Chapter 2: Deltamethrin induced toxic transgenerational effects on the development and repellency of *Callosobruchus chinensis*

2.1 Introduction

Transgenerational effects pertain to the occurrence in which environmental exposures or experiences exert an influence not only on the individuals who are immediately exposed, but also on their offspring and later generations (Brevik et al., 2018). These effects manifest in various ways, including altered phenotypes, physiological changes, behavioural modifications, and susceptibility to diseases or stressors (Xin et al., 2015; Castano-Sanz et al., 2022; Tamagno et al., 2023). Transgenerational effects occur through different mechanisms, such as epigenetic modifications changes in gene expression, alterations in germ cell development, or transfer of parental resources (Nilsson et al., 2022; Pan et al., 2023). These mechanisms lead to heritable changes in the phenotype and physiology of subsequent generations, even in the absence of continued exposure to the original environmental stressor (Ayyanath et al., 2013; Fitz-James and Cavalli, 2022).

In the realm of stored grain management, insecticides are frequently employed as a means of regulating the population of pests that afflict stored grains, including beetles, weevils, and moths. These pests pose a substantial threat to the integrity and quality of stored grains, hence necessitating the use of insecticides for effective control. These insecticides are designed to kill or inhibit the growth and reproduction of the pests. However, some insecticides can have unintended consequences and lead to transgenerational effects on the pests (Costa et al., 2023). Insecticide-induced transgenerational changes refer to the effects of insecticide exposure on individuals that persist across multiple generations, influencing the phenotype and physiology of subsequent offspring (Hanson and Skinner, 2016). The mechanisms underlying insecticide-induced transgenerational changes are not fully understood but may involve epigenetic modifications. Epigenetic alterations can affect gene expression patterns without changes to the underlying DNA sequence (Hu et al., 2020; Pompermaier et al., 2022). These alterations may affect various biological processes, including development, metabolism, reproduction, and responses to stress or toxins (Wang et al., 2022; Wu et al., 2022).

Some of the potential transgenerational effects of insecticides include:

1. **Resistance development:** Continuous exposure to insecticides can result into the development of resistance in subsequent generations. Some individuals within the population may possess genetic variations that confer resistance to the specific insecticide. Over time, these resistant individuals become more prevalent, reducing the efficacy of the insecticide in controlling the pest population **(Fig 2.1)**.

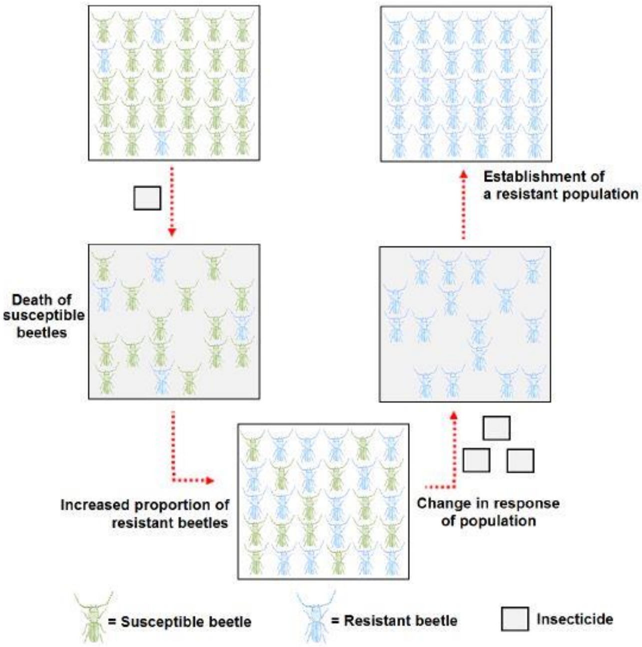


Figure. 2.1: Depicts how over the time resistance is developed in beetle due to exposure of insecticide (Napoleão et al., 2015).

2. **Altered growth and development:** Insecticide exposure can affect the growth and development with reference to alteration in their developmental rate, body size, or morphology. This change passes on to subsequent generations, leading to modified growth patterns or phenotypic variations.
3. **Behavioural changes:** Insecticides can also influence the behaviour. They may affect their feeding preferences, reproductive behaviours, or movement

patterns. Transgenerational effects of insecticides on behaviour can impact the ability of the pest to locate suitable food sources, find mates, or avoid detection, potentially altering their population dynamics.

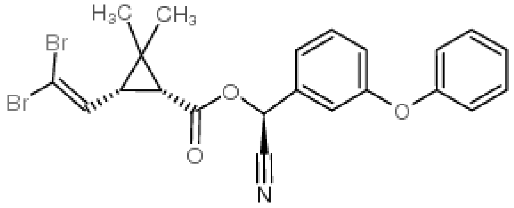
4. **Reproductive effects:** Insecticides may disrupt the reproductive capacity by interfering with mating, fertility, or egg-laying patterns. These effects can carry over to subsequent generations, affecting the overall reproductive success and population growth of the pest.
5. **Fitness consequences:** Fitness refers to an organism's ability to survive and reproduce. Transgenerational effects of insecticides can have fitness consequences by lowering their survival rates, reproductive output, or offspring viability. Leading to have an impact on population dynamics of the pests over time.

Olivares-Castro et al., (2021) have mentioned the transgenerational effects of carbaryl and permethrin, on aquatic insects and reported that insecticide exposure in one generation can have negative effects on the survival, growth, and reproduction of subsequent generations (Gross and Garric, 2019). Further, Jaffar et al., (2022) examined the transgenerational effects of two insecticides, imidacloprid and propoxur, on the reproductive traits of fruit flies (*Drosophila melanogaster*) and have reported reduced fecundity and altered offspring development in subsequent generations. Iftikhar et al., (2020) focused on the transgenerational effects of imidacloprid on the mealybug predator (*Cryptolaemus montrouzieri*) and have confirmed the influence on parental behavior, such as oviposition site selection, leading to negative effects on the offspring survival, development, and body size. Giving emphasis on the fitness, reproductive success, development and population dynamics, it is equally important to note that the specific effects can vary depending on the insect species, insecticide formulation, dosage, exposure duration, and other factors.

