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TRANSGENERATIONAL EFFECTS OF CHLORANTANILIPROLE ON BIONOMICS *OF HELICOVERPA ARMIGERA* (HÜBNER)

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ABSTRACT

The lethal and sublethal effects of chlorantraniliprole against *Helicoverpa armigera* were evaluated to review its impact on life table parameters and population dynamics. Exposure to lethal and sublethal concentrations (LC_{25} and LC_{50}) significantly disrupted critical developmental and reproductive behavior of *H. armigera*. The result demonstrated that in the treated groups the larval and pupal duration were remarkably shortened, while adult lifespan was also reduced in LC_{25} and LC_{50} exposure, respectively, in comparison with control. The treated population displayed a substantial reduction in key population dynamics parameters, specifically the net reproduction rate (*Ro*), intrinsic growth rate of increase (*rm*), and gross reproduction rate (*GRR*) with LC_{25} exhibiting more prominent suppression than LC_{50}. Conversely, the population doubling time (*T*) decreased significantly at both concentrations, indicating accelerated population rate. The study indicates that sublethal doses impair physiological and reproductive behavior in *H. armigera*, which potentially suppressing population over long-term periods. However, at low concentrations slight physiological damage may induce, driving to resistance evolution. The study emphasizes the dual role of chlorantraniliprole in Integrated Pest Management (IPM), while effective at reducing population growth, its sublethal effects impose to lessen spontaneous ecological harms and resistance development.

INTRODUCTION

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is widely recognized as one of the most significant agricultural pests globally (Tay et al., 2013). It has been documented as the most dangerous pest affecting 181 plant species from 45 different families (Srivastava et al., 2005). Currently, *H. armigera* has a widespread distribution across most parts of the world. It is recognized as a major agricultural insect pest (Yu et al., 2008) across diverse regions including central and southeastern Asia, the Middle East, Africa, and Southern Europe (Karim, 2000). The most destructive stage of *H. armigera* are larvae (Wackers et al., 2007). Larvae mostly feed on reproductive plant parts like flowers, buds, fruits, spikes, and inflorescence. While the first and second instars may superficially feed on leaf surface material, the damage caused during this stage is typically minimal (Tripathy et al., 1999). To mitigate the losses due to cotton bollworm and enhance the crop yield, the application of pesticides is utmost important. The most commonly used insecticides being used against *H. armigera* include pyrethroids, carbamates, organophosphates and organochlorines (McCaffery, 1998).

Evaluating the sub-lethal impacts of insecticides is essential for analyzing their ecological consequences, as these chemicals can harm arthropods such as via bio-control species through non-lethal disruptions to biological processes, reproductive capacity, physiological systems, or behavioral patterns (Desneux et al., 2007; Liu et al., 2022). While direct exposure often leads to immediate death, sub-lethal affects risk disruption of enzyme, and suppressing the population (Idrees et al., 2022) . It also changes the habitat of the key pest inducing sub lethal effects (Malaj & Morrissey, 2022; Tamilselvan et al., 2021). These all have impacts on integrated pest management (IPM). Currently, broad-spectrum insecticides remain the primary method for controlling lepidopteron pests like *H. armigera*, which has developed significant resistance to various chemical treatments (Yang et al., 2013). In 1998, Africa faced an outbreak of American bollworm in cotton field causing high yield loss. Reports from Pakistan and India showed a high resistance of insecticide in *H. armigera* with high yield losses alerts the cotton grower of Africa (Ahmad et al., 1995).

Scientist are working to develop methods for controlling agricultural insect pest that are safer for both environment and living beings (Ahmed et al., 2022). Chlorantraniliprole is a new chemistry insecticide from anthranilic diamides, a novel group with a distinct mode of action. When it activates the ryanodine receptors of insects, it imbalances the sarcoplasmic reticulum in muscle cells (Dinter et al., 2010),which cause abnormal functioning and finally death (Cordova et al., 2006). Chlorantraniliprole is very selective to beneficial insects (Liu et al., 2012) and have a low mammalian toxicity (Lahm et al., 2007a). Chlorantraniliprole proved to be very effective against insect pests specially lepidopteron insect pest (Hannig et al., 2009). Research has been done on the non-lethal effects of Coragen on biological agents and pest insects. The sub lethal effects of various chemicals have been documented. For instance, triazophos alters life table parameters in *Sogatella furcifera* (Liu et al., 2016). Studies show that sublethal doses of chlorantraniliprole reduce egg-laying capacity (fecundity) in pests like *Spodoptera frugiperda*, *S. cosmioides*, and *H. armigera*. Additionally, these pests experienced delayed development, ultimately leading to significantly slower population growth compared to untreated groups.

