Background

Annually, over 2 billion tonnes of grains are cultivated for sustenance and animal feed, making up approximately two-thirds of protein consumption from direct and indirect sources (Erenstein et al., 2022; Tiwari et al., 2022). Agriculture is the primary livelihood for 58% of India's population. In the 2019-20 crop year, a record-breaking food grain production of 295.67 million tonnes was expected. In the 2020-21 fiscal year, the Indian government aimed to achieve a food grain production of 300 million tonnes (APEDA, Union Budget 2020-21). For the 2022-23 crop year (July to June), India has set a target of 328 million tonnes, a 4% increase from the previous year's record of 315.7 million tonnes. However, the Food and Agriculture Organization (FAO) has cautioned that while there is potential to feed the global population with available land resources, much of this land is only suitable for limited crops. Food grains and pulses are crucial for addressing food insecurity, especially in tropical and sub-tropical regions.

In many countries, including India, substantial post-harvest grain losses occur, with 15% of food grains lost during or after harvest (Parfitt et al., 2010). The FAO assessed post-harvest grain loss in India at 40%, while post-harvest cereal loss was reported as 30% by the National Academy of Agricultural Sciences in the 2019 report "Saving the Harvest: Reducing Food Loss and Waste." Singh (2010) noted annual monetary losses exceeding Rs. 50,000 crores due to these losses. According to the Associated Chambers of Commerce of India, a substantial amount of food valued at 92,651 crore rupees is wasted during post-harvest operations before reaching consumers (PIB, February 2016). A nationwide study by Jha et al. (2016) found losses in various crop categories, including cereals (3.9% to 6%), pulses (4.3% to 6.1%), oilseeds (2.8% to 10.1%), fruits (5.8% to 18.1%), and vegetables (6.9% to 13%), occurring during harvesting, post-harvest activities, handling, and storage. These losses pose a substantial challenge to food security and sustainability.

Insect pests are recognized as the most significant biotic agents, causing substantial losses estimated at around 30-40% (Abass et al., 2014; Kumar and Kalita, 2017; Mesterházy et al., 2020). They play a major role in the degradation of stored food and agricultural commodities, resulting in annual losses estimated to range from 15% to 25% of the stored grain (Adu et al., 2014; Nayak and Solanki, 2021; Tanda et al.,

2022). Insects are particularly destructive due to their rapid reproductive rates and short generation periods. This issue not only involves the physical matter consumed by insects but also the food rendered unsuitable for human consumption due to contamination. There are around 600 insect species commonly associated with stored grain products, and approximately 100 kinds of insect pests are responsible for significant economic losses in stored products, according to the International Grain Research Institute (IGMRI, 2019).

Several stored grain insect pests, including *T. granarium, R. dominica, S. oryzae, S. cerealella, T. castaneum* and *C. chinensis*, have a significant detrimental impact, leading to annual losses of 5-10% or more. These losses are often attributed to inappropriate insecticide application (Ramzan et al., 2019). Research efforts have primarily focused on strategies to reduce losses, control methods that are cost-effective and integrated with production/marketing systems, and adaptive research evaluating the bio-efficacy of various plant products. Integrated pest management, combining various control techniques, has become a focus for research on stored products. Laboratory studies on pest biology have also been conducted intensively (Babendreier et al., 2020). A range of pest management strategies encompassing physical, mechanical, biological, and chemical approaches are accessible. Chemical-based methods such as fumigation, grain protectants, and aerosols continue to be the predominant grain management solutions. Insecticides are commonly used to control stored grain pests like beetles, weevils, and moths, which pose a significant threat to stored grains. These insecticides are designed to eliminate or hinder pest growth and reproduction. However, the use of insecticides can lead to unintended consequences, including transgenerational effects on the pests.

Numerous studies have investigated the transgenerational effects of insecticides on stored grain pests, given the importance of managing these pests to safeguard stored food commodities. Morrison et al. (2018) studied the transgenerational effects of deltamethrin on *R. dominica* and observed that deltamethrin exposure influenced the development, survival, and reproductive parameters of subsequent generations of the lesser grain borer. Nath et al. (2023) studied the transgenerational effects of phosphine, a commonly used fumigant, on three stored-product insects *T. castaneum, R. dominica* and *S. oryzae.* Their results showed that phosphine exposure influenced

the reproductive parameters and body weight of subsequent generations of these pests. These studies underscore that insecticide exposure can induce transgenerational effects in insects, impacting their fitness, reproductive success, development, and population dynamics. It's important to note that specific effects can vary depending on insect species, insecticide formulation, dosage, exposure duration, and other factors.

Over recent years, there has been a documented increase in insect species developing resistance to insecticides, with a total of 504 species showing resistance. This resistance extends to multiple classes of chemical compounds, including DDT, malathion, pirimiphos-methyl, deltamethrin, and permethrin. Insects have demonstrated adaptability through the development of physiological and behavioural resistance mechanisms. This issue of pesticide resistance in postharvest ecosystems is a significant concern, as it threatens the effectiveness of grain protectants and fumigants in preserving stored food (Karaağaç, 2012; Dara, 2013 and 2016; Zhu et al., 2016; Kortbeek et al., 2019; Jallow et al., 2017; Nansen et al., 2016; Dara, 2017; Hagstrum and Phillips, 2017; Jian 2019; Bajaracharya et al., 2013; Nguyen et al., 2015; Nayak et al., 2020).

Understanding the molecular mechanisms of resistance is crucial for sustainable control and resistance management (Le Goff and Nauen, 2021). Diagnostic techniques are essential for assessing pest vulnerability and guiding chemical agent selection. Bioassays are commonly used for resistance monitoring, but molecular markers linked to resistance are emerging. Genomic advances, including transcriptome and whole-genome sequencing, have expanded our understanding of resistance mechanisms, including metabolic resistance, penetration resistance, and knockdown resistance. Microbiome research investigates the role of bacteria, viruses, and fungi within insect hosts in mitigating the effects of insecticides (Dada et al., 2018; Shamjana and Grace, 2021; Wang et al., 2021). Functional genomics tools like CRISPR/Cas9 allow the study of resistance mechanisms in terms of multiple gene interactions. Resistance mechanisms can involve changes in target sites and enhanced detoxification. The genetic basis of resistance can vary between insect populations and even within populations, underscoring the need to understand the molecular basis of resistance for developing new pesticides and integrated pest management techniques (Wei et al., 2019; Zhang and Zhang, 2019).

The lab's previous work has made significant progress in understanding the mechanisms of insecticide toxicity in various insects and insect cell lines. They have successfully developed primary cell lines from stored grain pests like Sitophilus oryzae to assess pesticide toxicity (Thakkar et al., 2020). However, there is a gap in the existing knowledge, as the molecular mechanism for pesticide resistance in the stored grain pest *Callosobruchus chinensis* remains unexplored, highlighting the need for further research in this area.

Therefore, the present inventory was designed to understand the molecular mechanisms of insecticide resistance in stored grain pests (C. chinensis).

Chapter 1: Rearing and Host Preference of Callosobruchus chinensis (Coleoptera: Bruchidae) in the laboratory conditions

Pulses are a highly nutritious food source, rich in protein, carbohydrates, and dietary fiber, and are known for their valuable bioactive constituents. They are widely consumed globally and include various types of dried seeds like beans, lentils, peas, green gram, black gram, horse gram, and chickpeas (Maphosa and Jideani, 2017; Venkidasamy et al., 2019; Hussain et al., 2021). Losses during harvesting, threshing, and storage at farms and processing units are substantial and are primarily attributed to inappropriate and delayed harvesting and poor post-harvest management (Tibagonzeka et al., 2018; Vishwakarma et al., 2019 and 2020). Threshing and harvesting contribute significantly to total losses on farms, often due to the use of unsuitable machinery. Additionally, unscientific storage practices at households, farms, and warehouses further contribute to these losses (Tibagonzeka et al., 2018; Vishwakarma et al., 2019 and 2020). At the storage level, pulses are susceptible to degradation from various microbial and abiotic factors. Collectively, these factors result in a reduction of 25% in food grain production. Influential factors include high temperatures, moisture, microbes, mites, insects, and rodents (Singh et al., 2018; Mundhada et al., 2022; Sharma et al., 2023).

Regions with tropical and subtropical climates experience the greatest losses due to favourable conditions for rapid proliferation and production of organisms that harm food supplies. Storage insect pests, for instance, are responsible for approximately 30% of annual losses (Abass et al., 2013; Adu et al., 2014; Kumar and Kalita, 2017;

Mohapatra et al., 2017; Delouche et al., 2021). Various chemical and biological control measures have been implemented to combat *C. chinensis* (Lal et al., 2017; Liu et al., 2020; Dent and Binks, 2020; Takla et al., 2021; Pipariya et al., 2022). However, excessive pesticide use has led to resistance and increased survival rates among these pests (Daglish et al., 2014; Dara 2017; Fang et al., 2019; Kortbeek et al., 2019).

To address these challenges, the development of insect-specific insecticides is essential. Mass rearing and maintaining pure pest breeds under controlled conditions are crucial for assessing the efficacy of such insecticides and reducing stochastic variation. Understanding the dynamics of the insect's life cycle is integral to successful pest monitoring and management (Merville et al., 2014; Ribeiro et al., 2018; Arai et al., 2022). Host preference is a significant aspect of *C. chinensis* life history. The suitability of the host grain is vital for the entire life cycle, including mating, oviposition, and larval feeding. Numerous studies have explored the life cycle, host preference, and host suitability of *C. chinensis* on various pulse crops, including chickpeas, green gram, black gram, cowpea, moth bean, and multiple hosts (Chandel and Bhaudaria, 2015; Rana et al., 2020; Devi and Devi, 2014; Kumari et al., 2020; Gopi and Singh 2020; Dalal et al., 2020; Augustine and Balikai, 2018; Meghwal and Singh 2005; Patel et al., 2005; Hosamani et al., 2018; Jaiswal et al., 2019; Mehta and Negi, 2020).

Hence, the first objective was undertaken to investigate a suitable rearing protocol, life cycle as well as screening the preference of different stored pulses by C. chinensis in control laboratory conditions.

Infested grains from Vadodara were collected and insect pests like *T. castaneum, S. Oryzae, T. granarium* and *C. maculatus* were observed. *C. chinensis* was selected as the insect model for further study due to its dominance on various host grains. The Pulse Beetle, *C. chinensis*, was studied under laboratory conditions at The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat.