Daglish, (2008) studied transgenerational effect of methoprene an insect growth regulator in rice weevil (*Sitophilus oryzae*), and have confirmed that methoprene exposure influenced the reproductive output, developmental time, and population growth of subsequent generations. Cui et al., (2021) examined the transgenerational effects of phosphine, a commonly used fumigant, on fecundity and body weight in three stored-product insects: red flour beetle (*Tribolium castaneum*), lesser grain borer (*Rhyzopertha dominica*), and rice weevil (*Sitophilus oryzae*) and have proved that phosphine exposure manipulated reproductive parameters and body weight in subsequent generations. Further, Rösner et al., (2020) investigated the effects of deltamethrin on the reproductive fitness of *Tribolium castaneum*, found that it altered the reproductive performance and offspring development in subsequent generations. All these studies thus emphasize that insecticide exposure can induce transgenerational effects on insects, influencing their fitness, reproductive success, development, and population dynamics. The transgenerational effects of insecticides on stored grain pests can have both short-term and long-term implications (Guedes et al., 2016; Nyamukondiwa et al., 2022). In long term, the effects may be beneficial to the pests, allowing them to survive and reproduce in the presence of insecticides ultimately leading to the development of insecticide resistance and pose challenges for effective pest management strategies (Bueno et al., 2023).

Pyrethroids belong to a class of insecticides known for their neurotoxic effects on insects. Research has indeed investigated the transgenerational effects of pyrethroids on insect development. Breivik et al., (2018) have seen the transgenerational effects of deltamethrin exposure on the diamondback moth (*Plutella xylostella*) and have reported its negative effects on various life history traits and increased oxidative stress in initial generations. Later on, Valle et al., (2019) too have reported transgenerational effects of deltamethrin on the fitness traits of *Aedes aegypti* mosquitoes, the primary vector for dengue and other diseases and found that it affected larval development, adult body size, and reproductive parameters in various generations. Transgenerational toxicity of the esfenvalerate and bifenthrin on the freshwater crustacean *Daphnia magna* and terrestrial isopod *Porcellionides pruinosus* has also been well explored by Yang et al., (2018) and Kumar et al., (2023) respectively and have reported delayed development, reduced reproduction, and altered population dynamics in multiple generations. Additionally, Huang et al., (2022) investigated the transgenerational effects of deltamethrin on the cotton bollworm and have confirmed reduced survival, delayed development, and decreased reproductive capacity in initial generations. Previous studies have also reported that exposure to insecticides results in increased resistance or tolerance to the chemicals in subsequent generations due to genetic changes or adaptations that occur over time.

Table 2.1: Insecticide **(**Source: PPDB)

Chemical name	**Deltamethrin**
Mode of Action	The mechanism of action involves both direct contact and ingestion. The compound has the ability to exert its effects on neuronal membranes through the mechanism of delaying the closure of the activation gate of the sodium ion channel (Worthing and Walker, 1987).
CAS RN	52918-63-5
IUPAC name	[(S)-Cyano-(3-phenoxyphenyl)-methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1- carboxylate
Chemical formula	$C_{22}H_{19}Br_2NO_3$
Molecular structure	
Molecular weight (g/mol)	505.21
LogP (at 25°C)	1.5
Relative density	1.5g/cm^3
Henry’s Law Constant (Pa m^3 mol^{-1})	1.2 x 10^{-4}
Manufactures and suppliers of products	Sigma Aldrich

Deltamethrin (**Table 2.1**), a specific synthetic pyrethroid of class II, is widely recognised for its capacity to impact the sodium channel (Meunier et al., 2020).

The transgenerational effects of insecticides on stored grain pests have been a subject of study due to the importance of managing these pests to protect stored food commodities. Insects provide favourable attributes as model animals for the examination of transgenerational research due to their abbreviated generational span and the ease of maintaining substantial populations inside controlled laboratory settings (Mukherjee et al., 2015). Insects exhibit prompt responses to pesticides, leading to the emergence of diverse adaptive phenotypes over the course of a few generations. These phenotypes are heritable and linked to alterations in gene expression (Dubovskiy et al., 2013; Mukherjee and Vilcinskas, 2019). Overall, the transgenerational effects of insecticides on stored grain pests highlight the need for careful and responsible insecticide use. Integrated pest management approaches that combine multiple strategies, such as proper grain storage practices, biological controls, and judicious use of insecticides, can help minimize the development of resistance and mitigate the potential transgenerational effects on stored grain pests. Understanding insecticide-induced transgenerational changes is important for insecticide risk assessment, management strategies, and the development of sustainable pest control practices. Further research is needed to unravel the underlying mechanisms and assess the ecological implications of these transgenerational effects on insect populations and ecosystems.

Hence, the present study was undertaken to investigate the transgeneration effects of deltamethrin on development and repellency of the C. chinensis in control laboratory conditions.

2.2 Materials and Methodology

Experimental regime: Finding out the LC_{50}

A survey was conducted in different Insecticide shops and ware houses to find the usage of different insecticides. The unworked or least explored insecticide was taken into the account. A type II semisynthetic pyrethrin insecticide, technical grade deltamethrin (98% AI, Sigma Aldrich, Saint Louis, MO) was used. To determine contact toxicity of deltamethrin against *C. chinensis*, five concentrations of deltamethrin, 6.25, 12.50, 25, 50 and 100 ppm respectively were tested. These concentrations were obtained by dissolving 98% deltamethrin in acetone. A stock solution of 1,000 ppm was prepared from which other desired concentrations (serial dilutions) were prepared. There were three replicates for each treatment in addition to controls. One mL of each concentration was placed on the bottom of each Petri dish (9 cm diameter). After the acetone was evaporated, 10 pairs of adults of pulse beetle were placed into each dish. The same procedure was used for the control treated with acetone. Mortality percentages were recorded after 24hrs, 48hrs, 72hrs and 96 hrs of treatment. Thereafter Probit analysis was performed to obtain the LC_{50} value.