Keeping in view the harmful effects of *H. armigera* we are evaluating the lethal or sublethal effects of chlorantraniliprole using life table parameters for *H. armigera*. This will be useful for all cotton growing countries by developing management strategies of *H. armigera.*

Materials and Methods

Collection and rearing of *H. armigera*

A laboratory colony of *H. armigera* was established using larvae collected from *G. hirsutum* fields in Multan, Pakistan. The larvae were reared on an artificial diet formulated with chickpea flour under controlled environmental conditions, including a temperature of (26 ± 2°C), relative humidity of (75±5%), and a (14:10) hour light-dark photoperiod. To mitigate cannibalism, each larva was isolated in a 5 cm plastic petri dish. After pupation, pupae were transferred to (30×30×30 cm) plastic cages to allow adult emergence. Newly emerged moths were relocated to plastic jars (14×7×5 cm) at a 1:1 male-to-female ratio (6 males and 6 females per jar) to promote mating. A 10% honey solution, provided via cotton-soaked substrate, served as nourishment for the adults. Third instar larvae from the F_1 generation were subsequently selected for experimental use.

Insecticide bioassay

This experiment consisted of five treatments and the control, and each treatment was replicated three times. Each replication of experiment contained five larvae of *H. armigera* placed in petri dishes. Third instar larvae were exposed to Chlorantraniliprole at five concentrations (500µL, 250µL, 125µL, 62.5µL, and 31.25µL), along with a control group treated with distilled water. Using the immersion method, the larvae were treated and subsequently transferred individually into plastic Petri dishes. They were then provided with diet following the protocol outlined by Smith et al. (2023). The experiment was conducted over a period of three days, with larval mortality data assessed after 72 h.

Transgenerational effects of chlorantraniliprole on *H. armigera*

To evaluate the Transgenerational effects of the insecticide, chlorantraniliprole, we compared the life table traits across the parent (F_0) generation, treated (F_1) generation and control. 100 newly laid eggs were selected and after hatching the newly emerged larval instars were reared until the 2nd instar larvae, and then they were exposed with chlorantraniliprole to find out LC_{25} and LC_{50}. After 48 hours of treatment, the surviving larvae were counted and shifted to the untreated cotton leaf disc. The survival rates, development time and fecundity ratio were monitored until all the individuals died. For the F_1 generation 100 newly laid eggs were collected from the adults of F_0 generation. Then the newly hatched larvae were shifted to a small plastic container with cotton leaves for feeding. The larval development in the container was monitored on daily basis. The emerging adults were paired in a plastic jar with the available feeding environment and the development time, survival rates, and fecundity ratio were recorded as same as for the F0 adults.

Data Analysis

The LC_{25} and LC_{50} values were determined using the POLO-PLUS software and mortality means were compared with the help of Statistics 8.1.

The *H. armigera* cohort data were processed using the TWOSEX-MSChart software (Chi, 1988; Chi & Liu, 1985). Developmental time, lifespan, reproductive output, and population life table metrics were assessed with their respective variances and standard errors, estimated via the bootstrap method. Statistical comparisons were made using the paired bootstrap test (Chi et al., 2020). Figures of the age-stage survival rate (*sxj*), age specific maternity (*lxmx*), age-specific survival rate (*lx*), reproductive value (*vxj*) and age-stage-specific life expectancy (*exj*) were generated using Sigmaplot 15.

RESULTS

Determination of the sublethal concentrations

The third instar larvae susceptibility to chlorantraniliprole, evaluated via diet incorporation method, resulted in LC_{50} and LC_{25} values of 27.54 and 8.57 respectively Table 1.

The sublethal concentrations employed in the subsequent experiments were determined based on preliminary assessments. After application of lethal and sublethal doses, mortality rates of third instar, 90% and 60%, were recorded after 72 hours, compared to a 39% mortality rate observed in the control group. These outcomes indicate that the selected sublethal concentrations were appropriate for the study.