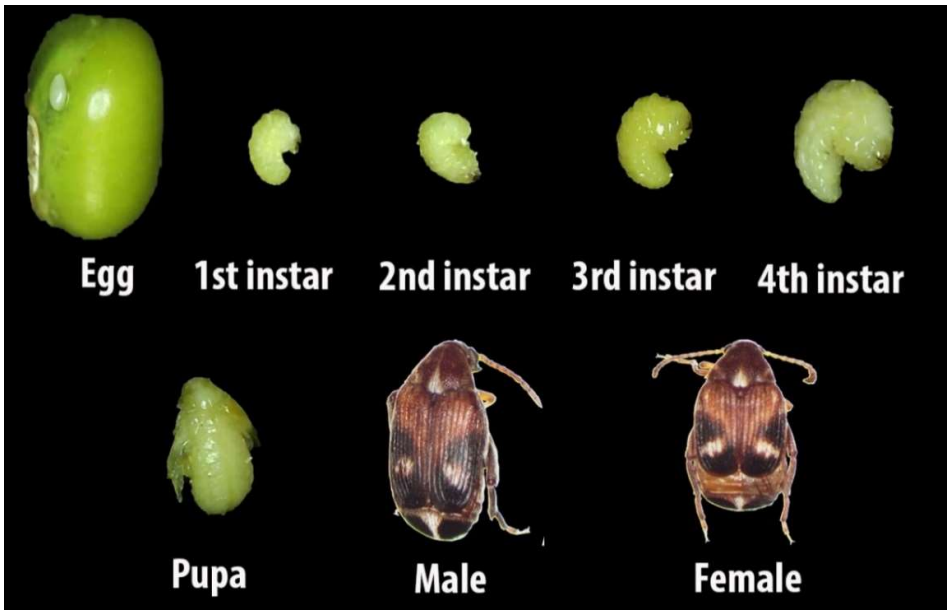

Figure: 1 Depicts the life cycle stages of *C. chinensis*

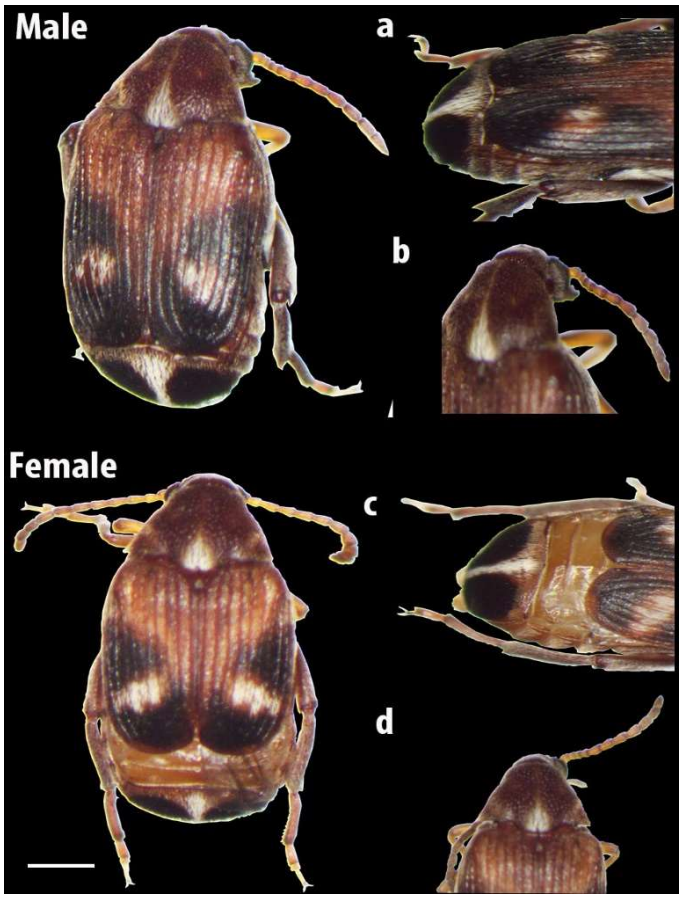

Figure: 2: Depicts the morphological characters of *C. chinensis* (a. male abdomen, b. male antenna; c. female abdomen, d. female antenna)

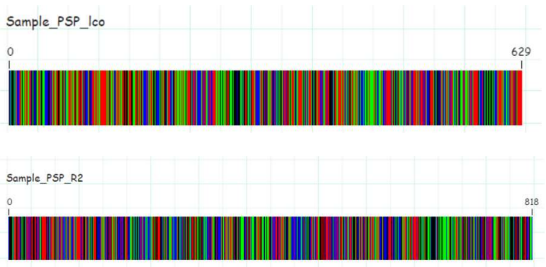

Figure 3: Depicts the barcode of the sequence (Molecular identification).

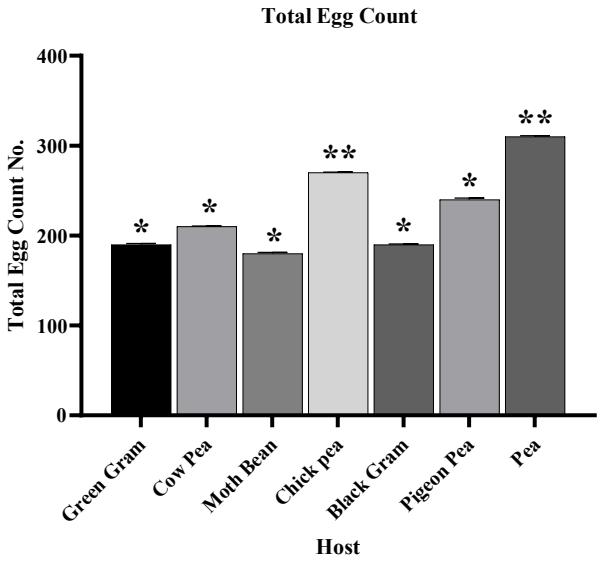

Figure:4: Total egg count of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

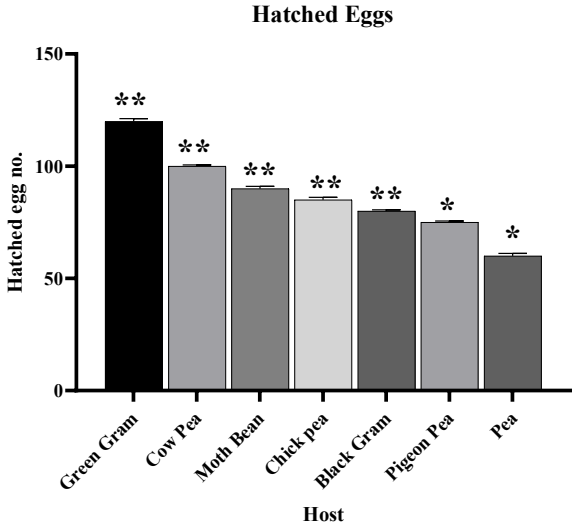

Figure:5 Total hatched eggs of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

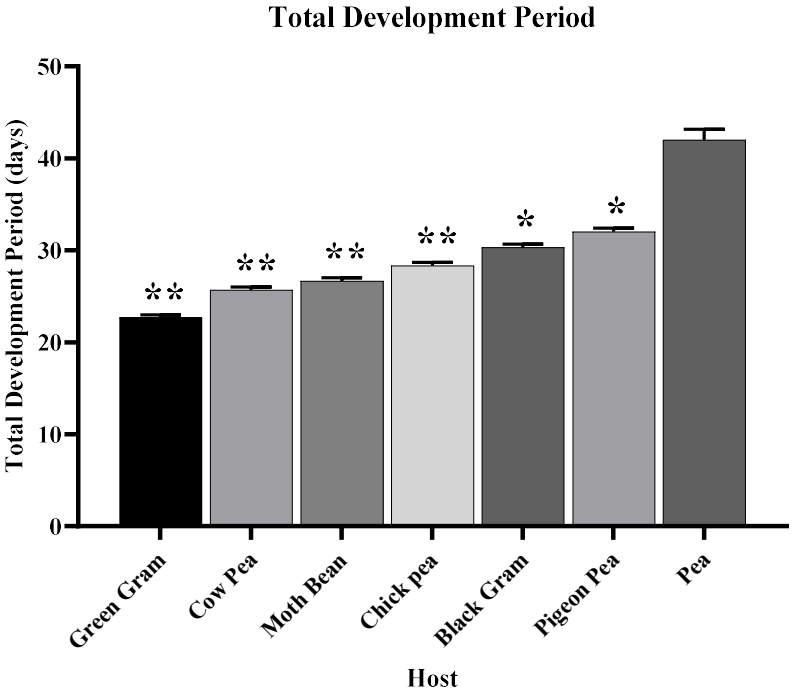

Figure:6: Total development period of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

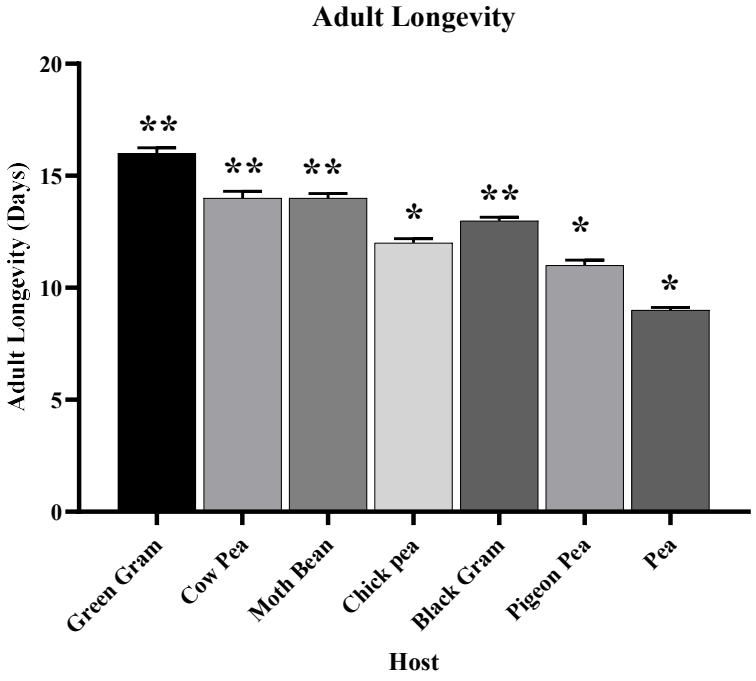

Figure:7: Adult longevity of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

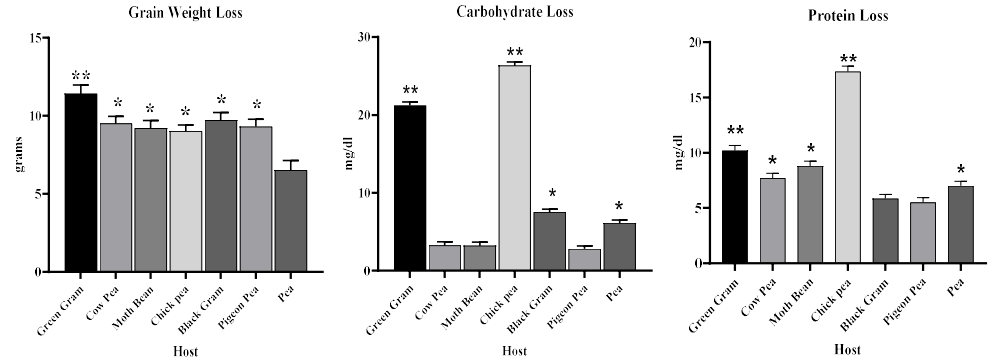

Figure8: Quantity and Quality losses by *C. chinensis* on different host (Grain Weight loss, Carbohydrate loss, Protein loss). Significant level *($p<0.05$); **($p<0.01$)

A crucial aspect of effective pest management is understanding the interaction between hosts and pests, particularly when dealing with a polyphagous pest like *C. chinensis*. The type of host significantly influences the pest's life cycle due to variations in seed texture, size, hardness, and nutritional composition (Singh et al., 2013; Mason et al., 2016; Sewsaran et al., 2019).

Previous studies have also highlighted the influence of seed characteristics on oviposition activity, with grain hardness, length, and width affecting the pest's choice of host (Padmasri et al., 2017; Adebayo and Ogunleke, 2016). The results of this study align with earlier reports (Jaiswal et al., 2019; Bidar et al., 2020; Nisar et al., 2021). While the highest number of eggs was laid on peas due to their large surface area, the highest hatching success was observed on green gram, which was also reported in previous studies (Sharma et al., 2016; Nisar et al., 2021). The hatching success appears to be influenced by the softness and hardness of the grains, with soft-coated grains like green gram allowing easy penetration by the 1st instar larvae. *C. chinensis* spends a considerable part of its life inside the grain kernel, affecting the endosperm amount (Keskin and Ozkaya, 2015; Thakkar and Parikh, 2018).

