General Consideration

Each year, over 2 billion tonnes of grains are grown for sustenance and animal feed, making up about two-thirds of the world's total protein consumption (Erenstein et al., 2022; Tiwari et al., 2022). In India, agriculture provides the main source of livelihood for approximately 58% of the population. In the 2019-20 crop year, there was an expected record-breaking food grain production of 295.67 million tonnes. In the 2020-21 fiscal year, the Government of India aimed for a food grain production of approximately 300 million tonnes (Agricultural and Processed Food Products Export Development Authority (APEDA), Union Budget 2020-21). For the crop year 2022-23 (July to June), the Indian government set a food grain production target of 328 million tonnes, which is a 4% increase from the previous year's record of 315.7 million tonnes. While there is sufficient land to feed the growing global population, the Food and Agriculture Organisation (FAO) has warned that much of this land is only suitable for specific crops, and food grains and pulses are crucial for addressing food insecurity.

According to the Food and Agriculture Organisation (FAO) projections, a global food shortage is looming if there is no increase in total world food production. The number of people experiencing hunger is expected to reach 702-828 million by 2021 due to factors like the COVID-19 pandemic, regional conflicts, natural disasters, and climate change (Fan et al., 2021; FAO et al., 2022; George et al., 2020; Janssens et al., 2020). About 33% of global food production is lost or wasted annually throughout the food supply chain, resulting in economic losses of approximately 936 billion USD and contributing to 8-10% of global greenhouse gas emissions (Buzby and Hyman, 2012; Chalak et al., 2016). In developing nations, food loss is a significant barrier to food security and sustainable development (Abbade, 2020; Xue et al., 2017). To meet the needs of the projected 9.1 billion global population by 2050, a 70% increase in food production is required. Developing countries are grappling with food insecurity and malnutrition, particularly among children, with hunger affecting one in six children in poor countries (World Health Statistics 2012; FAO, 2019).

One of the major challenges to achieving optimal food production is pre- and post-harvest issues that lead to significant grain losses. Food grains undergo multiple processes, including threshing, cleaning, drying, storage, processing, and transportation, before reaching consumers. Post-harvest food losses begin during harvesting and continue until the final marketing of the product. Technical challenges, inadequate storage facilities, subpar packaging practices, and poor infrastructure contribute to these losses. The FAO estimates that 1.3 billion metric tonnes of food, equivalent to 33% of total production, are lost during the post-harvest stage globally. If current practices persist, this loss is projected to reach 2.1 billion metric tonnes by 2030.

In India, post-harvest grain loss is a significant issue, with the FAO estimating a loss of 40% for food grains and 30% for cereals. Monetary losses in India exceed Rs. 50,000 crores annually, and a substantial amount of food, valued at 92651 crore rupees, is wasted during post-harvest operations. A nationwide study reveals crop losses in various categories, such as cereals, pulses, oilseeds, fruits, and vegetables, due to issues in harvesting, post-harvest activities, handling, and storage. Grain losses are primarily attributed to factors like insect infestation, poor storage practices, and the influence of environmental conditions like drought, heat, and cold. Insects are the most destructive pests in stored grain commodities, causing losses ranging from 15% to 25% of stored grain. Insect pests have a rapid reproductive rate and are responsible for extensive damage to agricultural and forestry commodities. There are two categories of storage insect pests: primary pests, which infest entire grains, and secondary pests, which consume previously damaged grains or processed goods. Primary pests are limited in their dietary preferences, while secondary pests have a broader spectrum of hosts. Pest management strategies include physical, mechanical, biological, and chemical approaches. Fumigation, grain protectants, and aerosols are commonly used chemical-based methods. The use of methyl bromide has been phased out due to its environmental impact.

Grain production, food security, and food loss are critical global challenges. Increasing production and reducing post-harvest losses, particularly from insect pests, are key factors in addressing food insecurity and ensuring a sustainable future.