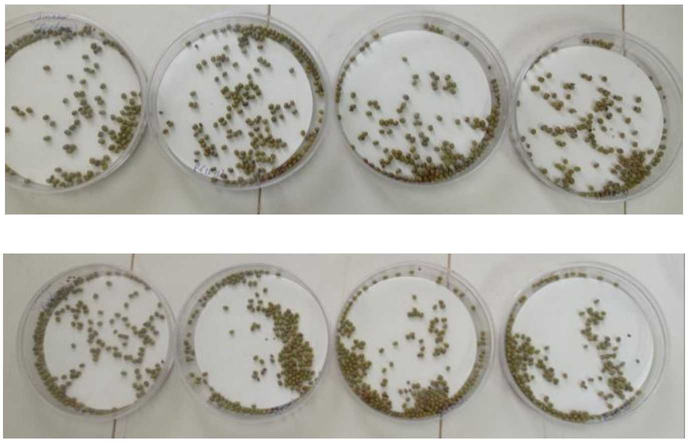


Figure 2.2: Experimental setup for obtaining LC_{50}

Transgenerational effect of insecticide on the development of *C. chinensis*

The experiment performed on three groups. Group I: Control and the obtained LC_{50} value were divided into Group II: Low Concentration (L_{Lc50}) ($1/5^{th}$) and Group: High Concentration (H_{Lc50}) ($1/20^{th}$) for further studies and acetone was used as control.

One mL of L_{Lc50}, H_{Lc50} was placed on the bottom of each Petri dish (9 cm diameter). After the acetone was evaporated, 10 pairs of freshly emerged (1-2 days old) *C. chinensis* were released in glass petri dish containing 50g host. These petri dishes were maintained at 26-28 °C, 60-70% RH and 12-hour photo period, and they were allowed to mate for 7 days, whole set up was replicated three times. The same procedure was used for the control treated with acetone. After the period of seven days insects were scarified for further studies.

Statistics of the following parameters was analysed till the termination of the experiment.

Total number of eggs: To determine the total number of eggs, the eggs laid on different group were counted using magnifying glass and recorded for 7 days.

Hatching percentage: It was calculated by the formula

$$\text{Hatching (\%)} = \frac{\text{No. of eggs hatched}}{\text{No. of eggs laid}} \times 100$$

Incubation period: The duration from egg laying to emergence pf 1st instar larva was recorded.

Larval + Pupal period: The duration from the 1st instar larva to the emergence of adult was recorded.

Total Development period: The period from the egg laid to adult emergence in each treatment was recorded.

Total adult emergence: Male and Female emerged adults were counted separately and the sum of male and female was calculated as Total adult emergence.

Adult longevity: The number of days that the emerged adult survive were recorded.

Susceptibility Index: The susceptibility index is determined by using formula below (Schöller et al., 2018 and Ngom et al., 2021)

$$SI = \text{Log}F/DME \times 100$$

Where,

Log: Logarithm
F: Total number of F1 progenies
DME: generation development time.
The SI ranged from 0-11

0 - 3 = maximum effect
4 - 7 = moderately effect
8 - 10 = susceptible
11 = highly susceptible

Transgenerational effect of insecticide on the repellency of *C. chinensis*

Filter papers (9 cm diameter Whatman No. 41) was cut in half and each labelled “C” for control and “L_{Lc50}” for low dose similarly it was done for Control “C” and high dose “H_{Lc50}”. The treatment half was treated with 1 mL of one dose and allowed to air dry for 2 min. The control half was treated with 1 mL of acetone only. Both halves were re-joined with clear adhesive tape and placed with the taped side down in a 9 cm petri dish. Twenty seeds of green gram were evenly distributed in the petri dish and five pairs of newly emerged adult beetles were placed in the centre of the filter paper and the dishes sealed tightly with Parafilm® to prevent escape. The dispersion of the beetles on each side (treatment and control) was noted 0, 1, 2, 4, 8, 12 and 24 h. The experiment was a randomized block design with three replicates per treatment.

The Percent Repellency (PR) (Nerio et al. 2009) was

calculated based on the formula:

$PR= [(N_C-N_T)/(N_C+N_T)]X100$

Nc = number of insects on control half of filter paper after required exposure interval

N_T = number of insects on treated half of filter paper after required exposure interval.

The Repellent Index (RI) of Kogan and Goeden (1970) based on the formula was calculated:

$RI = 2G/G+P$

Where G = number of insects on treatment side

P = Number of insects on control side

The standard deviations (SD) of the mean values of the RI were calculated and insecticide at different group classified based on whether it was an attractant (RI > 1+SD), was indifferent (= neutral) (RI between 1 – SD and 1 + SD) or was a repellent (RI < 1–SD).

Statistical Analysis

This study was in a Completely Randomized Design (CRD) by three replications. Statistical analysis was done by analysis of variance (ANOVA) with GraphPad prism 9.0v followed by multiple comparison test (Tukey’s). Results are presented as Mean±SEM. The level of significance was set as *$p<0.05$, ** $p<0.01$.

2.3 Results

Determining LC_{50} value of Deltamethrin

LC_{50} value of Deltamethrin was determined using Probit Analysis after 96 hours of exposure of pulse beetles. The 50% probit mortality ranged between 6.25 to 100 ppm concentration (**Table 2.2**). LC_{50} value was obtained as 22.93 ppm from the dose response curve (**Fig. 2.3**). Further, the sub-lethal concentrations: Low concentration (L_{Lc50})-1/20th of LC_{50}, and High concentration (H_{Lc50})-1/5th of LC_{50} (**Table 2.3)** were used to understand the effects of Deltamethrin on pulse beetles.