Table 1. LC_{25} and LC_{50} values of chlorantraniliprole against 3rd instar of *H. armigera*

Insecticide	LC_{50}	LC_{25}	$\chi 2$	*df*	Slop ± SE	*p*
Chlorantraniliprole	27.54	8.57	1.26	4	0.87±0.27	0.68

$\chi 2$=chi square, P=probability, *df*= degree of freedom

Table 2. Impact of chlorantraniliprole on F_0 of *H. armigera*

	LC_{50}	LC_{25}	Control
Female Longevity(days)	42.27±0.61a	46.34±0.41[a]	48.71±0.65[c]
Male Longevity(days)	37.20±0.34[a]	42.36±0.27[a]	45.71±0.35[b]
Fecundity	290.23±6.21[a]	320.33±12.3[a]	340.21±12.21[b]

Impact of chlorantraniliprole on life history traits of F_0 generation

The life table aspects of *H. armigera* were decreased significantly in F_0 generation. The reduction in male and female longevity was observed in all treated insects compared to the untreated. Females exposed to LC_{25} (46.34 days) and LC_{50} (42.27 days) displayed significant decline in life span while in males exposed to LC_{25} (42.36 days) and LC_{50} (37.20 days) concentrations also showed same trend (Table 2). Additionally, egg hatching rates were lower in treated *H. armigera*, with LC_{25} (320.33 eggs) and LC_{50} (290.23 eggs) exhibiting significant reductions (Table 2).

Impact of chlorantraniliprole on life history traits of F_1 generation

The larval developmental period was shorter in the treated groups compared to the untreated, with notable variations observed among the sublethal concentrations. Additionally, significant variations in larval development were detected in LC_{25} 22.24 days and LC_{50} 19 days concentrations relative to the control 23.06 days (Table 3). Moreover, the pupal stage duration also showed significant differences, with pupal development being shorter in the LC_{50}-treated population 9.32 days than in the LC_{25}-treated group 9.9 days (Table 3).

H. armigera longevity was reduced significantly in all treated insects compared to the untreated. Female longevity decreased after consuming the insecticide-treated diet, particularly in the LC_{25} (44.25 days) and LC_{50} (39.1 days) treated concentration. Similarly, males exposed to LC_{25} 41.17 days) and LC_{50} concentrations also showed a significant decline in lifespan (Table 3). Additionally, egg hatching rates were lower in treated *H. armigera*, with LC_{25} (308.42 eggs) and LC_{50} (277.9 eggs) exhibiting significant reductions (Table 3).

Table 3. Life history (Mean ± SE) of *H. armigera* after chlorantraniliprole treatment

Stages	LC_{50}	LC_{25}	Control
Egg	2.54 ± 0.08^{ab}	3 ± 0.05^{a}	3.24 ± 0.06^{b}
L1	2.66 ± 0.08^{b}	3.13 ± 0.05^{a}	3.26 ± 0.07^{c}
L2	2.92 ± 0.08^{a}	3.5 ± 0.08^{a}	3.56 ± 0.07^{b}
L3	2.85 ± 0.08^{ab}	3.55 ± 0.08^{a}	3.64 ± 0.07^{b}
L4	2.97 ± 0.08^{b}	3.54 ± 0.08^{a}	3.74 ± 0.17^{b}
L5	3.03 ± 0.03^{b}	3.61 ± 008^{ab}	3.73 ± 0.07^{b}
L6	4.65 ± 0.08^{b}	4.95 ± 0.09^{a}	5.11 ± 0.09^{c}
Pupa	9.32 ± 0.09^{b}	9.9 ± 0.12^{a}	10.04 ± 0.13^{c}
Total larval duration (days)	19 ± 0.19^{b}	22.24 ± 0.19^{a}	23.06 ± 0.19^{c}
Female longevity (days)	39.1 ± 0.46^{a}	44.25 ± 0.3^{a}	45.71 ± 0.44^{a}
Male longevity (days)	36.56 ± 0.23^{b}	41.17 ± 0.29^{a}	42.84 ± 0.29^{c}

N= Numbers of exposed insects. Mean ± SE were measured by 10,000 bootstrap resampling. Statistical comparisons between treatments were performed using the paired bootstrap test ($P < 0.05$).

Transgenerational effects on population parameters

Chlorantraniliprole significantly impacted the growth parameters of *H. armigera* (Table 4). Both the net reproduction rate (R_o) and intrinsic rate of increase (r_m) were notably reduced in exposed larvae compared to the untreated. Furthermore, these parameters were significantly lower at the LC_{50} concentration than at LC_{25}. The doubling time (T) was also significantly shorter at LC_{50} and LC_{25} concentrations relative to the untreated. Additionally, the gross reproduction rate (GRR) at LC_{50} showed a marked decline compared to the untreated population (Table 4).