Regarding the incubation period, it ranged from 3 to 6 days, with the shortest incubation period observed on green gram and the longest on peas. This variation is likely due to feeding responses, nutritional factors, and physical properties of the host (Naseri and Shadi, 2022). These findings are consistent with previous research conducted on *C. chinensis* (Hosamani et al., 2018; Jaiswal et al., 2019; Dalal et al., 2020; Satish et al., 2020; Sekender et al., 2020). The development periods also varied

among different hosts, with the longest period recorded on peas, followed by pigeon peas, and the shortest on green gram, followed by cowpea. This variation in developmental periods is attributed to the nutritional composition of the host, with green gram providing a more favourable nutritional content. These findings are consistent with previous studies (Fabres et al., 2014; Hosamani et al., 2018; Jaiswal et al., 2019; Nisar et al., 2021; Bidar et al., 2022).

The study investigated adult emergence, longevity, and weight loss of *C. chinensis* on different host grains. Maximum adult emergence was observed in green gram, followed by cowpea, with the least emergence in pea and black gram. The lower emergence in pea and black gram may be attributed to the hard seed coat, which can reduce egg hatchability, as reported in previous studies (Padmasri et al., 2017). The chemical composition of the seeds plays a vital role in the adaptation of host-host relationships (Soumia et al., 2017; Kébé et al., 2020). The observations on adult longevity of *C. chinensis* correlate with previous findings by Hosamani et al. (2018) and Mehta and Negi (2020). Host significantly influenced adult longevity, with the longest recorded on green gram, likely due to easy penetration and high hatchability. Cowpea followed green gram in longevity, attributed to its larger surface area and curvature. Pea and pigeon pea had the shortest adult longevity, consistent with earlier studies (Nisar et al., 2021; Bidar et al., 2021).

The study also examined the weight loss caused by *C. chinensis* infestation on different host grains. Maximum weight loss occurred in green gram, while the least weight loss was recorded in peas. *C. chinensis* infestations lead to deterioration, reducing moisture content, and affecting food quality and calorie supply. Losses in nutritional values are primarily attributed to stored insect pests, which preferentially feed on grain embryos. Pulses are a significant source of proteins and carbohydrates. The infested grains exhibited a significant decrease in protein and carbohydrate content, with severity of infestation negatively correlated with nutrient content. The observed reduction in protein and carbohydrate content can be attributed to the feeding activities of *C. chinensis* larvae and the measures taken to remove eggs, egg cases, excretory products, and life stages of the pest before analysis. These findings align with previous research by Bamaiyi et al. (2006) and highlight the impact of *C. chinensis* infestation on grain quality and nutritional content. The study reveals that *C.*

chinensis host preference affects hatching percentage, adult emergence, longevity, and weight loss. Host size, texture, and surface area affect offspring survival and oviposition. Understanding *C. chinensis* biology is crucial for finding alternative grain protection methods and assessing resistance mechanisms to insecticide exposure.

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

Transgenerational effects refer to the phenomenon in which environmental exposures or experiences impact not only the individuals immediately exposed but also their offspring and subsequent generations (Brevik et al., 2018). These effects can result in various outcomes, including altered physical traits, physiological changes, behavioural adjustments, and increased vulnerability to diseases or stressors (Xin et al., 2015; Castano-Sanz et al., 2022; Tamagno et al., 2023). Transgenerational effects are brought about by diverse mechanisms, such as epigenetic modifications, changes in gene expression, alterations in germ cell development, or the 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 when there is no ongoing exposure to the original environmental stressor (Ayyanath et al., 2013; Fitz-James et al., 2022).

In stored grain management, insecticides are commonly used to control pests such as beetles, weevils, and moths, which can jeopardize the quality of stored grains (Costa et al., 2023). However, the use of insecticides can lead to unintended transgenerational effects on these pests, causing persistent changes in their phenotype and physiology across generations (Hanson and Skinner, 2016). These effects are not fully understood but may involve epigenetic modifications, which can impact gene expression without altering the DNA sequence (Hu et al., 2020; Pompermaier et al., 2022). Such alterations can influence various biological processes, including development, metabolism, reproduction, and responses to stress or toxins (Wang et al., 2022; Wu et al., 2022).

Pyrethroids, known for their neurotoxic effects, have been found to affect larval development, adult body size, and reproductive parameters in subsequent generations. Deltamethrin, a neurotoxic insecticide, has been found to have negative effects on

insect development in subsequent generations (Yang et al., 2018 and Kumar et al. 2023). These effects can be carried over to future generations, potentially impacting the pests' population dynamics. Additionally, exposure to insecticides can result in increased resistance or tolerance due to genetic changes or adaptations over time (Huang et al., 2022).

Understanding the mechanisms underlying insecticide-induced transgenerational changes holds vital implications for insecticide risk assessment, pest management strategies, and the development of sustainable practices for pest control. Moreover, further research is required to unravel the precise mechanisms involved and assess the ecological consequences of these transgenerational effects on insect populations and ecosystems. Insects present favourable characteristics as model organisms for studying transgenerational effects due to their short generational cycles and the ease of maintaining substantial populations within controlled laboratory settings.

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

A survey was conducted in insecticide shops and ware houses to explore various insecticides, including technical grade deltamethrin. Five concentrations of deltamethrin were tested against *C. chinensis*, and mortality percentages were recorded after 24 hours, 48 hours, 72 hours, and 96 hours of treatment. Probit analysis was performed to obtain the LC_{50} value. The experiment involved three groups: Control, Low Concentration (L_{Lc50}) and High Concentration (H_{Lc50}) groups.

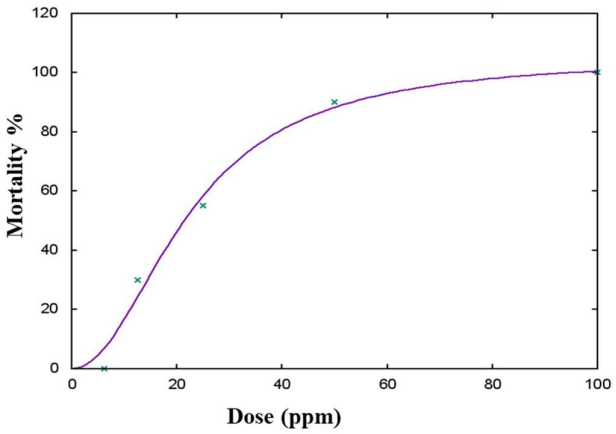

Figure: 9: Dose response curve for the LC_{50}

Table 1: 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

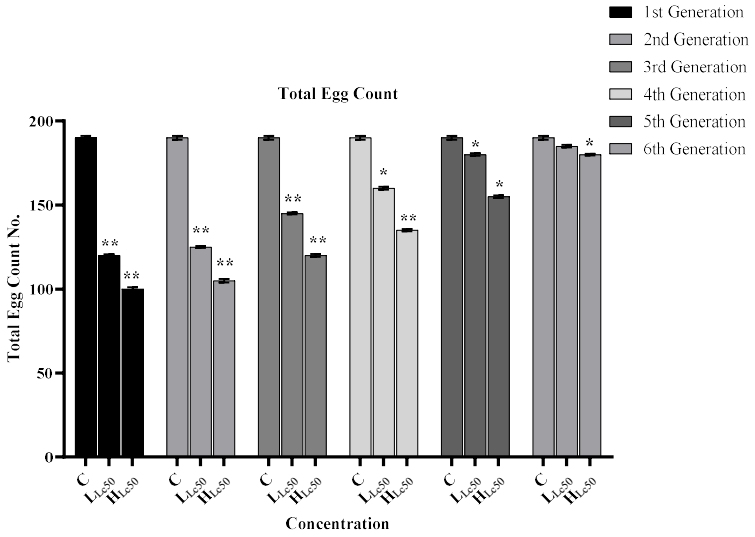

Figure 10: Transgenerational effect of deltamethrin on the total egg count of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

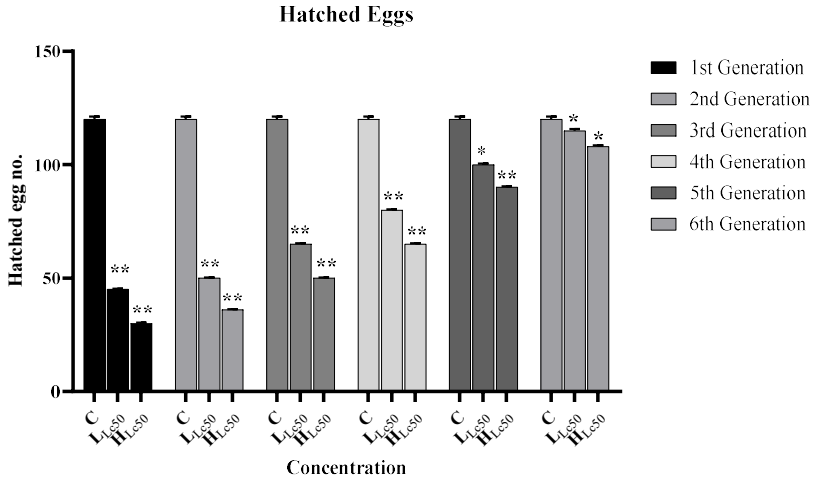

Figure: 11: Transgenerational effect of deltamethrin on the total hatching of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

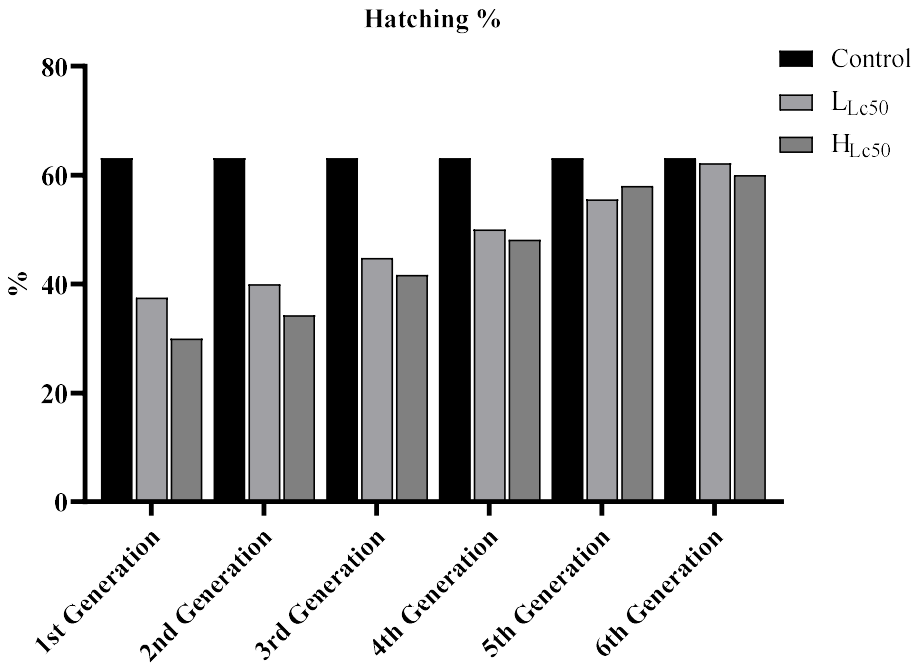

Figure: 12: Transgenerational effect of deltamethrin on the hatching% of *C. chinensis.* Significant level *(p<0.05); **(p<0.01)