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

The agricultural sector plays a crucial role in India's economy, contributing approximately 14% to the country's GDP and 11% to its export value (Jha et al., 2015; Wagh, and Dongre, 2016). Nearly two-thirds of India's population depends on agriculture for their livelihood (Manohar, 2016; Dolli and Divya, 2020). India leads the world in pulse production, accounting for 29% of the global area and 19% of the global pulse production (Shukla and Mishra, 2020; Palai et al., 2019; Bhat et al., 2022). Pulses are a vital source of protein, especially for India's large vegetarian population, earning them the nicknames "poor man's meat" and "rich man's vegetable" (Singh et al., 2015; Shukla and Mishra, 2018; Tripathi et al., 2019). Pulses offer significant nutritional and health benefits (Venkidasamy et al., 2019; Ferreira et al., 2021) due to their protein, carbohydrate, dietary fibre, and bioactive content.

Pulses, including various beans, lentils, peas, and more, are a cornerstone of the Indian diet, providing a major source of both protein and energy (Venkidasamy et al., 2019; Hussain et al., 2021). In a country where a significant portion of the population is economically disadvantaged, pulses are a crucial source of affordable dietary protein (Minocha et al., 2017; Chakrabarti et al., 2018). India is the world's largest producer, consumer, and importer of pulses, playing a pivotal role in ensuring food security (Ahlawat et al., 2016; Dizon and Herforth, 2018; Abraham and Pingali, 2021; Singh et al., 2022). In recent decades, India has seen rapid economic growth, coupled with demographic shifts, increasing the demand for a diverse range of food groups (Nagarajan et al., 2016; Agarwal et al., 2020). Meeting the dietary needs of the growing population in the face of changing food habits, modernization, and rising awareness presents a significant challenge (Kumar et al., 2014; Changan et al., 2017; Langyan et al., 2022b). The central question is how to provide nutritious food to an expanding population without depleting the Earth's finite resources (Langyan et al., 2022a).

Food loss and waste are prevalent globally, with higher losses in high-income countries downstream in the food chain and in low-income countries occurring upstream (FAO 2013; Dou et al., 2016; Cattaneo et al., 2021; Mokrane et al., 2023). Substantial losses during harvesting, threshing, and storage are primarily due to inappropriate practices (Tibagonzeka et al., 2018; Vishwakarma et al., 2020. Storage insect pests, such as Coleoptera (beetles) and Lepidoptera (moths and butterflies), are responsible for substantial post-harvest losses (Yahia et al., 2019; Demis and Yenewa, 2022; Suleiman and Rosentrater, 2022). *C. chinensis*, a member of the Bruchidae family, poses a significant threat to pulses, causing substantial damage and rendering seeds unfit for consumption or planting (Kumar, 2017; Ahmad et al., 2021). Traditional chemical and biological control measures have been employed, but overuse of pesticides has led to resistance issues (Daglish et al., 2014; Dara 2017; Fang et al., 2019; Kortbeek et al., 2019). Insecticide resistance is a growing problem, with over 500 insect species now reported as resistant (Naqqash et al., 2016; Du et al., 2020).

To address this, insect-specific insecticides are needed, but these require a comprehensive understanding of the insect's life cycle, host preference, and behaviour (Ribeiro et al., 2018; Arai et al., 2022). Host preference plays a crucial role in the life cycle of *C. chinensis*, affecting mating, oviposition, and larval development (Thakkar and Parikh, 2018). Many studies have explored host preferences for *C. chinensis* on various pulses (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 et al., 2018; Meghwal and Singh 2005), and competition for host preference has been observed (Athanassiou et al., 2017). The suitability of the host varies based on factors like seed size, germ layer, moisture, and nutritional values (Ojo and Omoloye, 2016; Akhter et al., 2017).