Table 4. Population parameters (Mean ± SE) of *H. armigera* after chlorantraniliprole treatment

Parameters	LC_{50}	LC_{25}	Control
APOP (days)	2.2 ± 0.2^{a}	2.25 ± 0.13^{a}	2.36 ± 0.17^{a}

TPOP (days)	32.8±0.42^{b}	37.42±0.42^{a}	38.5±0.39^{c}
O_d(days)	6.3±0.15^{a}	6.83±0.24^{a}	7.21±0.24^{b}
F	277.9±7.96^{b}	308.42±13.69^{a}	329.29±14.42^{c}
R_0 (offspring/individual)	55.58±15.90^{b}	74.02±18.97^{a}	92.2±21.18^{c}
r (per day)	0.1112±0.008^{b}	0.1052±0.0069^{a}	0.1071±0.005^{b}
λ (per day)	1.1176±0.009^{b}	1.1110±0.0076^{a}	1.1130±0.006^{b}
GRR	193.16±29.04bc	230.02±41.73a	246.27±33.11c
T (day)	36.12±0.415^{b}	40.881±0.398^{a}	42.224±0.433^{c}

Mean ± SE were measured by 10,000 bootstrap resampling. Statistical comparisons between treatments were performed using the paired bootstrap test ($P < 0.05$).