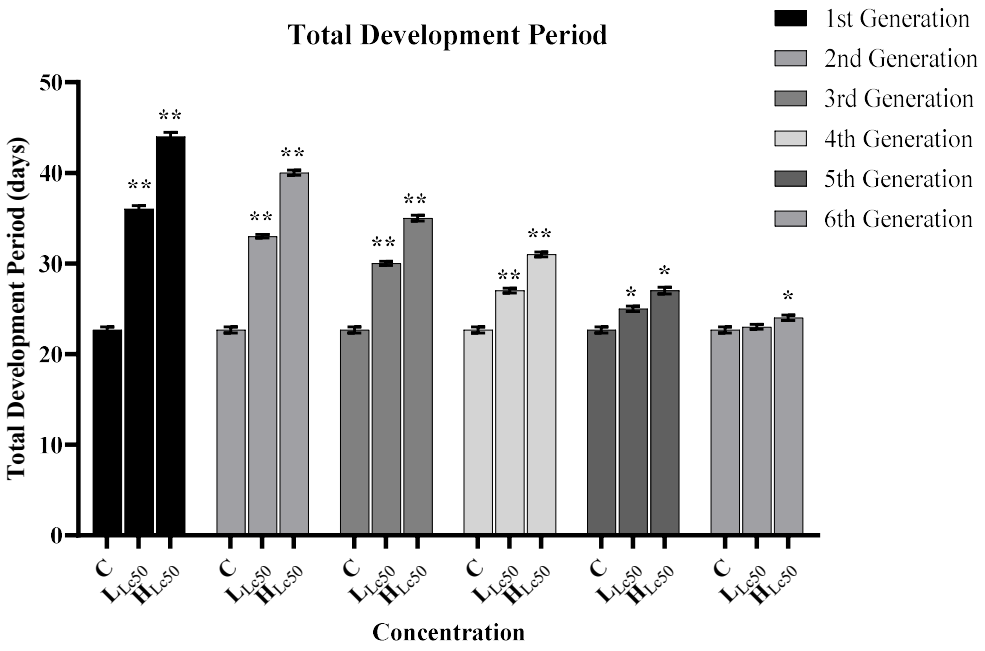

Figure: 13: Transgenerational effect of deltamethrin on the total development period of *C. chinensis.* Significant level *(p<0.05); **(p<0.01).

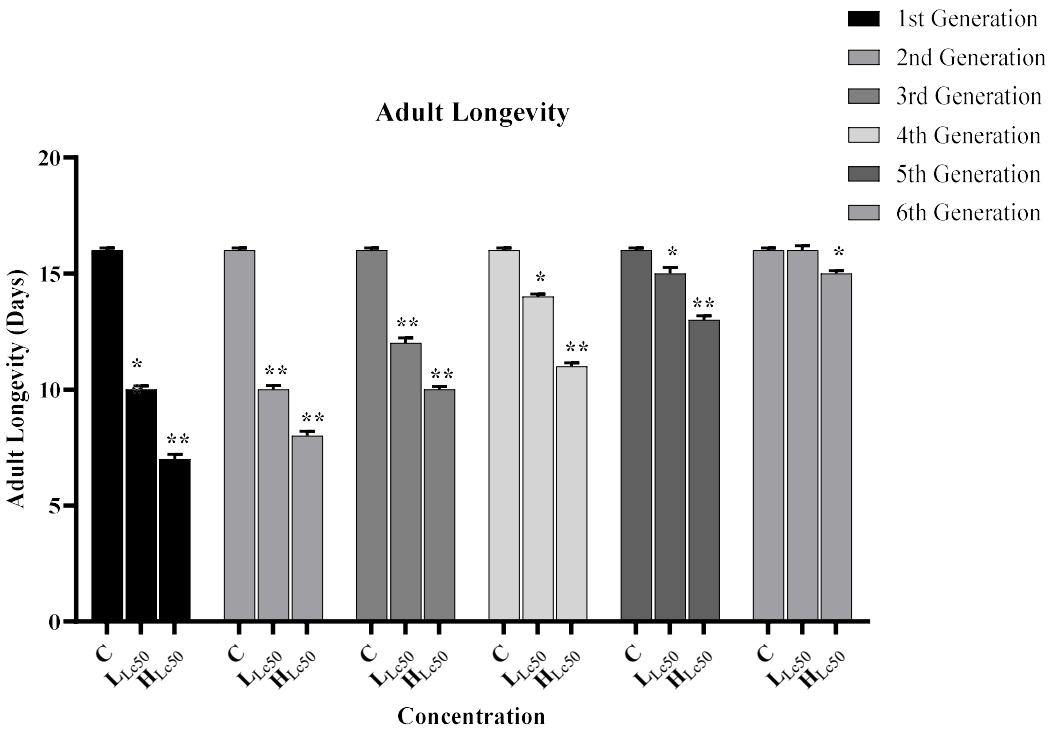

Figure: 14: Transgenerational effect of deltamethrin on the adult longevity of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

Table 2: Transgenerational alteration in the Susceptibility index of *C. chinensis.*

Susceptibility Index			
Generations	**Control**	**Low Concentration**	**High Concentration**
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

Table: 3: 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 4: 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 5: 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 6: 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

Deltamethrin is a synthetic pyrethroid insecticide designed to replicate the properties of naturally occurring pyrethrins derived from Chrysanthemum flowers. It effectively paralyzes insects' nervous systems, causing rapid incapacitation and mortality by altering nerve membrane ion permeability (Velki et al., 2014; Paudiyal et al., 2016 & 2017). This study aimed to demonstrate the effectiveness of higher deltamethrin dosages on various insect species, including *C. chinensis*, with observations showing increased mortality at high concentrations (Paudiyal et al., 2016). Notably, the present study recorded a 50% mortality rate at 22.9ppm, a lower concentration compared to other studies due to the use of technical grade deltamethrin (Gupta, 2019).

Research on insecticide effects often focuses on intragenerational and intergenerational impacts, but transgenerational consequences remain poorly understood (Margus et al., 2019). The current study investigated the transgenerational effects of sub-lethal doses of deltamethrin on *C. chinensis*, assessing parameters like egg count, hatching rate, development period, and adult longevity. It found that low (1.15ppm) and high (4.5ppm) concentrations of LC_{50} had significant negative effects on the initial generations compared to the control. However, subsequent generations showed a reduced impact, indicating an increased tolerance to deltamethrin over generations (Brevik et al., 2018).

The present study assessed the transgenerational effects of sublethal concentrations of deltamethrin on egg count and hatching in *C. chinensis*. In the initial generation (F1), both deltamethrin concentrations significantly reduced egg count and hatching rates compared to the control group. However, subsequent generations (F2 onward) showed less pronounced effects, eventually resembling the control group. This pattern aligns with existing literature demonstrating the impact of various insecticides on reproduction in different species, such as cycloxaprid and spinetoram on *Aphis gossypii* and *Plutella xylostella* (Qu et al., 2017; Xu et al., 2019; Tamilselvan et al., 2021); spinetoram on *R. dominica, P. truncatus* and *S. granaries* (Vassilakos et al., 2012 & 2015; Rumbos et al., 2018); methylthio-diafenthiuron on *P. xylostella* (Su and Xia 2020); cyantraniliprole on *H. assulta* (Dong et al., 2017); and flupyradifurone on *A. gossypii* (Liang et al., 2019). Furthermore, Ali et al. (2017) reported reduced fecundity and hatchability in generations of *S. furcifera* after buprofezin treatment.

In the F1 generation, exposure to deltamethrin extended the total development period and decreased adult longevity, suggesting delayed development and shorter lifespan. However, over subsequent generations (F5, and F6), the insects developed tolerance to the insecticide, returning to normal development times and adult longevity comparable to the control group. This trend is consistent with previous research, as exposure to spinetoram has been found to prolong the developmental duration of *P. xylostella* F1 progeny (Lai and Su 2011; Zhu et al. 2012; Guo et al. 2013; Xu et al. 2016). Sublethal concentrations of Spinosad and chlorantraniliprole have also increased the duration of *P. xylostella* and *H. armigera*'s F1 generation (Yin et al. 2008; Zhang et al. 2013). Similarly, adult longevity in F1 *C. chinensis* significantly

decreased after exposure to sublethal doses of deltamethrin, consistent with the findings of Ali et al. (2017) for *S. furcifera* after buprofezin exposure, and Deng et al. (2019) for *R. padi* adults of the F1 generation after dinotefuran exposure.

The study investigated the transgenerational effects of deltamethrin on *C. chinensis*, focusing on the susceptibility index, which correlated with egg production and development duration. In the F1 generation, there was a decrease in population size, indicating a pronounced impact of deltamethrin and reduced offspring. In contrast, the F5 and F6 generations showed an increased population size, suggesting higher susceptibility, reduced deltamethrin effectiveness, and increased sensitivity of *C. chinensis*. This trend reflects changes in susceptibility over generations. Understanding these effects on developmental parameters and repellency is crucial for assessing resistance or susceptibility to insecticides. The F1 generation exhibited the lowest susceptibility, while the F5 and F6 generations had the highest (Ngom 2021; Tenrirawe et al., 2023).

The impact of insecticide persistence on the repellency of *C. chinensis* across generations remains uncertain and significant. Understanding the transgenerational consequences of insecticide exposure is limited, with a gap in research on transgenerational repellency effects in stored grain pests. This study is the first of its kind to report deltamethrin-induced transgenerational effects on *C. chinensis* repellency. The findings reveal that deltamethrin's repellent performance varies with time and generation. Repellency diminishes as exposure time lengthens, continuing across subsequent generations. In comparison, Muntaha et al. (2017) found deltamethrin to exhibit high repellency, at 85% in the initial generation, suggesting limited insect tolerance. However, in later generations, a declining trend in repellency is observed, reaching 35% in the F6 generation, indicating an increased capacity in *C. chinensis* to withstand deltamethrin effects. It is important to note that when insects are exposed to sublethal insecticide concentrations over multiple generations, they may develop resistance (Deng et al., 2019).

The transgenerational effects of sublethal deltamethrin exposure on *C. chinensis* initially caused a decrease in egg count, hatching rate, longer development time, and shorter adult longevity in the F1 generation. Subsequent generations, however, exhibited increased tolerance, and pronounced repellency was noted. Repellency

depended on both time and generation, with decreasing repellency as time and generations increased. The findings suggest a strong link to resistance in the F6 generation. To further understand this resistance, comparative transcriptome analysis between the treatment and control groups will be conducted, opening up new avenues for target-based research in developing next-generation insecticides.