Table 2.2: Probit Mortality obtained after 96 hours of exposure to Deltamethrin

Concentration (ppm)	log Concentration	% Mortality	Probit Mortality
6.25	0.795880017	0	0
12.5	1.096910013	33	4.53
25	1.397940009	70	5.52
50	1.698970004	100	8.09
100	2	100	8.09

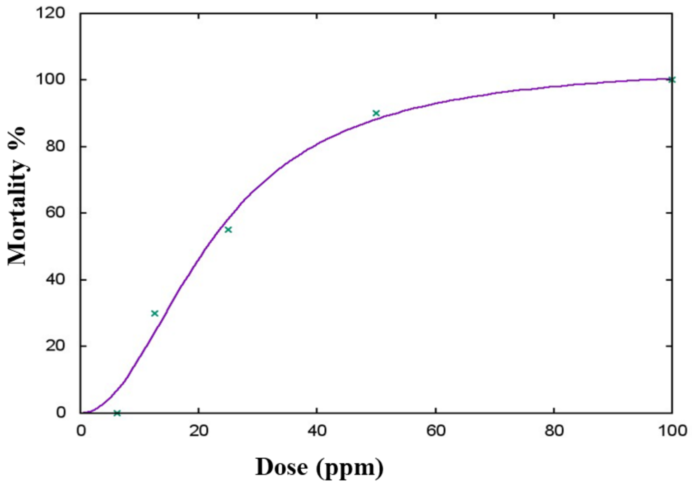


Figure: 2.3: Dose response curve for the LC_{50}

Table 2.3: LC_{50} value obtained and the sub-lethal concentrations selected for further studies

LC_{50}	22.93 ppm
Low Concentration (L_{LC50})	**1.15 ppm**
High Concentration (H_{LC50})	**4.59 ppm**

100% mortality at higher concentration (50 ppm and 100ppm) and 0% mortality at (6.25ppm). The mortality was concentration dependent as the concentration increases it increases the mortality.

Transgenerational effect of insecticide on the development of *C. chinensis*:

There is a statistically significant variation ($p<0.05$ and $p<0.01$) in the total egg count observed across the groups throughout several generations **(Table 2.4)**. The first generation demonstrates a noticeable impact of deltamethrin, as seen by a significant drop in the overall egg count as compared to the control group (190±1.10). Specifically, the low concentration group exhibited a count of (120±0.7) eggs, while the high concentration group had a count of (100±0.4) eggs. In subsequent generations, there is a gradual increase in the total egg count compared to the first generation. By the sixth generation, the total egg count of the control group (190±1.10), low dose group (185±0.6), and high dose group (180±0.7) is nearly identical. This suggests that the pulse beetle population at the sixth generation has developed tolerance to the effects of deltamethrin **(Fig. 2.4)**. The overall hatching rate has a comparable pattern to the total egg count, and it demonstrates significant ($p<0.05$ and $p<0.01$) among both the groups throughout several generations **(Fig. 2.5)**. The first generation demonstrates a noticeable impact of deltamethrin, as seen by a substantial decrease in overall hatching rates compared to the control group (120±1.10). Specifically, the low dosage group had a reduction of (45±0.5) hatching events, while the high dose group experienced a reduction of (30±0.4) hatching events, both of which were statistically significant. In subsequent generations, there is a gradual increase observed in the total hatching rate when compared to the first generation. By the sixth generation, the total hatching rates of the control group (120±1.10), low dosage group (115±1.0), and high dose group (108±0.7) were found to be in close proximity. The observed trend in hatching % **(Fig. 2.6)** exhibited considerable variance between various groups and generations. The study observed a progressive rise in both the total egg count and hatching rate throughout subsequent generations. Specifically, the hatching percentage rose from 37.5% and 30% in the first generation to 62.2% and 60% in the sixth generation in both the treated groups respectively **(Table 2.4).**

Table 2.4: Total eggs count, hatched eggs and hatching % of *C. chinensis* in various generations.

Generations	Total Eggs Count			Hatched Eggs			Hatching %		
	Control	L_{Lc50}	H_{Lc50}	Control	L_{Lc50}	H_{Lc50}	Control	L_{Lc50}	H_{Lc50}
F1	190±1.1	120±0.7	100±0.4	120±1.1	45±0.5	30±0.4	63.16	37.5	30
F2	190±1.1	125±0.8	105±0.5	120±1.1	50±0.5	36±0.4	63.16	40	34.3
F3	190±1.1	145±0.5	120±0.7	120±1.1	55±0.8	50±0.7	63.16	44.8	41.6
F4	190±1.1	160±0.7	135±0.8	120±1.1	80±0.6	65±0.5	63.16	50	48.2
F5	190±1.1	180±0.9	155±0.8	120±1.1	100±0.9	90±0.8	63.16	55.5	58.1
F6	190±1.1	185±0.6	180±0.7	120±1.1	115±1.0	108±0.7	63.16	62.2	60

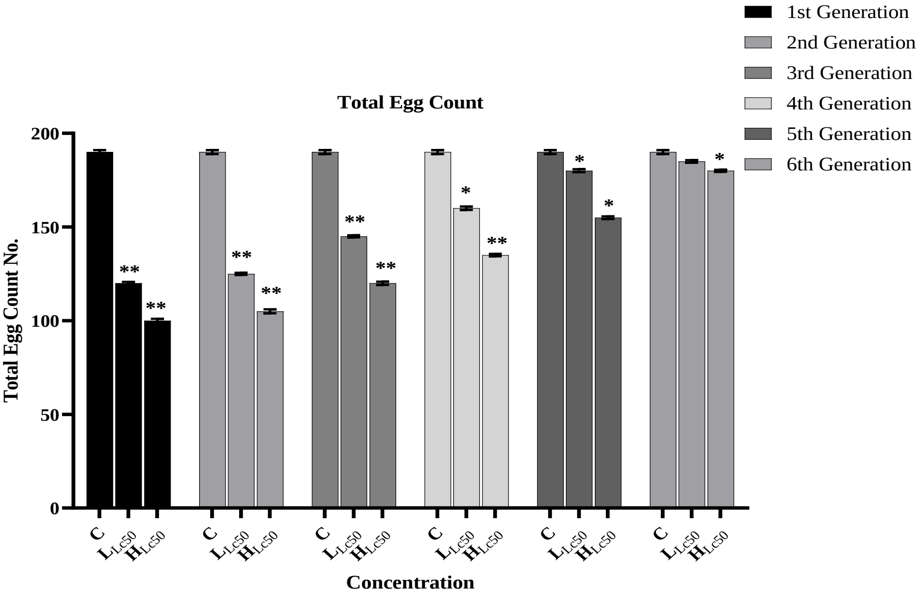


Figure 2.4: Transgenerational effect of deltamethrin on the total egg count of *C. chinensis*. Significant level *($p<0.05$); **($p<0.01$)

