that the pupal weight of Apis mellifera was increased at low Cu²⁺ concentrations and decreased at high Cu²⁺ concentrations. Montezano et al. (2019) reported that pupal weight was positively correlated with the fecundity of Spodoptera frugiperda, which was also observed in Helicoverpa armigera (Liu et al. 2017). Jiang and Yan (2017) found that Lymantria dispar exposed to Pb and Cd had a decrease in pupal weight followed by a reduction in fecundity. Although the correlation between pupal weight and fecundity of S. litura was not verified in this study, the fecundity was higher when the pupal weight was heavier at low Cu²⁺ concentrations.

Higher Cu²⁺ concentration (32 mg/kg) shortened adult longevity of S. litura, prolonged APOP and TPOP, and significantly reduced fecundity. Low Cu²⁺ concentrations increased fecundity, which were not significantly different from the control group. Our results are in agreement with those of Su et al. (2021), who found that the fecundity of S. exigua was increased at low Cd concentrations and significantly decreased at high Cd concentrations. High concentrations of heavy metals affect fecundity, which may be related to heavy metal accumulation (Jiang and Yan 2017) and inhibition of vitellogenin (Vg) synthesis (Shu et al. 2009). Vg synthesis and absorption have a major impact on insect reproduction (Zhou et al. 2021). Ye et al. (2009) found that Cu indirectly affected the fecundity of Nasonia vitripennis by inhibiting Vg synthesis in host pupae. Shu et al. (2009) also demonstrated that excess Zn down-regulated Vg gene expression and reduced yolk protein accumulation in S. litura, resulting in reduced fecundity. Therefore, the effect of high Cu²⁺ concentrations on the fecundity of S. litura may be related to the blocked Vg synthesis. However, the relevant molecular mechanisms (how Cu²⁺ concentrations influence the fecundity of S. litura) are unclear and require future study.

The age-stage, two-sex life table method is a reliable tool for determining the effects of environmental changes on insect populations (Tuan et al. 2014). The population growth of S. litura was significantly accelerated in the Cu²⁺ concentrations range of 2–8 mg/kg but decreased under 32 mg/kg Cu²⁺ treatment. Additionally, λ (finite rate of increase), r (intrinsic rate of increase), and Rₙ (net reproductive rate) accurately reflect the population growth rate and fecundity (Wang et al. 2017, Cao et al. 2021). We found that the λ, r, and Rₙ were maximum in the 4 mg/kg Cu²⁺ treatment, indicating that this concentration is suitable for the development and reproduction of the S. litura population. In contrast, λ, r, and Rₙ began to decrease when the Cu²⁺ concentration was ≥16 mg/kg. Although similar results were found in the previous studies, there were some significant differences (Huang et al. 2012). According to Huang et al. (2012), r and λ showed an increasing trend in the range of 0–50 mg/kg, which may be related to feeding conditions and diet quality (Xie et al. 2021).

In conclusion, a series of life table parameters of S. litura is affected under different Cu²⁺ concentrations. Our results demonstrated that the Cu²⁺ concentration of 4 mg/kg in the diet provided an excellent food supply for the rapid development and reproduction of S. litura. When the Cu²⁺ concentration exceeded 16 mg/kg, population expansion was controlled, and its size was reduced. These results provide some reference values on the probable causes of this pest outbreak and the assessment of the ecological risk of Cu²⁺ contamination.

**Acknowledgments**

We would like to express our gratitude to Hsin Chi for his assistance with the data analysis. Thanks to the reviewers and editor for their valuable comments and suggestions on this manuscript. This work was supported by the National Natural Science Foundation of China (31772168) and the Key Science and Technology Program of Hubei Tobacco Company (027Y2020-003).

**Author Contributions**

Y. Yang, X. H. Li and C. R. Li came up with the initial idea and designed the work. Y. Yang conducted the experiment and wrote the manuscript. J. W. Qi and Z. L. Wang assisted in the use of software and data analysis. C. W. Zhao reared insects for the experiment. Z. L. Wang, Z. X. Zhou and X. L. Dong revised the manuscript.

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