Chapter 3: Understanding the mechanism of insecticide resistance in Callosobruchus chinensis via transcriptomic approach

Recent research has demonstrated that sublethal concentrations of insecticides can have profound effects on insect reproduction, development, and chemical susceptibility, potentially leading to pest resurgence (Zhou et al., 2017). Insects employ a three-phase detoxification process: phase I, phase II (involving metabolizing enzymes), and phase III (involving transporters) (Xu et al., 2005). Key enzymes involved in phases I and II of detoxification are P450 monooxygenase, glutathione S-transferase (GST), and carboxylesterase (CarE) (Xiao et al., 2018), while phase III primarily relies on ATP-binding cassette (ABC) transporters (Ferreira et al., 2014). Detoxifying enzymes such as CarE, GST, and MFO (mixed-function oxidases) are crucial components of insect resistance mechanisms, and their activity needs to increase during insecticide metabolism (Qi et al., 2016). Previous studies have shown that sublethal exposure to abamectin led to elevated levels of GST and MFO activity in the pea aphid *Acyrthosiphon pisum* (Wang and Liu, 2014). Similarly, the activities of CarE, GST, and MFO increased significantly in *Tetranychus urticae* after exposure to abamectin (Ru et al., 2017). In the case of *Sogatella furcifera* exposed to avermectin, there was a significant induction of CarE, GST, and MFO activities at different time intervals (6, 12, and 24 hours), indicating that insects can adapt to the stress caused by avermectin by activating their detoxifying enzymes.

In recent years, advancements in sequencing technologies, particularly in increased sequence reading length, and the development of de novo transcriptome assembly software tools have allowed for the assembly of transcriptomes without relying on a reference genome. This approach has been applied to the de novo assembly of transcriptomes in several beetle species. In the context of this study, Illumina paired-end sequencing was employed to sequence samples from *C. maculatus* larvae, pupae,

and adults, with subsequent assembly of the sequences using Trinity, a de novo assembly software.

It's worth noting that the transcriptome of *C. maculatus* has been previously sequenced and made available (Sayadi et al., 2016). This resource facilitates gene expression analysis and the functional characterization of genes involved in various biological processes at a molecular level in *C. maculatus*. Investigating the molecular mechanisms associated with insecticide resistance, particularly those related to metabolic processes, provides valuable insights into the genetic, physiological, and biochemical changes that occur in insects. This research not only enhances our understanding of how insects develop resistance to insecticides but also opens doors to improving pest control strategies.

Therefore, this objective aimed to explore the molecular mechanism of insecticide resistance through a transcriptomic approach.

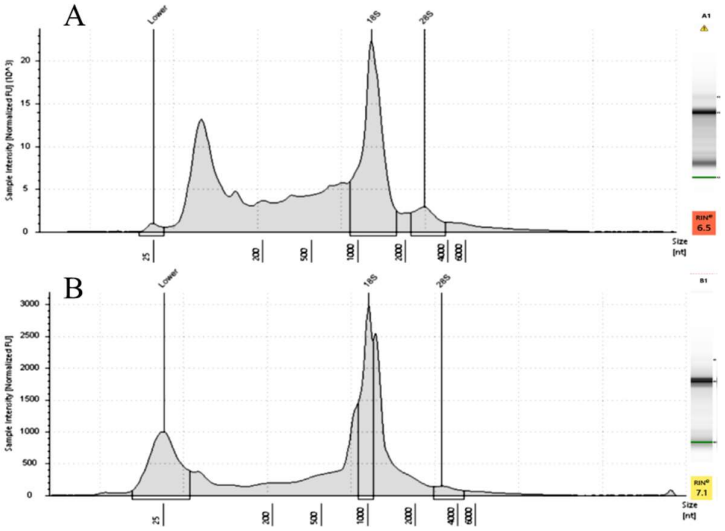

Figure 15: Depicts QC of Extracted RNA Samples on Agilent TapeStation

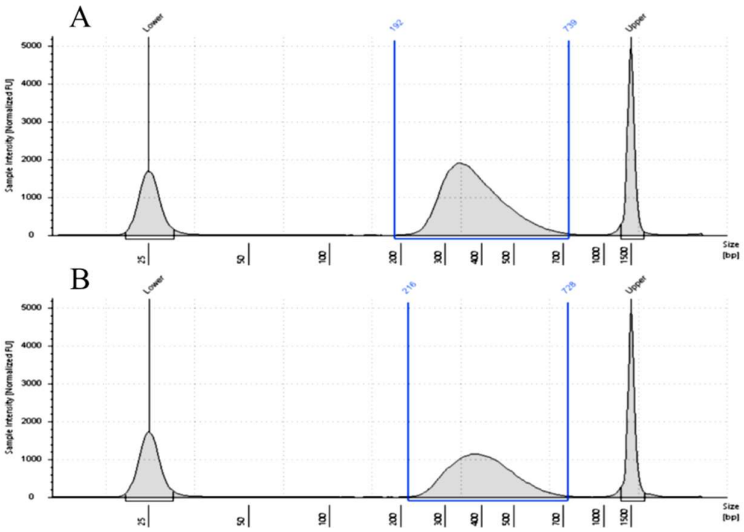

Figure 16: Depicts Library Profile of Samples: Control and Treated on Agilent TapeStation

Table 7: Transcript (Pooled) summary

Description	**Transcripts**
No. of Transcripts	58,120
Total transcript length (bp)	64,282,882
N50 (bp)	1,760
Length of the longest transcript (bp)	20,096
Length of the shortest transcript (bp)	301
Mean transcript length (bp)	1,106

Table 8: CDS (Pooled) Statistics

Description	**CDS**
No. of CDS	13,614
Total CDS length (bp)	16,206,501
Length of the longest CDS (bp)	17,022
Length of the shortest CDS (bp)	255
Mean CDS length (bp)	1,190

Table 9: BlastX Data Distribution Statistics

Sample Name	Total no. of CDS	No. of CDS with Blast Hit	No. of CDS without Blast Hit
Pooled CDS	13,614	12,629	985

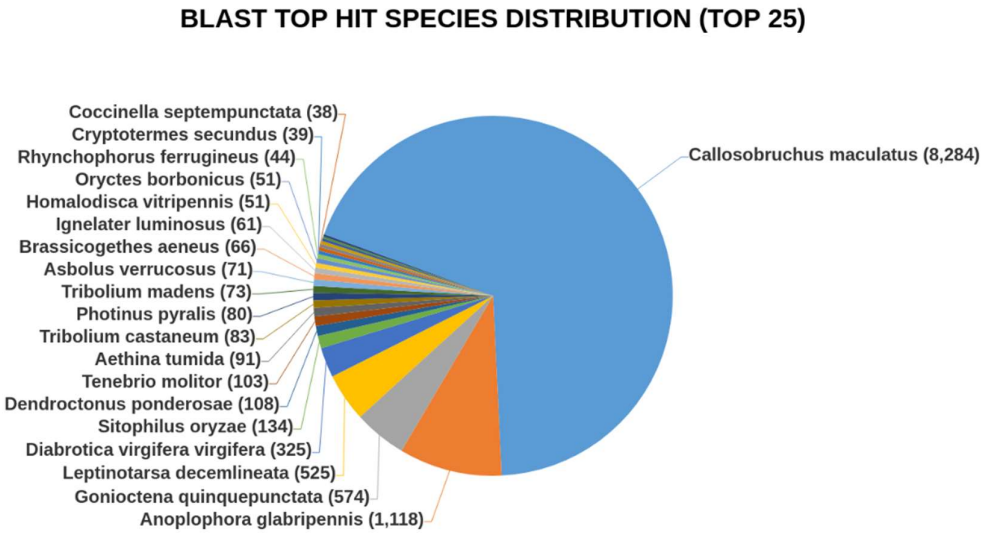

Figure 17: Depicts Top Blast Hit Species Distribution of pooled CDS.

Table 10: GO category distribution of CDS

Sr. No.	Sample Name	Total No. of CDS	Total No. of Annotated CDS	Biological Process	Cellular Component	Molecular Function
1	Control	6,596	3,403	2,115	2,048	2,458
2	H_{LC50}	11,622	5,654	3,608	3,504	4,112