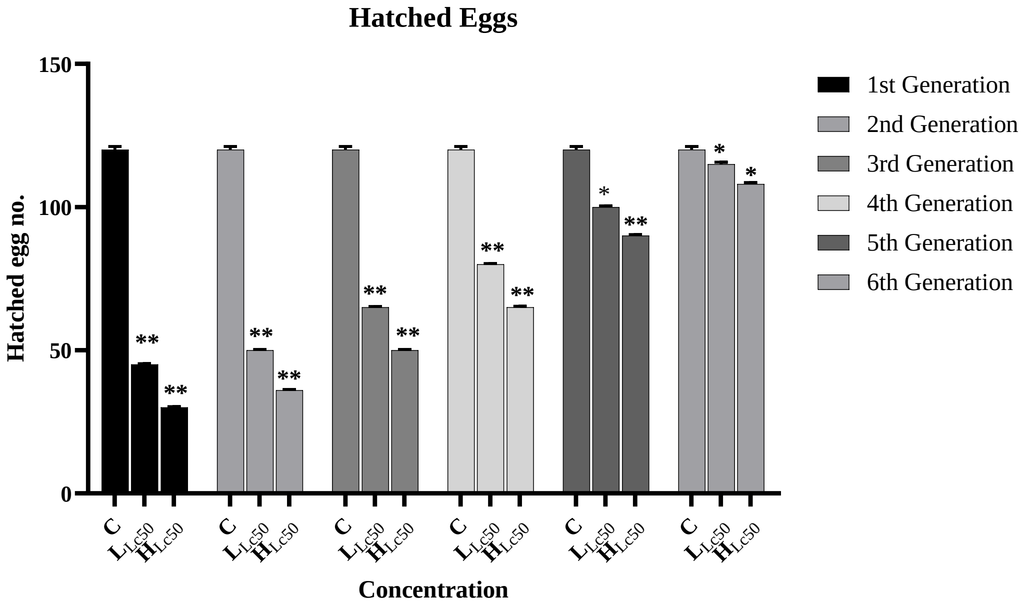


Figure: 2.5: Transgenerational effect of deltamethrin on the total hatching of *C. chinensis.* Significant level *(p<0.05); **(p<0.01)

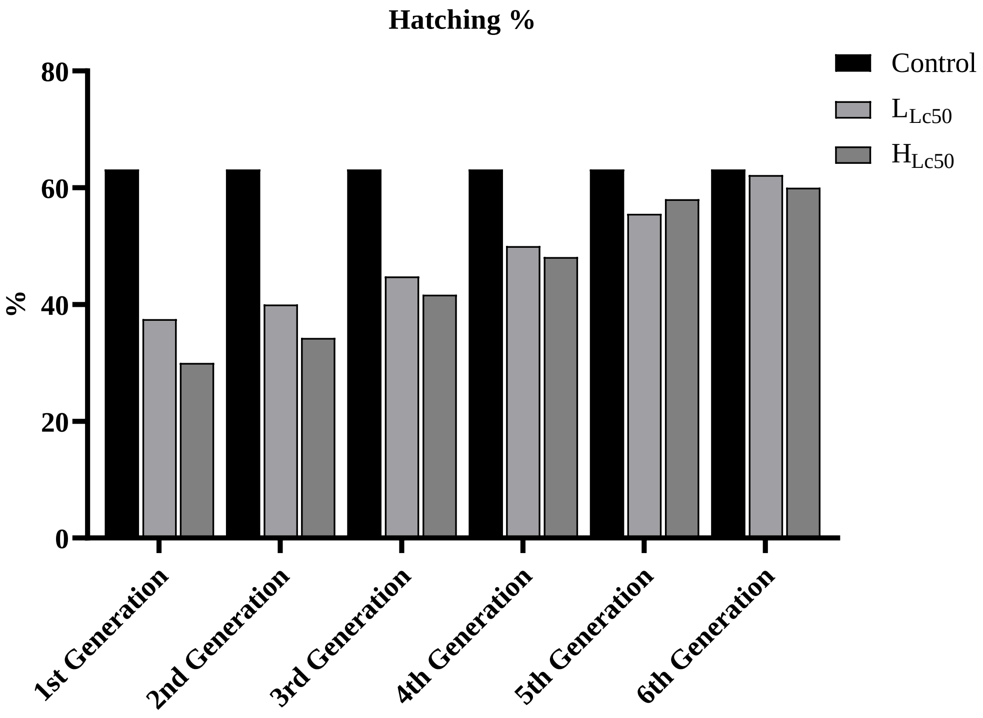


Figure: 2.6: Transgenerational effect of deltamethrin on the hatching% of *C. chinensis.* Significant level *(p<0.05); **(p<0.01)

In the first generation, the duration of complete development period **(Fig. 2.7)** was found to be 44±0.43 days in the high concentration group and 36±0.37 days in the low concentration group, which was substantially longer ($p<0.01$) compared to the control group with a duration of 22.67±0.33 days. In subsequent generations, there was a shift in the observed pattern, wherein the total duration of the developmental period exhibited a decreasing tendency in both the low dose and high dosage groups. Specifically, in the 6th generation, the low concentration group displayed a total development time of 23±0.26 days, while the high concentration group exhibited a total development period of 24±0.30 days, which was nearly comparable to the control group **(Table 2.5).**

Table 2.5: Total Development Period (Days) in various generations of *C. chinensis*

Generations	**Total Development Period (Days)**		
	Control	**L_{Lc50}**	**H_{Lc50}**
F1	22.67±0.33	36±0.37	44±0.43
F2	22.67±0.33	33±0.20	40±0.29
F3	22.67±0.33	30±0.23	35±0.33
F4	22.67±0.33	27±0.28	31±0.27
F5	22.67±0.33	25±0.30	27±0.32
F6	22.67±0.33	23±0.26	24±0.30