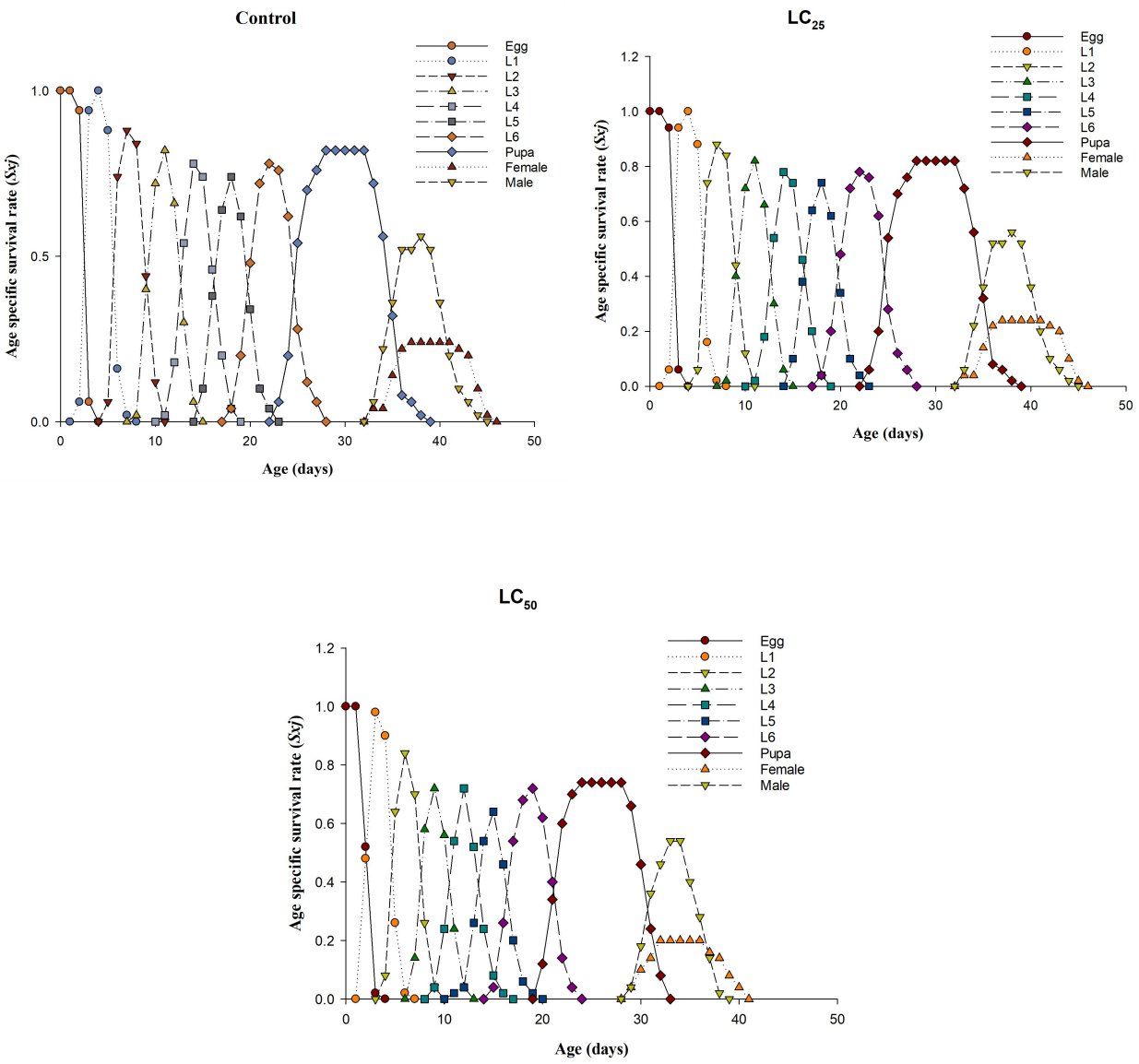

Figure 1. Age specific survival rate (*sxj*) of the F1 generation in *H. armigera.*
Analysis of *sxj* in the F1 generation revealed a significant reduction in lifespan for both LC_{50}- and LC_{25}-exposed populations relative to the control (Figure 1).

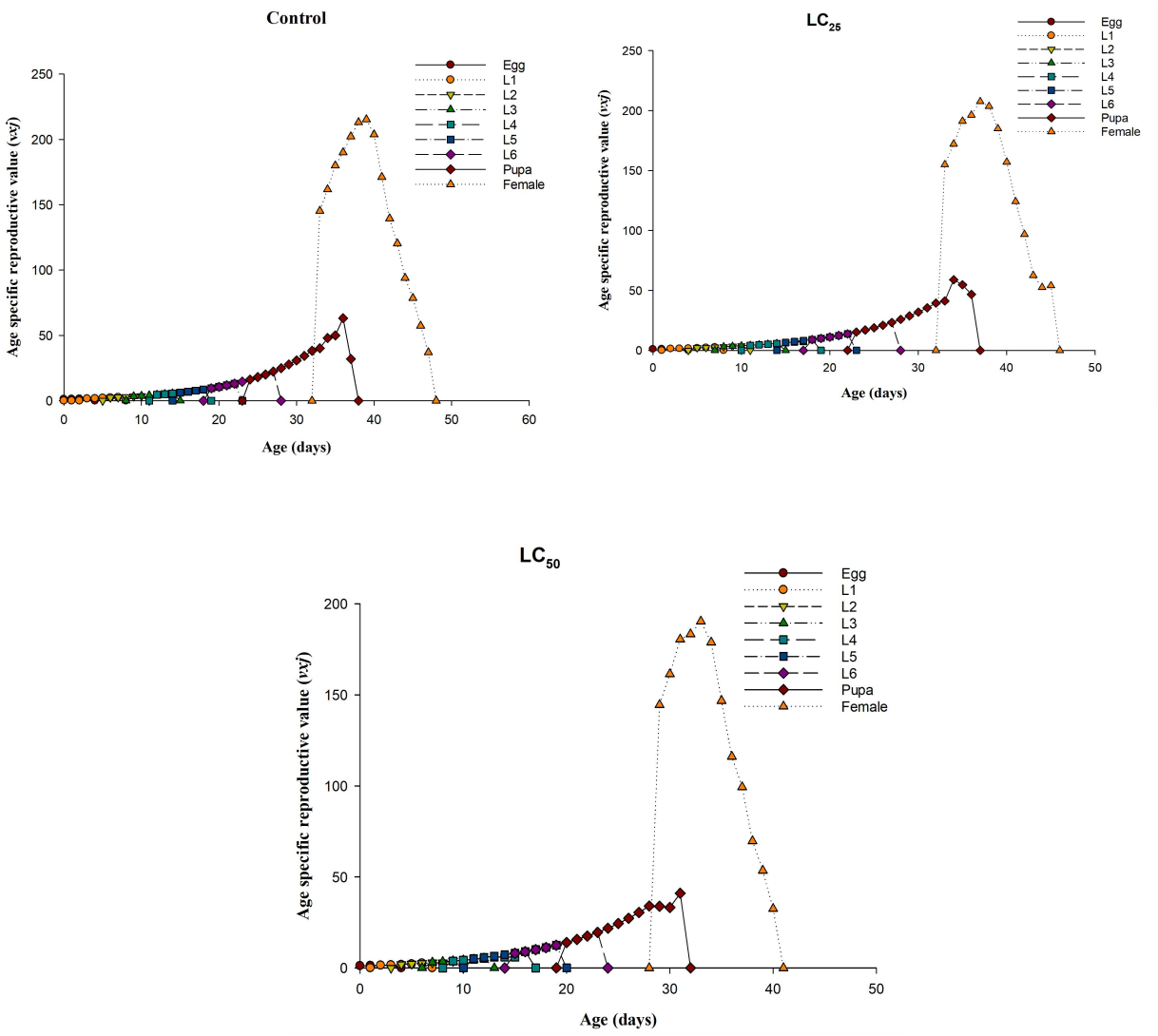

Figure 2. Age stage reproductive value (*vxj*) of the F1 generation in *H. armigera.*
The *vxj* values of the F1 generation indicated a reduced overall reproductive rate in LC_{50}-treated insects, with LC_{25}-treated insects also showing lower reproductive rates compared to the control (Figure 2).