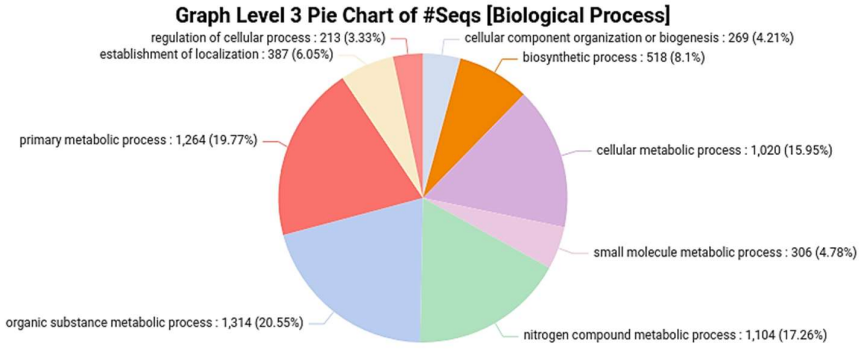

Figure 18: Depicts Biological Process GO term distribution for Control sample

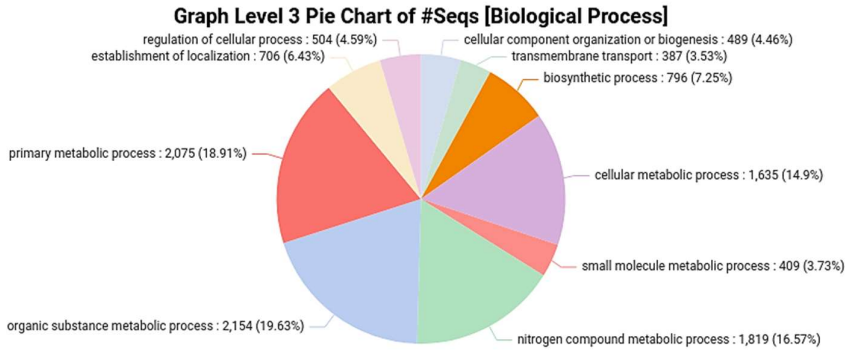

Figure 19: Depicts Biological Process GO term distribution for H_{LC50} sample

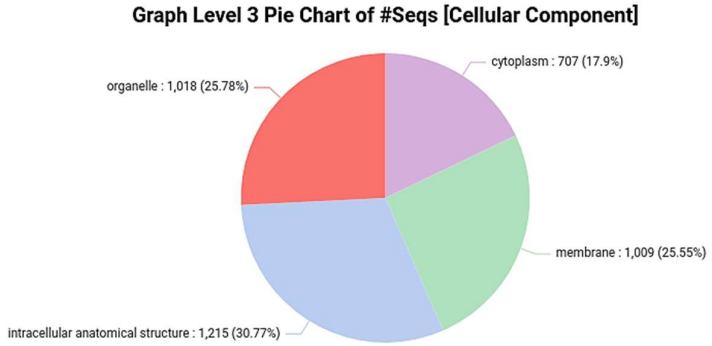

Figure 20: Depicts Cellular Component GO term distribution for Control sample

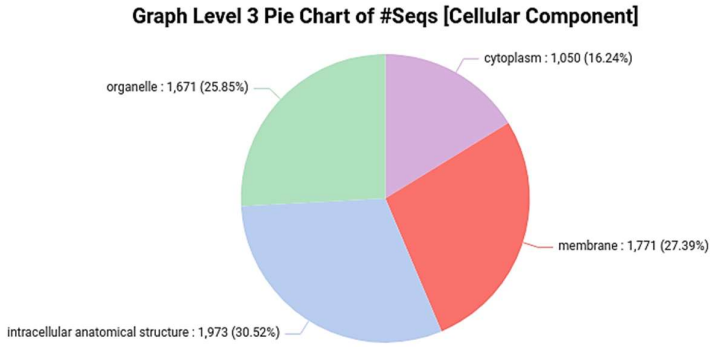

Figure 21: Depicts Cellular Component GO term distribution for H_{LC50} sample

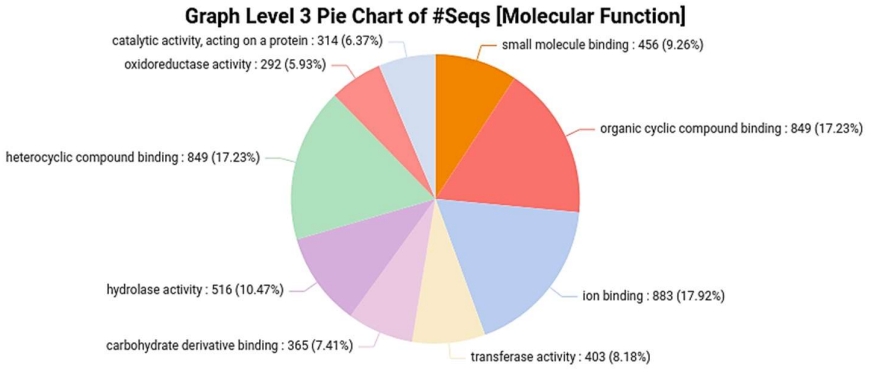

Figure 22: Depicts Molecular Function GO term distribution for Control sample

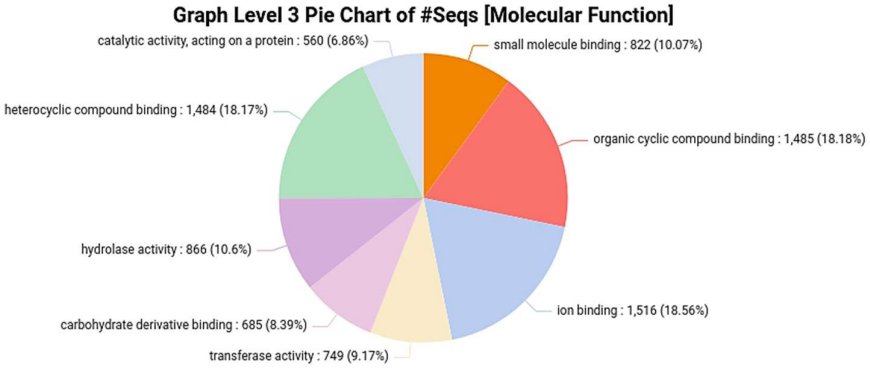

Figure 23: Depicts Molecular Function GO term distribution for H_{LC50} sample

Table 11: KEGG Pathway annotation summary of CDS

Sample Name	No. of Identified CDS	No. of Annotated CDS	No. of Annotated Categories
Control	6,596	1,992	31
H_{LC50}	11,622	3,037	31

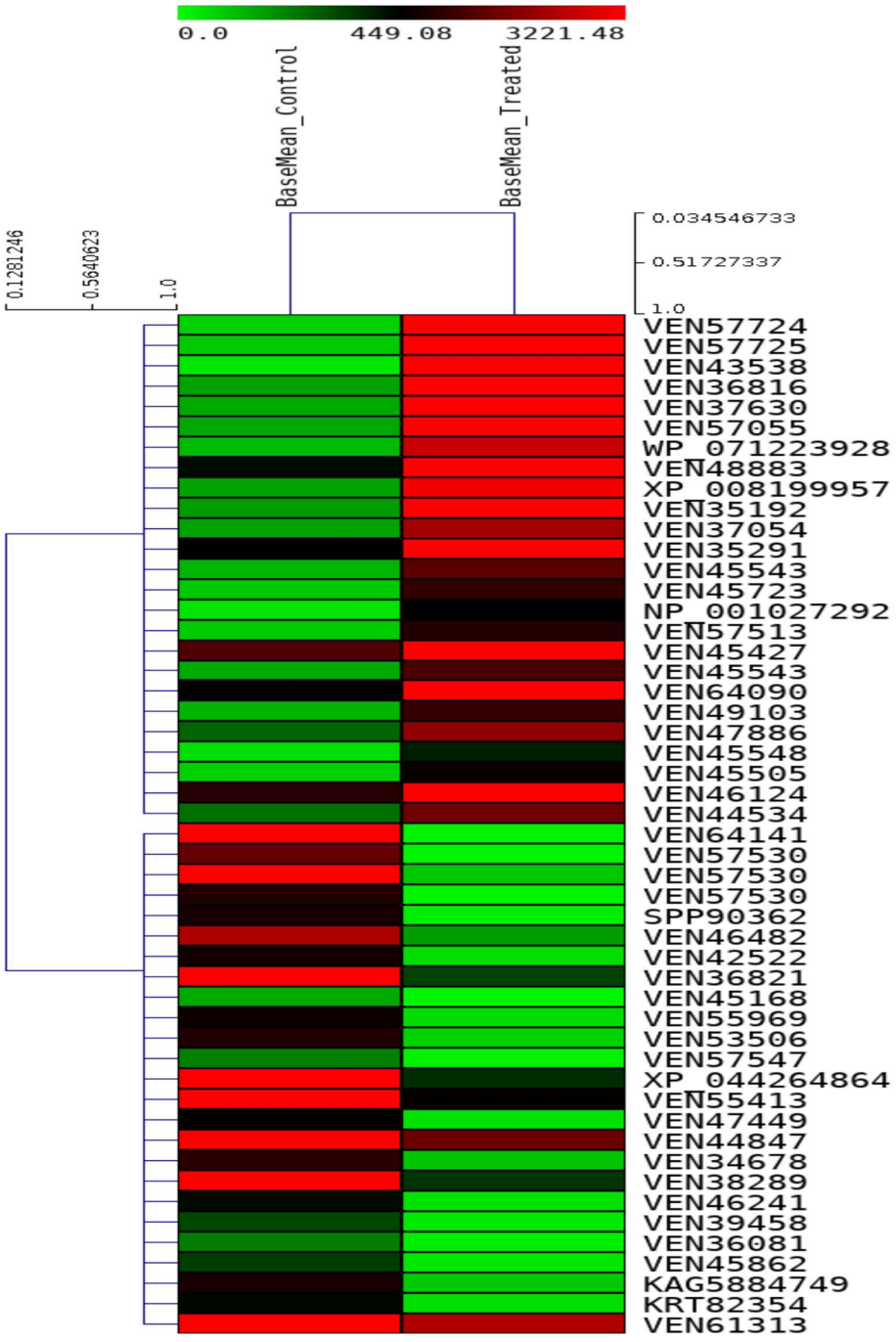

Figure 24: Depicts Heat map depicting the top 50 differentially expressed genes (significant); Basemean_Control represents the normalized expression values for Control sample and Basemean_Treated represents the normalized expression values for H_{LC50} sample for DGE Combination.

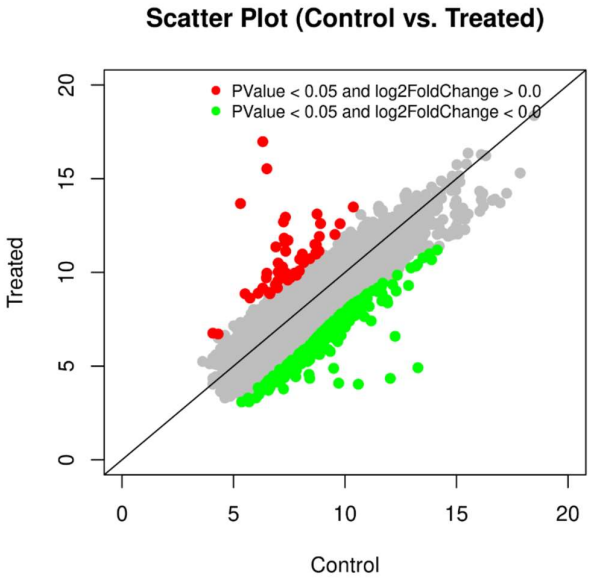

Figure 25: Depicts Scatterplot of differentially expressed genes; green dots represent the downregulated (significant) and red dots represent the upregulated (significant) genes in DGE Combination 1.

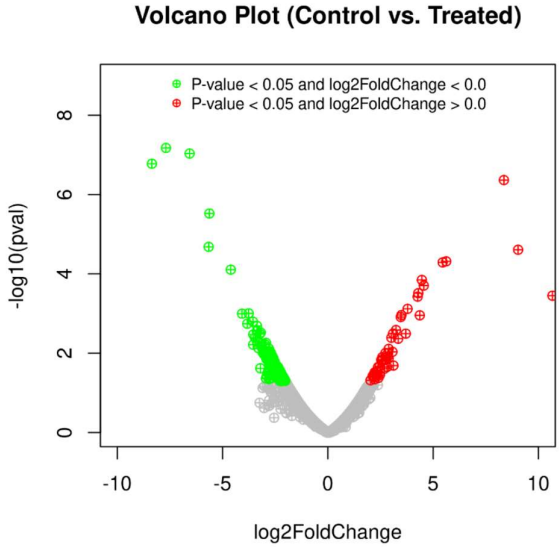

Figure 26: Depicts Volcano plot of differentially expressed genes; green dots represent the downregulated (significant) and red dots represent the upregulated (significant) genes for DGE Combination 1.

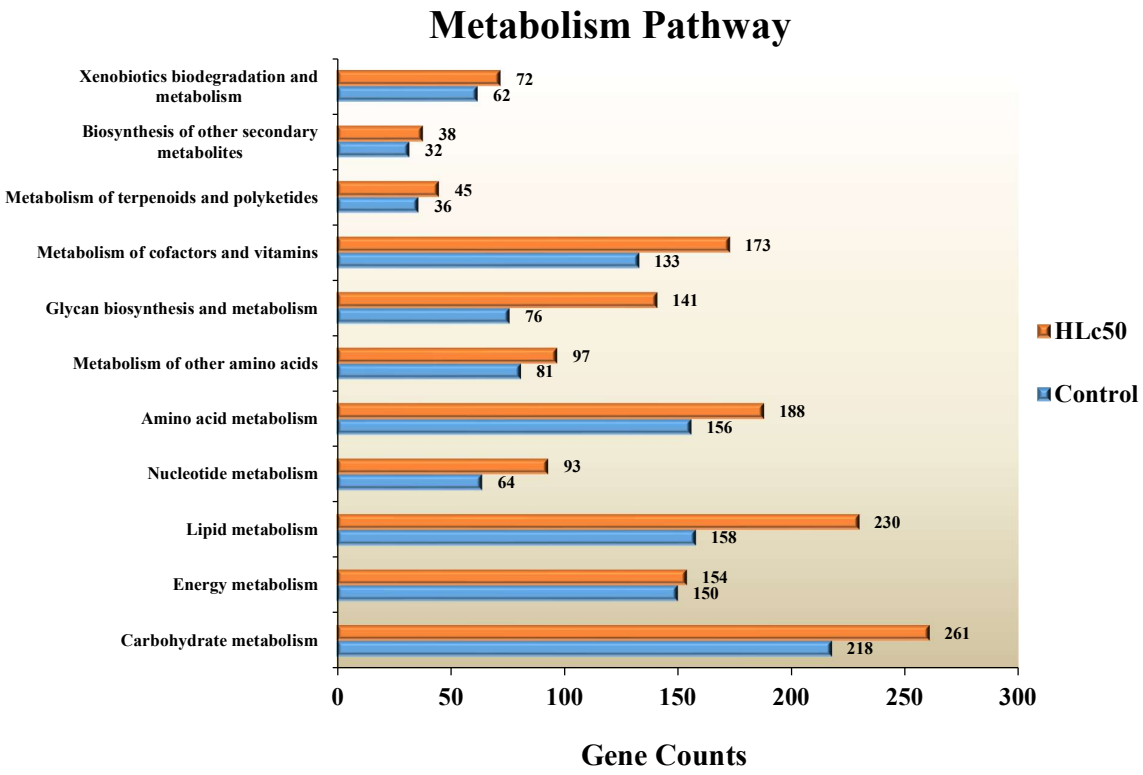

Figure 27: Depicts the total gene counts which are involved in different metabolic pathways.