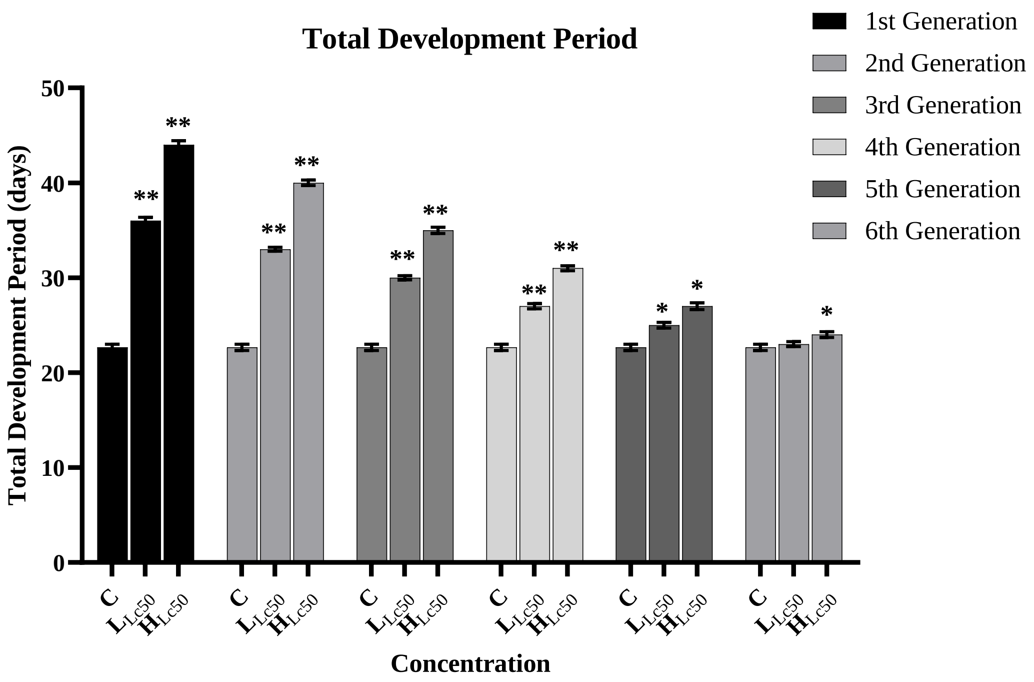


Figure: 2.7: Transgenerational effect of deltamethrin on the total development period of *C. chinensis*. Significant level *(p<0.05); **(p<0.01).

The duration of survival for the hatched adult individuals exhibits a statistically significant difference (p<0.01) **(Table 2.6)**. Specifically, the control group had a mean survival time of 16±0.25 days, the low concentration group had a mean survival time of 10±0.25 days, and the first-generation group had a mean survival time of 7±0.12 days. The duration of survival in consecutive generations exhibits an increasing trend. Specifically, at the 6th generation, the low concentration group demonstrated a survival period of 16±0.18 days, while the high concentration group exhibited a survival period of 14±0.22 days. These values were found to be comparable to the control group, as seen in **(Fig. 2.8)**.

Table 2.6 Adult Longevity (Days) in various generations of *C. chinensis*

Generations	**Adult Longevity (Days)**		
	Control	**L_{Lc50}**	**H_{Lc50}**
F1	16±0.25	10±0.25	7±0.12
F2	16±0.25	10±0.15	8±0.15
F3	16±0.25	12±0.20	10±0.20
F4	16±0.25	14±0.22	11±0.18
F5	16±0.25	15±0.15	13±0.25
F6	16±0.25	16±0.18	14±0.22

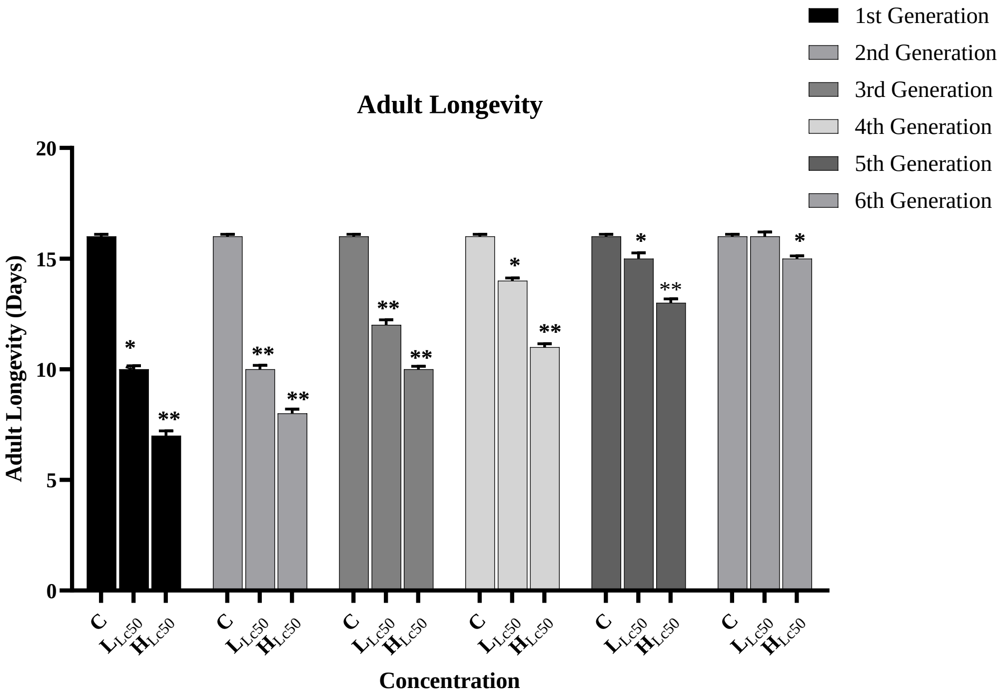


Figure: 2.8: Transgenerational effect of deltamethrin on the adult longevity of *C. chinensis.* Significant level *(p<0.05); **(p<0.01)

The susceptibility index, as indicated in **Table 2.7**, provides evidence that the initial generation of pulse beetles exhibited higher susceptibility, particularly when exposed to higher concentrations of the insecticide. However, over subsequent generations, the susceptibility demonstrated a declining tendency, with the 6th generation displaying significantly reduced susceptibility.

Table 2.7: Transgenerational alteration in the Susceptibility index of *C. chinensis.*

Susceptibility Index			
Generations	**Control**	**L_{Lc50}**	**H_{Lc50}**
F1	9.16	4.58	3.36
F2	9.16	5.15	3.89
F3	9.16	6.03	4.86
F4	9.16	7.03	5.84
F5	9.16	8	7.22
F6	9.16	8.96	8.25

Transgenerational effects of the insecticide on the repellency behaviour of *C. chinensis*

Tables 2.8 and **2.9** provide an account of the repellent percentage, while **Tables 2.10** and **2.11** present the repellent index of deltamethrin against *C. chinensis*. The data reveals that *C. chinensis* exhibited the highest level of repellency in the 1st generation, with a subsequent decrease in repellency found in subsequent generations. A same pattern was found in the repellent index, whereby it initially exhibited repellent properties but then transitioned to a neutral state with regard to attractant characteristics throughout subsequent generations. The efficacy of deltamethrin as a repellent was shown to vary depending on the duration of exposure and the specific generation being tested. The level of repellency exhibits a decline as the duration of exposure and subsequent generations progress.