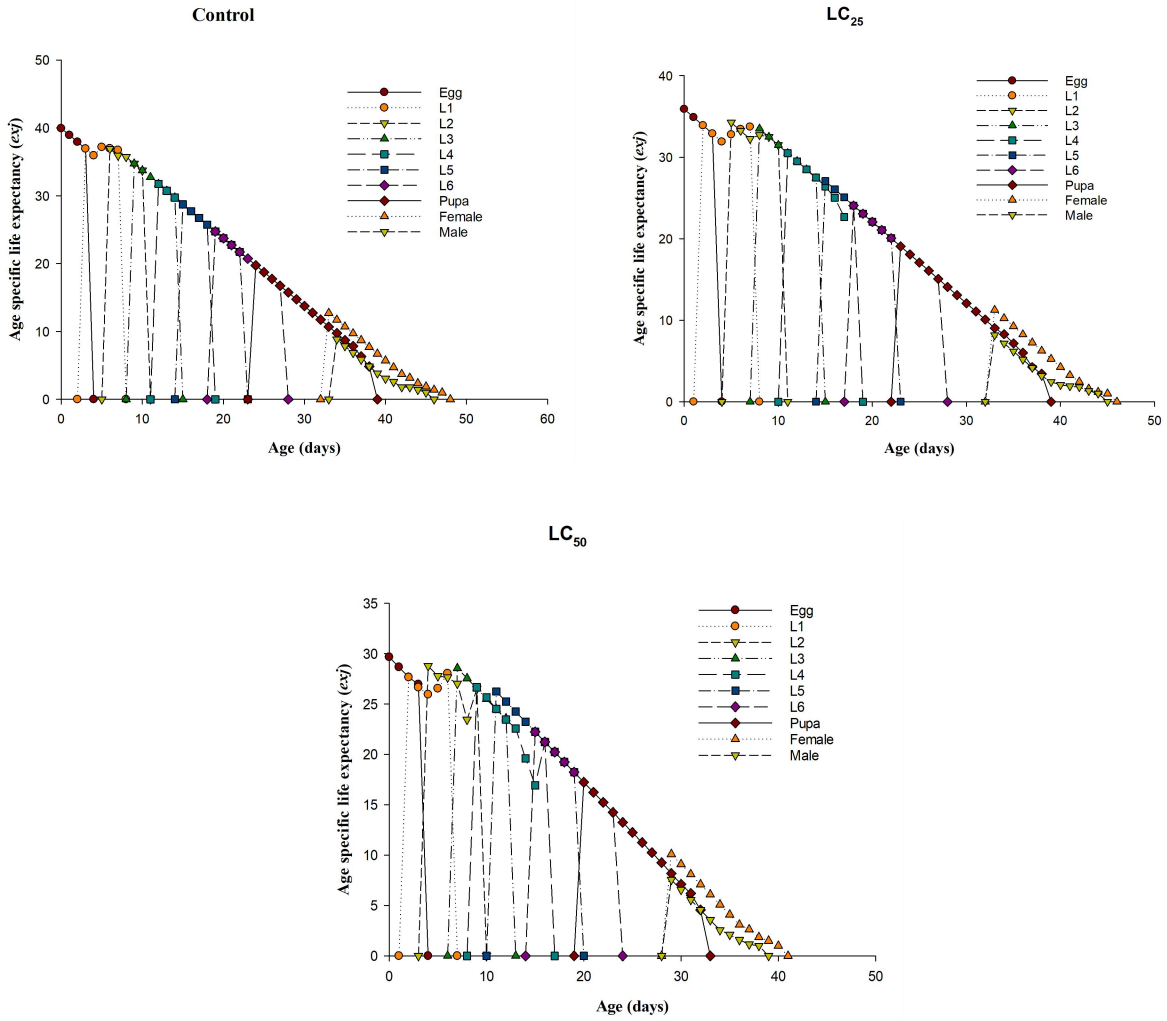

Figure 3.
Life expectancy (*exj*) of the F1 generation in *H. armigera.*
H. armigera treated with LC_{50} and LC_{25} exhibited lower *exj* values than untreated individuals (Figure 3).