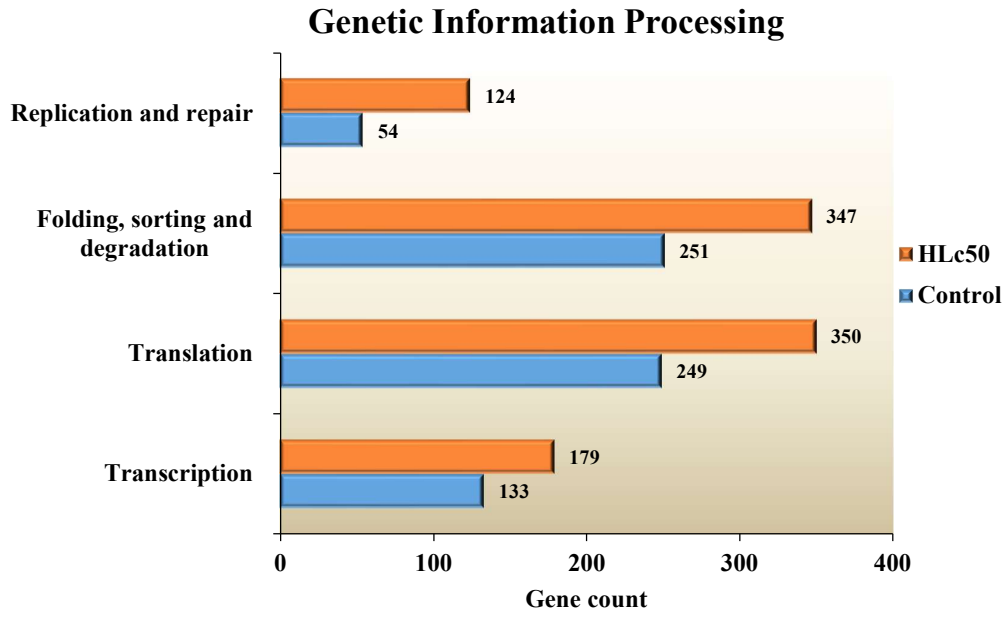

Figure: 28: Depicts gene count involved in different pathways of the genetic processes.

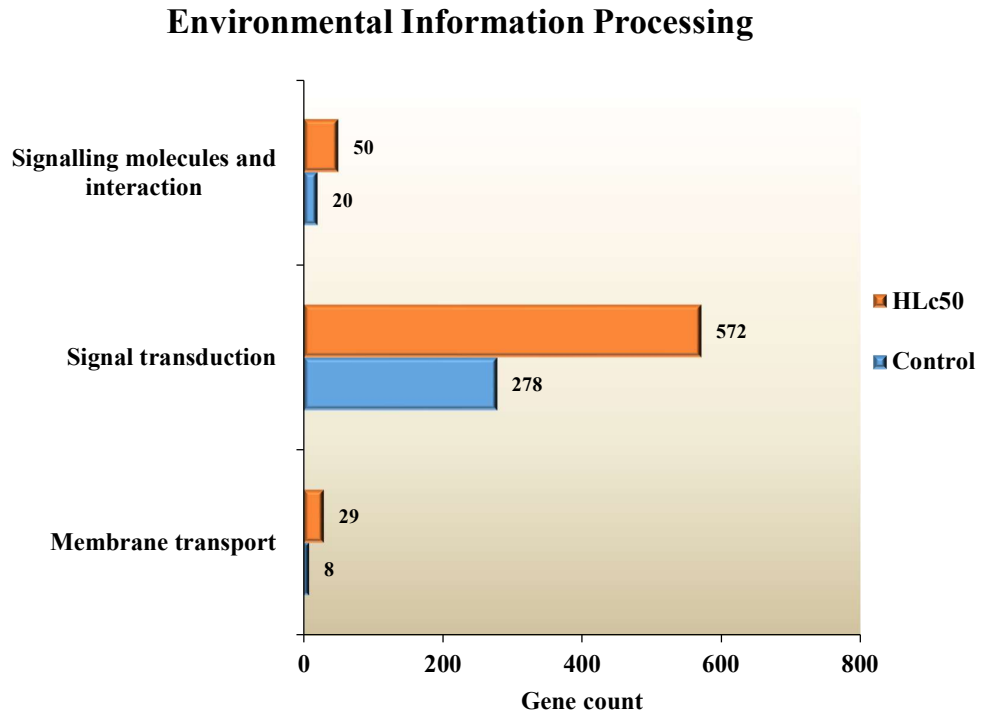

Figure:29: Depicts the gene count involved in the Environmental Information Processing

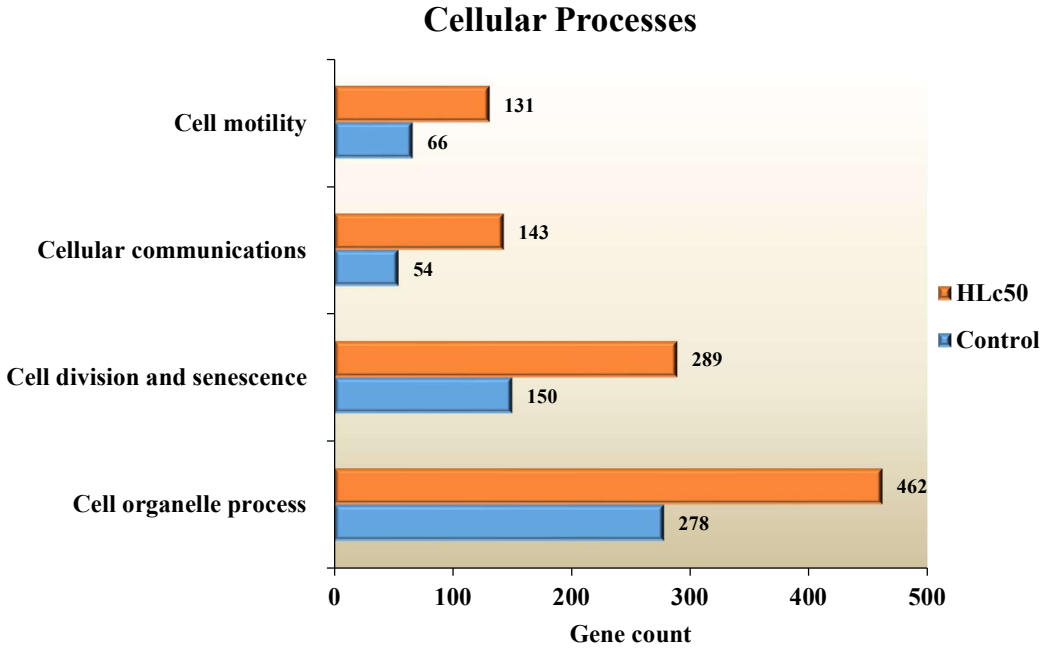

Figure: 30: Depicts the gene count involved in cell organelle process

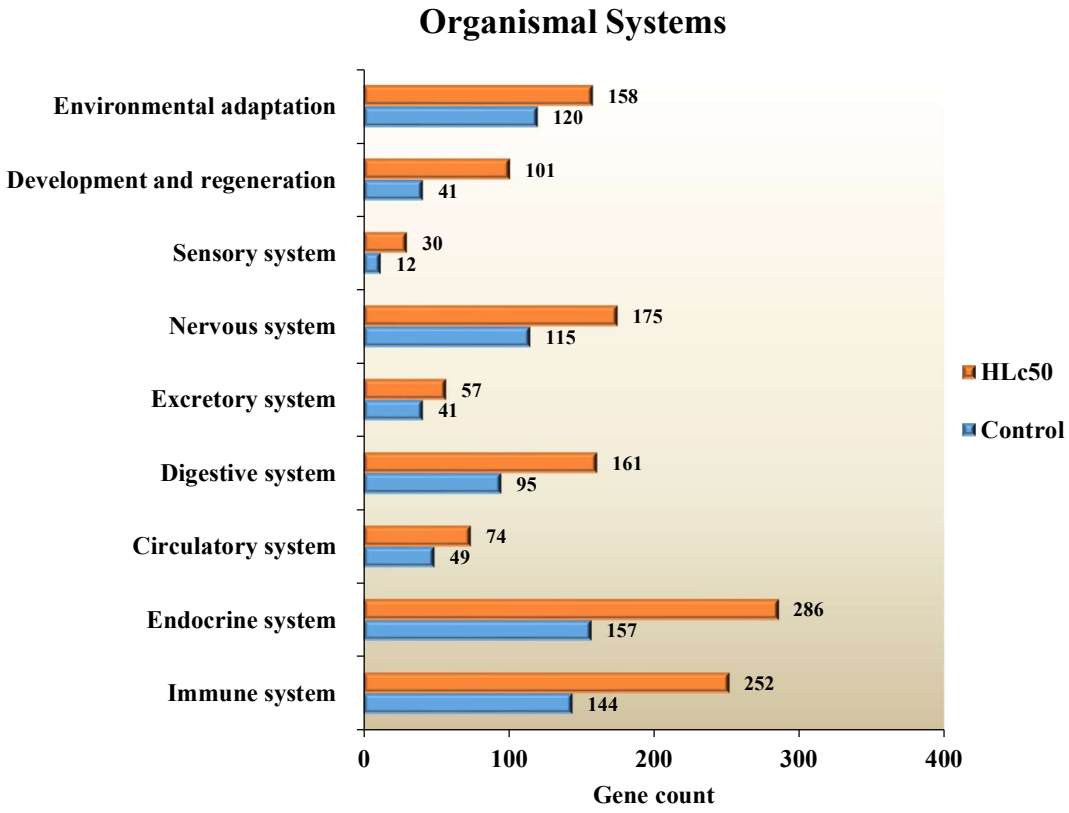

Figure 31: Depicts the gene count involved in the different organismal system.