Table: 2.8: Percent repellency of deltamethrin against *C. chinensis* on L_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	**0**	**1**	**2**	**4**	**8**	**12**	**24**
	L_{Lc50} Mean Repellency %						
F1	100	90	85	75	70	55	40
F2	100	85	75	68	60	45	35
F3	95	70	65	55	45	30	20
F4	90	55	50	40	35	25	5
F5	86	45	40	30	22	15	-10
F6	80	35	25	20	15	5	-20

Table 2.9: Percent repellency of deltamethrin against *C. chinensis* on H_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	**0**	**1**	**2**	**4**	**8**	**12**	**24**
	H_{Lc50} Mean Repellency %						
F1	100	92	90	82	75	60	50
F2	100	90	84	80	66	52	40
F3	100	75	72	62	50	40	30
F4	94	65	55	50	40	28	15
F5	90	60	50	34	30	20	10
F6	85	50	35	25	20	14	-10

Table 2.10: Repellent Index (RI) of deltamethrin against *C. chinensis* on L_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	**0**	**1**	**2**	**4**	**8**	**12**	**24**
	L_{Lc50} Repellent index						
F1	-	0.10±0.06	0.15±0.02	0.25±0.15	0.30±0.10	0.45±0.04	0.60±0.10
F2	-	0.15±0.10	0.25±0.06	0.32±0.10	0.40±0.06	0.55±0.10	0.65±0.16
F3	-	0.30±0.08	0.35±0.12	0.45±0.10	0.55±0.18	0.70±0.08	0.80±0.22
F4	0.05±0.01	0.45±0.12	0.50±0.15	0.60±0.15	0.65±0.20	0.75±0.16	0.95±0.12
F5	0.14±0.08	0.55±0.15	0.60±0.10	0.70±0.08	0.78±0.14	0.85±0.2	1.10±0.10
F6	0.20±0.10	0.65±0.18	0.75±0.15	0.80±0.2	0.85±0.16	0.95±0.08	1.20±0.23

Table 2.11: Repellent Index (RI) of deltamethrin against *C. chinensis* on H_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	**0**	**1**	**2**	**4**	**8**	**12**	**24**
	H_{Lc50} Repellent index						
F1	-	0.08±0.02	0.10±0.0.4	0.18±0.12	0.25±0.14	0.40±0.2	0.50±0.12
F2	-	0.10±0.04	0.16±0.0.6	0.20±0.08	0.34±0.05	0.48±0.12	0.60±0.16
F3	-	0.25±0.1	0.28±0.1	0.38±0.12	0.50±0.08	0.60±0.06	0.70±0.04
F4	0.06±0.02	0.35±0.1	0.45±0.08	0.50±0.18	0.60±0.12	0.72±0.06	0.85±0.08
F5	0.10±0.06	0.40±0.12	0.50±0.2	0.66±0.1	0.70±0.06	0.80±0.2	0.90±0.1
F6	0.15±0.06	0.50±0.08	0.65±0.15	0.75±0.2	0.80±0.2	0.86±0.1	1.10±0.23

2.4 Discussion

Deltamethrin is a chemically synthesised compound that replicates the properties of pyrethrins, which are naturally derived from the dried flowers of *Chrysanthemum* plants (Shrivastava et al., 2011; Bhanu et al., 2011). This compound is a synthetic pyrethroid insecticide with a wide range of applications for the control of stored-product insect pests in various regions globally (Vayias et al., 2010; Trostanetsky et al., 2023). The mechanism of action is the paralysis of the insects' nervous system, resulting in rapid incapacitation, impaired coordination, and ultimately, mortality (Velki et al., 2014).

The neurotoxic effect of deltamethrin in insects is attributed to its capacity to impair axonal transmission of nerve impulses by modifying the ion permeability of nerve membranes (Paudiyal et al., 2016 and 2017). The anticipated outcome of this investigation was the demonstrated efficacy of greater dosages of deltamethrin in *C. chinensis*, attributed to its quick knockdown effects on a range of insects, including coleopterans. In a study conducted by Paudiyal et al., (2016), it was observed that the mortality rates of adult *Tribolium castaneum*, *Sitophilus oryzae*, and *Rhyzopertha dominica* significantly increased when exposed to a high concentration of deltamethrin. Studies by Jacob et al., (2014) have reported 50% mortality at 250ppm after exposure to a commercial grade deltamethrin in adult *S. zeamais*, while in the present study 50% mortality was recorded on 22.93ppm. The high mortality at low concentration in the present study is possibly due to technical grade used and further confirms the sensitivity of technical grade deltamethrin against *C. chinensis* and thus more suitable for monitoring of insecticide resistance in stored grain pests (Gupta, 2019).

The persistence of insecticide effects from one generation to the next are unknown but have important consequences. The primary emphasis of research is often placed on intragenerational and intergenerational effects, rather than transgenerational consequences, as highlighted by Margus et al., (2019). The understanding of the potential transgenerational consequences resulting from exposure to insecticides is currently limited and lacks comprehensive knowledge. In the current study we explore the transgenerational effect of sub lethal dose of the deltamethrin on the developmental parameters *viz.* total egg count, total hatching, hatching %, total development period and adult longevity. The present study demonstrated that sub lethal concentrations *viz.* Low Concentration (1.15ppm) of LC_{50} (L_{Lc50}) and High Concentration (4.5ppm) of LC_{50} (H_{Lc50}) of deltamethrin when exposed to *C. chinensis*, it shows a significant negative effect on the development parameters of the initial generations with respect to the control. A more comprehensive analysis of the generational differences reveals a notable decline in the adverse impact of the insecticide on developmental parameters as subsequent generations were observed. This suggests that the transgenerational effect of deltamethrin exhibits a discernible trend wherein the insects gradually develop a greater tolerance towards it (Brevik et al., 2018).