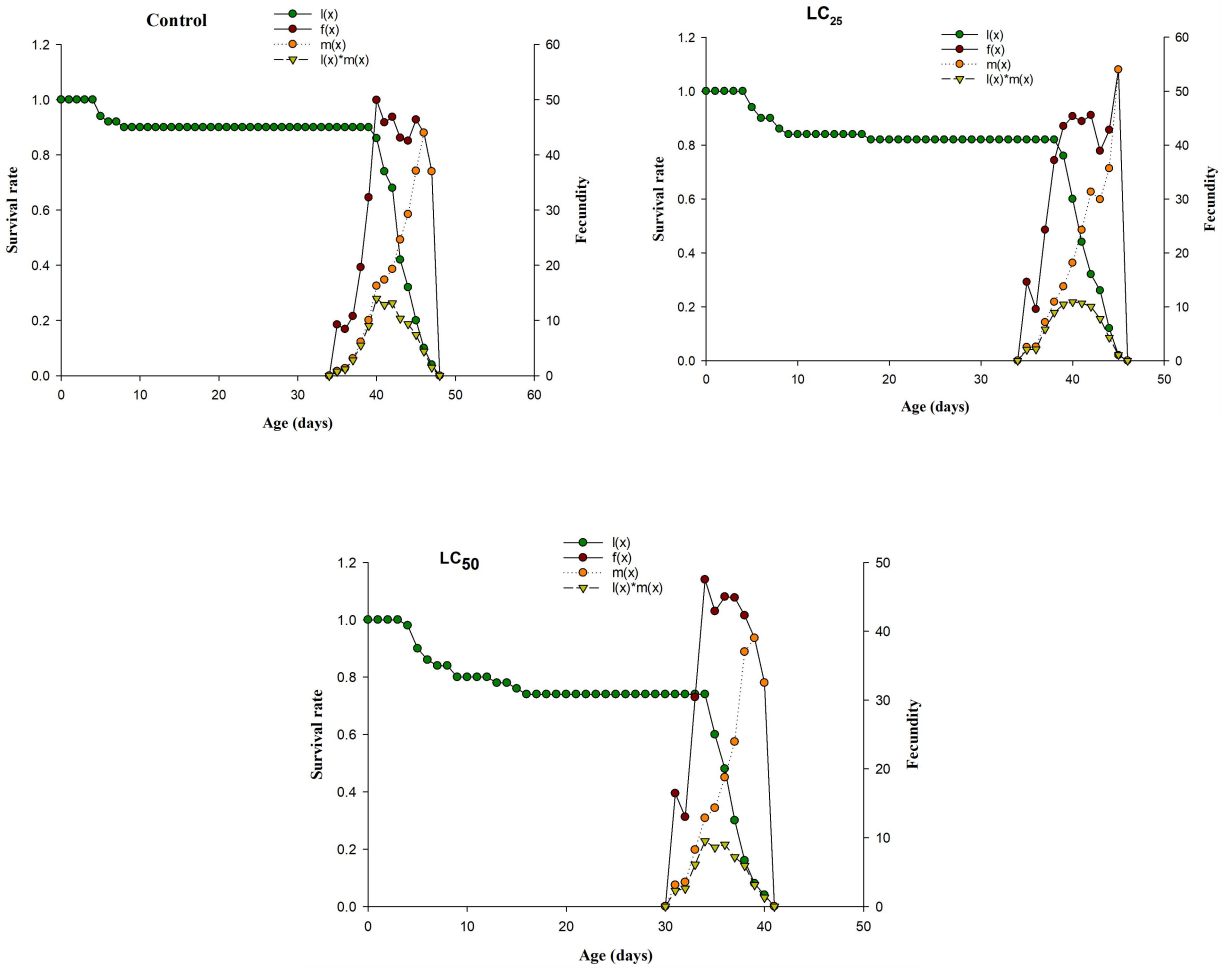

Figure 4. Survival rate and fecundity of the F1 generation in *H. armigera.*
Survival rate and fecundity of *H. armigera* was reduced significantly in LC_{50} treated population in comparison with control (Figure 4).