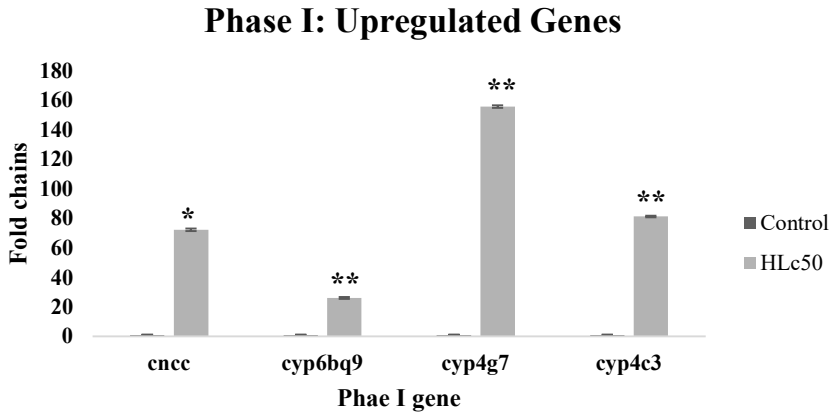

Figure 32: Depicts gene expression of the Phase I upregulated genes Significant level *($p<0.05$); **($p<0.01$)

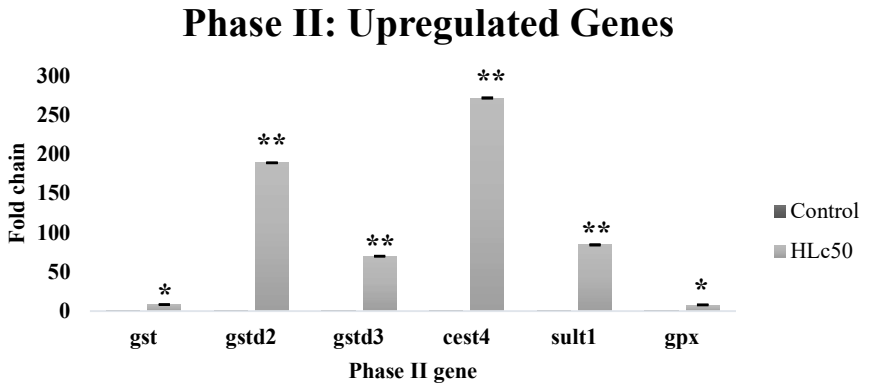

Figure 33: Depicts gene expression of the Phase II upregulated genes Significant level *($p<0.05$); **($p<0.01$)

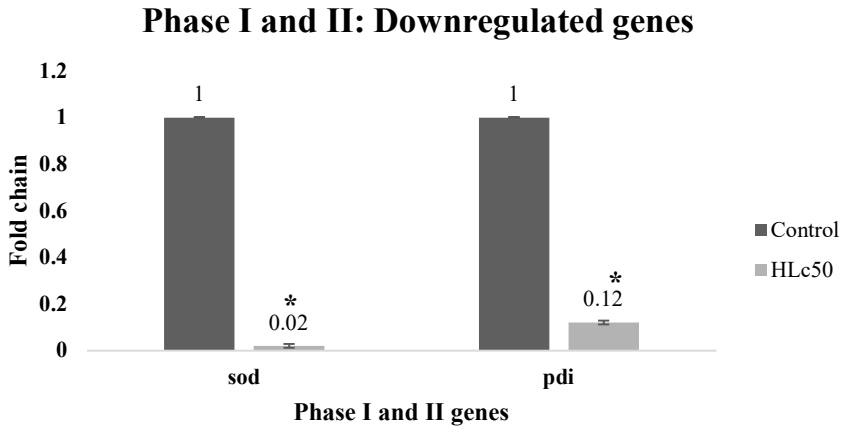

Figure 33: Depicts gene expression of the Phase I and II downregulated genes Significant level *($p<0.05$); **($p<0.01$)

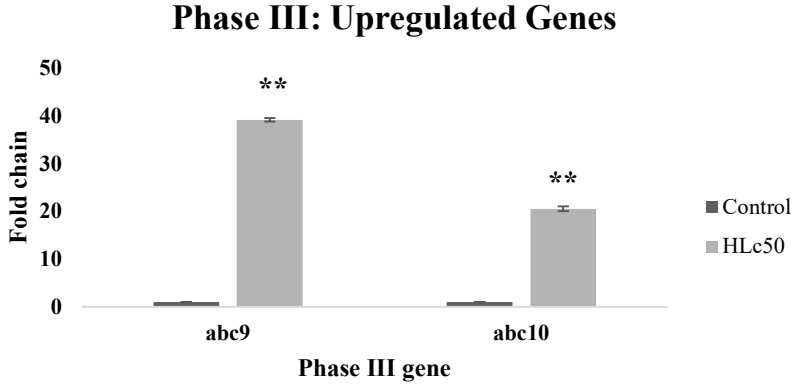

Figure 34: Depicts gene expression of the Phase III genes Significant level *($p<0.05$); **($p<0.01$)

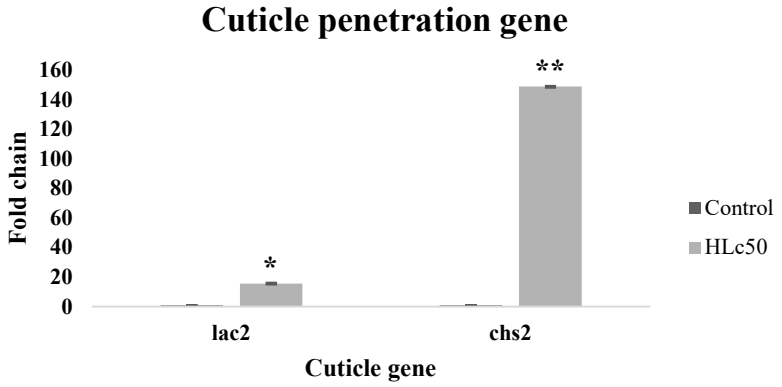

Figure 35: Depicts gene expression of the cuticles Significant level *($p<0.05$); **($p<0.01$)

In a comparative transcriptome study, gene expression profiles of treated and control *C. chinensis* were analyzed. The examination of the *C. chinensis* transcriptome led to the identification of a total of 58,120 transcripts, with 25,343 of them classified as unigenes. Among these, 13,614 transcripts were identified as the final coding sequences. Functional categorization of these sequences was performed based on their homologous blast results in publicly available databases. Intriguingly, the results revealed that 82% of the coding sequences (CDS) showed a high degree of similarity with the CDS of *C. maculatus*, a closely related species. This finding is of significance as it provides a valuable reference point for future investigations into the functional characterization of these genes (Sayadi et al., 2016).

The discovery and characterization of DEGs related to deltamethrin action and detoxification in *C. chinensis* provide a molecular foundation for understanding the toxic effects of insecticide-induced physiological changes. GO analysis revealed significant enrichment in "metabolic processes" in response to deltamethrin exposure. Additionally, KEGG analysis predicted multiple important metabolic pathways affected by these DEGs, including carbohydrate, amino acid, lipid, energy, and xenobiotic metabolism. The correlation study between GO and KEGG emphasized the significant association between key pathways and the hazardous effects of deltamethrin. Notably, changes in gene counts related to signal transduction and post-translational modifications under the "information processing" category were observed.

Carbohydrate and energy metabolism are crucial for insect activities, and the impact of insecticides on these processes can vary. Some studies have reported both upregulation and downregulation of key players in carbohydrate metabolism upon insecticide exposure, as seen in studies of insecticide effects on Meng et al. (2019) and Gao et al. (2020). In the current study, carbohydrate metabolism is suggested to play a vital role in defense against deltamethrin stress. Additionally, energy metabolism, influenced by ATPase expression, is considered a contributor to insecticide resistance. Sagri et al. (2014) suggested that increased energy metabolism in resistant olive flies facilitates detoxification, indicating a link between energy metabolism and insecticide resistance. ATPase, NADH dehydrogenase, and COX are involved in energy metabolism and are impacted by insecticide treatment. This study aligns with Meng et al. (2019), which reported a decrease in ATPase, NADH dehydrogenase, and COX upon insecticide exposure, negatively affecting insect growth.

Amino acid metabolism contributes to protein synthesis and cellular energy provision. This study showed enrichment in pathways related to glycine, serine, threonine, arginine, proline, and others. These pathways involve enzymes such as dehydrogenase and serine protease, and their differential expression might be linked to insecticide resistance, similar to the findings of Meng et al. (2019) in *C. suppressalis*. Lipid metabolism plays a role in energy generation and is crucial for insect growth, development, and reproduction. Alterations in lipid metabolism can impact these processes. In this study, KEGG analysis revealed enrichment in pathways directly associated with lipid metabolism. The findings suggest that deltamethrin exposure significantly alters lipid metabolism, potentially affecting insect growth, development, and reproduction.

Insects have complex detoxification mechanisms to metabolize xenobiotics like insecticides. These mechanisms encompass three phases, with the initial stage involving cytochrome P450 monooxygenases (P450s) and esterases targeting lipophilic xenobiotics. Phase II includes the conjugation of intermediate metabolites by uridine diphosphate (UDP)-glycosyltransferases (UGTs) and glutathione S-transferases (GSTs). The present study identified DEGs related to these processes, primarily P450 enzymes, in pathways related to "metabolism of xenobiotics by

cytochrome P450" and "drug metabolism-cytochrome P450." Several genes, from phase I, phase II and phase III were upregulated or downregulated. These findings are consistent with previous studies that have reported upregulation or downregulation of metabolic detoxification genes (Miah et al., 2018; Simma et al., 2019; Nagar et al., 2020).

In *C. chinensis* exposed to deltamethrin, the expression of receptors and kinases associated with signal transduction was altered. These differentially expressed genes (DEGs) primarily fell into the domain of "environmental information processing" and were enriched in pathways like the "mTOR signaling pathway and MAPK signaling pathway-fly." The Mitogen-Activated Protein Kinase (MAPK) pathway, a fundamental mechanism for signal transmission, is known to mediate stress responses, including those triggered by insecticides (Hotamisligil and Davis, 2016).The exposure to deltamethrin also influenced "posttranslational modifications," "protein turnover," and "chaperones." Posttranslational modifications are crucial for protein functionality, protein turnover is essential for maintaining optimally functioning proteins through continuous synthesis and degradation, and chaperones assist in protein folding and assembly while maintaining cellular homeostasis. The study identified several heat shock proteins (Hsps) as molecular chaperones, which are rapidly synthesized in response to various environmental stressors, including pesticides (Lu et al., 2017).

Chitin synthase 2 (chs2) is specifically involved in chitin synthesis in the peritrophic matrix (Zhang et al., 2012; Arakane et al., 2005; Khajuria et al., 2010; Liu et al., 2012). In the present study, chs2 was significantly overexpressed indicating its role in the development. These findings align with previous research and established functions of chs2 in the development of the peritrophic membrane, which is vital in insect resistance against external factors. This highlights the importance of studying chitin synthase genes across different insect taxa to gain a comprehensive understanding of their biological mechanisms.

Insects develop resistance to external factors, such as insecticides, by modifying the thickness and composition of their cuticular barriers (Lilly et al., 2016; Balabanidou et al., 2018). They also alter their cuticles through the abundant presence of cuticular proteins, with the laccase enzyme playing a role in cuticle defense by promoting the

synthesis of a thicker cuticle that hinders insecticide penetration (Dubovskiy et al., 2013; Rösner et al., 2019). The upregulation of laccase2 (lac2) is associated with changes in cuticle composition, enhancing insects' ability to resist insecticides (Ye et al., 2021; Li et al., 2023). Previous research demonstrated the correlation between cuticle tanning and the expression profile of laccase2 throughout various developmental stages.

Overall, RNA-seq technology has proven to be an effective instrument for investigating the molecular pathways involved in the adverse effects of sublethal pesticide dosages in *C. chinensis*. Transcriptome analysis revealed that differentially expressed genes (DEGs) were enriched in pathways related to metabolism and information processing, suggesting their involvement in toxic mechanisms. The study findings highlight how sublethal exposure to deltamethrin can lead to the upregulation or downregulation of key detoxification-related genes, including P450s, ESTs, GSTs, and ABC transporters. These results significantly contribute to our understanding of the activities of detoxification-related genes in *C. chinensis* and provide insights into the systematic toxicity processes triggered by deltamethrin. This knowledge is crucial for evaluating the environmental management, hazards, and risks associated with this particular insecticide.