The present study evaluated the transgenerational effects of the sublethal concentrations of deltamethrin on the overall egg count and hatching of *C. chinensis*. The findings of this study demonstrate that both concentrations of deltamethrin significantly reduced the total number of eggs and the hatching rate in the initial generation, as compared to the control group. However, in subsequent generations, the observed effects were not as pronounced and were almost found to be similar to that of the control group. Our results are in accordance with the existing literature, which has provided evidence that the egg laying and hatching of *Aphis gossypii* and *Plutella xylosteichlla*, are affected by the insecticides cycloxaprid and spinetoram (Qu et al., 2017; Xu et al., 2016; Tamilselvan et al., 2021); spinetoram inhibitory effect on the reproductive capacity of *Rhyzopertha dominica* (Fabricius), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), and *Sitophilus granaries* (Linnaeus) (Coleoptera: Curculionidae) (Vassilakos et al., 2012 and 2015; Rumbos et al., 2018); methylthio-diafenthiuron effects the reproductive capacity of F1 offspring of *P. xylostella* (Su and Xia, 2020); cyantraniliprole had adverse effects on the fecundity of *H. assulta* (Dong et al., 2017); flupyradifurone adversely impacted the reproductive capacity of *A. gossypii* (Liang et al., 2019) as well as studies by Ali et al., (2017) have significant reduction in the fecundity and hatchability for generations of *Sogatella furcifera* after buprofezin treatment.

According to the findings of our research, exposure to deltamethrin in the F1 generation has a negative impact on the immediate subsequent generation (F2). In this generation, the total development period was significantly increased, which indicates that the insect took a longer amount of time to develop. This was also reflected in the adult longevity, which significantly decreased. However, with time, the insect is exhibited tolerance to the insecticide in later generations (F5 & F6) where it was observed that the *C. chinensis* was able to develop in normal time and adult longevity was observed to be at par with control. The results are in agreement with the earlier work where, exposure of spinetoram has been known to prolong the developmental duration of F1 progeny of *P. xylostella* after exposure to F_0 generation with a parallel increase in the larval duration (Zhu et al., 2012; Guo et al., 2013; Xu et al., 2016) similarly sublethal concentration of Spinosad and chlorantraniliprole has been reported to significantly increase the duration of *P. xylostella* and *H. armigera* F1 generation (Yin et al., 2008; Zhang et al., 2013). The adult longevity of the F1 *C. chinensis* was significantly reduced compared to control after exposure of the sublethal doses of the deltamethrin, (Ali et al., 2017) reported that adult longevity significantly decreased for generations of *S. furcifera* after buprofezin exposure. Similarly, Deng et al., (2019) reported that the administration of sub lethal concentration of dinotefuran on *R. padi* adults of the F1 generation reduced adult longevity.

The number of adult emergences correlates with the number of eggs laid in each generation and is dependent on the oviposition. The susceptibility index exhibited significant correlations with variables including the number of progenies produced in each generation and the total duration of the development phase. In the present study the susceptibility index was performed to know the effect of deltamethrin at transgenerational level. The decreased population size observed in the F1 generation suggests that deltamethrin has a pronounced impact on initial generations, resulting in a reduced number of offspring. Conversely, the increased population size observed in the F5 and F6 generations indicates a higher number of progenies in these generations. This trend in population growth suggests that the susceptibility index is higher, indicating a reduction in the effectiveness of deltamethrin and an increased sensitivity of *C. chinensis*. One approach to examine the resistance from the insecticide is to understand the effect of insecticide on the developmental parameters and repellency. These may be linked to the resistance or susceptibility of insects to insecticides and might potentially influence the interaction between deltamethrin and insects. The F5 and F6 generations had the greatest level of susceptibility, whereas the F1 generation showed the lowest level of susceptibility (Ngom, 2021; Tenrirawe et al., 2023).

The extent to which the repellency of *C. chinensis* is influenced by the persistence of insecticide effects through successive generations is uncertain, however it has significant implications. The understanding of the potential transgenerational consequences resulting from exposure to insecticide is currently limited and lacks comprehensive knowledge of the mechanisms involved. Based on the current literature, there appears to be a noticeable gap in the research pertaining to the transgenerational repellency effects of insecticide on the store grain pests. The currents study is 1st of its kind which reports deltamethrin induced transgenerational effect on the repellency of *C. chinensis.* The findings of this study indicate that the performance of deltamethrin as a repellent exhibited variability in relation to both time and generation. The degree of repellency diminishes as the time of exposure lengthens, and this pattern persists in following generations. In a study conducted by Muntaha et al., (2017), the repellent properties of pyrethroids on *C. chinensis* were investigated. The results indicated that deltamethrin exhibited the highest repellency at 92%, followed by cypermethrin at 90% and bifenthrin at 89%. In the current study, it was found that the initial generation exhibited a repellency rate of 85%, even after a 24-hour period. This observation implies that the insects may possess limited tolerance towards the insecticide. However, in subsequent generations, a declining trend in the repellency and was at a rate of 35% observed in the F_6 generation. The aforementioned finding indicates that the *C. chinensis* have gradually acquired an enhanced capacity to withstand the effects of the deltamethrin. However, when insects are exposed to sublethal concentrations of insecticide over multiple generations, it has been observed that they may develop resistance (Deng et al., 2019).

2.5 Conclusion

The transgenerational effects of deltamethrin suggests that sublethal concentration effects has influenced the development of *C. chinensis*, where significant decrease in the egg count, hatching rate, with prolonged the development period, as well as shortening of the longevity was observed in adults of the F1 generation. However, in subsequent generations, the insects were found to have developed tolerance and were able to overcome these effects. Consequently, in successive generation pronounced repellency was noted. Furthermore, it is observed that the repellency was dependent upon both time and generation i.e., with an increase in time and generation leading to a decrease in repellency. The cumulation of all the findings have suggested a strong link between the resistance in F6 generation, hence to confirm the findings comparative transcriptome analysis between the treatment and control group will elucidated to understand the mechanism of resistance and will open up new avenues for target-based research for developing new generation insecticides.