DISCUSSION

Chlorantraniliprole is a highly effective, broad-spectrum insecticide widely used to manage lepidopteran pests. When applied at field-recommended concentrations, it induces sublethal effects on the larval stages of various lepidopteran species, including *H. armigera*, *Plutella xylostella*, *Spodoptera exigua*, and *S. cosmioides*. These effects negatively influence pest population dynamics, as documented in previous studies (Han et al., 2012; Lai & Su, 2011; Lutz et al., 2018). Chlorantraniliprole belongs to the anthranilic diamide class of insecticides and acts as a potent activator of insect ryanodine receptors (RyRs), as described by Cordova et al. (2006). The activation of these receptors disrupts calcium homeostasis within insect cells, leading to feeding cessation, lethargy, muscle paralysis, and eventual death, as reported by Lahm et al. (2007b). This mode of action likely explains the adverse effects observed on *H. armigera* in this study, including reduced reproductive success and shortened lifespan.

This study explores the effects of lethal and sublethal doses of chlorantraniliprole at various life stages *H. armigera*. The results demonstrate that sublethal exposure to chlorantraniliprole led to varying impacts on developmental durations in the treated population. Adults exposed to LC_{50} and LC_{25} concentrations exhibited reduced longevity. These findings emphasize the significant influence of sublethal insecticide exposure on pest population regulation, suggesting the necessity for additional studies to evaluate its long-term ecological and pest management implications.

Previous studies on *S. exigua* neonate larvae exposed to sublethal concentrations of chlorantraniliprole for 72 hours documented several adverse effects, including increased larval mortality, delayed larval development, and reduced pupation success (Lai & Su, 2011). Similar responses were observed in the present study on *H. armigera* following exposure to sublethal doses. However, unlike Lai & Su (2011), who did not examine transgenerational effects, this study found a slight reduction in fecundity when both parental generations were treated with LC_{25} chlorantraniliprole.

Comparable findings were reported by Adamski et al. (2009) in beet armyworm exposed to a different insecticide, where sublethal doses disrupted reproductive performance. Additionally, research by Smagghe & Degheele (1994) demonstrated that tebufenozide exposure impaired ovulation in *S. exigua* females, with even low concentrations inducing significant effects (Adamski et al., 2009). These observations suggest that the pronounced offspring effects at lower concentrations may stem from differing selection pressures. While high insecticide doses eliminate susceptible individuals, leaving only resistant survivors, low doses may not exert such strong selection. Instead, they could inflict subtle physiological damage, particularly during embryonic development, leading to delayed growth and reduced reproductive capacity in subsequent generations.

Research on *P. xylostella* demonstrated no significant impact of sublethal chlorantraniliprole concentrations on pupal duration in offspring (Han et al., 2012). However, our study revealed contrasting results in *H. armigera*, where LC_{25} treatment significantly reduced pupal duration. Furthermore, we observed decreased adult emergence rates at LC_{50} concentrations, consistent with previous reports of inhibited emergence and reduced longevity following exposure to various insecticides (Hui et al., 2010; Mahmoudvand et al., 2011; Wang et al., 2009). The physiological mechanisms responsible for these emergence alterations remain unclear. The observed interspecific variations may be attributed to differences in insect species sensitivity, exposure methodologies, and concentration effects (Han et al., 2012). Our findings corroborate earlier work by Knight (2007) and Zhang et al. (2021), demonstrating that adult

longevity in *H. armigera* was significantly affected by both LC_{25} and LC_{50} chlorantraniliprole exposures, with transgenerational effects evident in offspring populations.

Previous studies have demonstrated variable effects of sublethal insecticide exposure on reproductive parameters. (Dong LiXia et al., 2011) found that emamectin benzoate reduced both the net reproductive rate (R_0) and intrinsic rate of increase (r_m) in *H. armigera*. In the current study, exposure to LC_{50} chlorantraniliprole significantly decreased R_0, r_m, and gross reproduction rate (*GRR*) in *H. armigera* compared to the control when both mated adults were treated. These reductions were associated with lower survival rates, shortened longevity, and diminished fecundity.

CONCLUSION

The present study demonstrates that chlorantraniliprole exhibits the transgenerational effects on the life-table parameters of *H. armigera*. Our findings reveal that this compound significantly impacts the biological performance of both parental and offspring generations, including reduced adult emergence rates and impaired reproductive capacity. Given its novel mode of action and high efficacy against lepidopteran pests, chlorantraniliprole represents a valuable tool for field applications. However, its sublethal effects must be carefully considered when developing integrated pest management strategies. Further research should investigate whether these sublethal effects persist across multiple generations, as such transgenerational impacts could influence the development of insecticide resistance in field populations.

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