The present study focuses on developing a rearing protocol, understanding the life cycle, and investigating the preference of *C. chinensis* for various stored pulses under controlled laboratory conditions. Understanding the host-pest interaction is critical for effective pest management. Host characteristics, such as seed texture, size, and nutritional composition, significantly influence the life cycle of pests like *C. chinensis* (Singh et al., 2013; Mason-D'Croz et al., 2016; Sewsaran et al., 2019). In our study, *C. chinensis* displayed a clear preference for specific hosts. The texture and surface area of the seed coat played a crucial role in this preference, with smooth-coated seeds with larger surface areas being favoured. Hatching was highest in green gram, attributed to its soft coat allowing easy larval penetration. The incubation period ranged from 3 to 6 days, influenced by host nutritional and physical properties (Naseri and Majd-Marani, 2022). Larval development and pupation periods were longest in pea and shortest in green gram, in line with previous research (Hosamani et al., 2018; Jaiswal et al., 2019; Nisar et al., 2021). Adult emergence was highest in green gram and cowpea, lowest in pea and black gram, potentially due to hard seed coats (Padmasri et al., 2017). Hosts affect adult longevity, with the longest observed on green gram, likely due to ease of penetration and high hatchability (Hosamani et al., 2018; Mehta and Negi, 2020). Grain weight loss was greatest in green gram and least in pea, indicating varying susceptibility among host grains. Host-induced variation in nutritional content may explain this (Soumia et al., 2017; Kébé et al., 2020).

In conclusion, our study reveals C. chinensis preference for green gram and emphasizes the importance of host characteristics in its survival and oviposition. This knowledge is valuable for developing alternative grain protection methods and studying insecticide resistance through mass rearing.

Chapter 2: Deltamethrin induced toxic transgenerational effects on the development and repellency of C. 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 and Cavalli., 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).

Potential transgenerational effects of insecticides include: (Brevik et al., 2018)

Resistance Development: Prolonged exposure to insecticides can lead to the development of resistance in subsequent generations. Certain individuals within the population may possess genetic variations that make them resistant to the specific insecticide. Over time, these resistant individuals become more prevalent, reducing the effectiveness of the insecticide in controlling the pest population.

Altered Growth and Development: Insecticide exposure can affect growth and development, resulting in changes in developmental rates, body size, or morphology. These alterations can be passed on to subsequent generations, leading to modified growth patterns or phenotypic variations.

Behavioural Changes: Insecticides may influence pest behaviour, affecting feeding preferences, reproductive behaviours, or movement patterns. Transgenerational effects of insecticides on behaviour can impact the pest's ability to find food sources, mates, or avoid detection, potentially altering their population dynamics.

Reproductive Effects: Insecticides may disrupt reproductive capacity by interfering with mating, fertility, or egg-laying patterns. These effects can carry over to subsequent generations, affecting overall reproductive success and the population growth of the pest.

Fitness Consequences: Fitness refers to an organism's ability to survive and reproduce. Transgenerational effects of insecticides can have consequences for fitness by lowering survival rates, reproductive output, or offspring viability, thereby affecting the population dynamics of pests over time.

Olivares-Castro et al. (2021) found that carbaryl and permethrin exposure in aquatic insects can lead to transgenerational effects, negatively impacting the survival, growth, and reproduction of subsequent generations. Similarly, Jaffar et al. (2022) investigated the impact of imidacloprid and propoxur on fruit flies (Drosophila melanogaster) and observed reduced fecundity and altered offspring development in later generations. Iftikhar et al. (2020) focused on imidacloprid's transgenerational effects on the mealybug predator (Cryptolaemus montrouzieri), noting changes in parental behaviour, such as oviposition site selection, leading to adverse effects on offspring survival, development, and body size. It is essential to recognize that the specific effects of insecticides on different insect species can vary depending on factors like insecticide formulation, dosage, exposure duration, and other ecological considerations. These variations can significantly influence fitness, reproductive success, development, and population dynamics (Gross and Garric, 2019).

The transgenerational effects of insecticides on stored grain pests have both short-term and long-term consequences (Guedes et al., 2016; Nyamukondiwa et al., 2022). In the long term, these effects can be advantageous for the pests, enabling them to thrive and reproduce despite the presence of insecticides. This, in turn, can lead to the development of insecticide resistance, posing significant challenges for effective pest management strategies (Bueno et al., 2023). Insecticides' transgenerational effects on stored grain pests are essential to study, given the need for effective pest management in safeguarding stored food (Mukherjee et al., 2015). Insects are ideal for transgenerational research due to their short generational cycles and suitability for controlled lab studies. Insects rapidly adapt to pesticides, leading to heritable, gene expression-related traits within a few generations (Dubovskiy et al., 2013b; Mukherjee and Vilcinskas, 2019). Further research is needed to uncover the underlying mechanisms and ecological consequences of these effects on insect populations and ecosystems. Insects serve as valuable model organisms for studying transgenerational effects due to their short generational spans and the ease of maintaining controlled laboratory populations.

Research has indicated the efficacy of deltamethrin against a range of insects, including coleopterans. For instance, studies by Paudyal et al. (2016) revealed significantly increased mortality rates in adult *T. castaneum, S.oryzae* and *R.dominica* when exposed to high concentrations of deltamethrin. Jacob et al. (2014) reported 50% mortality at 250ppm in adult *S. zeamais* after exposure to commercial grade deltamethrin, whereas your study observed 50% mortality at 22.93ppm. This variation may be attributed to the type of deltamethrin used and underscores its sensitivity against *C. chinensis*, making it suitable for monitoring insecticide resistance (Gupta, 2019).

The findings of the current work indicate that sublethal concentrations of deltamethrin, both low (1.15ppm) and high (4.5ppm) LC_{50} levels, have a significant negative impact on the developmental parameters of the initial generations when compared to the control group. However, a more detailed examination of generational differences reveals that the adverse effects of the insecticide diminish in subsequent generations. This suggests that *C. chinensis* gradually develops greater tolerance to deltamethrin (Brevik et al., 2018). Deltamethrin is a synthetic pyrethroid insecticide that replicates the properties of natural pyrethrins derived from *Chrysanthemum* flowers (Shrivastava et al., 2011; Bhanu et al. 2011). This widely used compound is employed globally for the management of stored-product insect pests, effectively paralyzing their nervous systems and causing rapid incapacitation and mortality (Velki et al. 2014). The neurotoxic effects of deltamethrin in insects result from its ability to disrupt axonal transmission of nerve impulses by altering ion permeability in nerve membranes (Paudyal et al., 2016 & 2017).

The transgenerational effects of insecticide exposure are relatively understudied compared to intragenerational and intergenerational effects (Margus et al., 2019). The potential transgenerational consequences of insecticide exposure are not comprehensively understood. Our study aims to address this gap by investigating the transgenerational effects of sublethal doses of deltamethrin on *C. chinensis*. The research focuses on developmental parameters, such as total egg count, total hatching, hatching percentage, total development period, and adult longevity.

Our research also evaluated the impact of sublethal concentrations of deltamethrin on the total egg count and hatching of *C. chinensis*. The results show that both concentrations of deltamethrin significantly reduced the total number of eggs and hatching rates in the initial generation compared to the control group. However, these effects were less pronounced in subsequent generations and approached levels similar to the control group. Similar effects have been observed in other studies involving different insecticides and species, where egg laying and hatching were affected (Vassilakos et al., 2012 & 2015; Ali et al., 2017; Dong et al., 2017; Qu et al., 2017; Rumbos et al., 2018; Liang et al., 2019; Su and Xia 2020 and Tamilselvan et al., 2021).

In the present study, it is evident that exposure to deltamethrin in the F1 generation negatively impacts the immediate subsequent generation (F2). The F2 generation exhibited a significantly increased total development period, indicating delayed development. This effect also affected adult longevity. However, as subsequent generations (F5 & F6) were observed, the insect developed tolerance to the insecticide, and normal development and adult longevity were noted. These findings align with previous studies involving different insecticides (Zhu et al. 2012; Guo et al. 2013; Xu et al. 2016; Yin et al. 2008; Zhang et al. 2013; Deng et al., 2019). The number of adult emergences correlates with the number of eggs laid in each generation and is dependent on oviposition.

The susceptibility index, indicating the effectiveness of deltamethrin, showed that the F1 generation had a smaller population due to the pronounced impact of deltamethrin, while the F5 and F6 generations had larger populations. This suggests increased sensitivity to deltamethrin in the initial generations and growing resistance over time and generations (Ngom 2021; Tenrirawe et al., 2023). Regarding the repellency of *C. chinensis*, the study reveals that the efficacy of deltamethrin as a repellent varies with time and generation. The repellency diminishes as exposure lengthens, with the F6 generation exhibiting the lowest repellency rate. This suggests that *C. chinensis* gradually develops an enhanced capacity to withstand deltamethrin's effects over multiple generations. Studies have shown that insects exposed to sublethal concentrations of insecticides over multiple generations may develop resistance (Deng et al., 2019).

In conclusion, the research highlights the transgenerational effects of deltamethrin, where sublethal concentrations influence the development of C. chinensis. While initial generations experience adverse effects, subsequent generations develop tolerance. Additionally, the findings indicate that the repellency of deltamethrin diminishes over time and generations, with a correlation between susceptibility and population size. These results suggest the potential development of resistance in the F6 generation, necessitating further research, such as comparative transcriptome analysis, to understand the underlying mechanisms and develop more effective insecticides.

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

The use of synthetic chemical pesticides has been essential for managing insect pests in agriculture, but it comes with significant drawbacks, including the development of insecticide resistance, environmental damage, and harm to non-target species. For example, methyl bromide and phosphine, known for their effectiveness in pest control, have faced challenges due to ozone layer depletion and insect resistance. Alternative pesticides like sulfuryl fluoride, ethyl formate, and hydrogen cyanide have limitations. Therefore, there is a growing interest in adopting less toxic and environmentally friendly methods for pest control. Exposure to sub-lethal levels of insecticides can impact insect reproduction, development, and susceptibility. Insects detoxify these chemicals in three phases: phase I, phase II, and phase III. Phase I involves initial metabolic reactions, phase II involves metabolizing enzymes (P450 monooxygenase, glutathione S-transferase, and carboxylesterase), and phase III involves transporters.

These detoxification enzymes, especially carboxylesterase (CarE), glutathione S-transferase (GST), and cytochrome P450 monooxygenase, are critical for insect resistance. Studies have shown that their activity increases in response to exposure to sublethal doses of pesticides like abamectin, avermectin, thiamethoxam, and buprofezin, allowing insects to cope with pesticide stress. However, the timing and magnitude of enzyme activation vary among different insect species. Insects with resistance often show elevated levels of P450 monooxygenases, which play a key role in catalyzing reactions involving toxic substances. Transferase genes, particularly in the delta and epsilon classes, are involved in sequestering and detoxifying various chemicals, including insecticides.

The primary mechanism of pesticide resistance in insects involves enhanced detoxification through cytochrome P450 enzymes, particularly transcription factors cncc play a crucial role in activating cyp genes, contributing to deltamethrin resistance. In other studies, similar mechanisms were found to regulate gene expression related to detoxification processes (P450s, GSTs, and ABC transporters). Glutathione S-transferases (GSTs) are essential enzymes in detoxification, facilitating the breakdown of insecticides through conjugation reactions with glutathione. They are categorized into various classes, and the epsilon class is known for its role in degrading pesticides. Delta-class GSTs have been linked to defense against toxic substances and resistance to insecticides. Carboxylesterases (CarE) play a crucial role in detoxifying xenobiotics, including esters, and their levels can significantly affect resistance. Increased CarE activity has been observed in insect species exposed to various insecticides. ABC transporters are a diverse group of proteins involved in transporting substances, including xenobiotics. Studies have shown a correlation between the upregulation of ABC transporters and pesticide resistance in various insects.

The current study on *C. chinensis* exposed to deltamethrin has the potential to shed light on the molecular mechanisms underlying insecticide resistance. Transcriptome sequencing and de novo assembly provide a valuable resource for understanding gene functions and responses to pesticides in this species. However, limited research has explored the toxic effects of pyrethroids, necessitating an understanding of their mechanisms of action and potential resistance. While studies have investigated the impact of pyrethroids on pests like *Tribolium*, *R. dominica* and *S. oryzae* but there's a significant gap in literature regarding pest infesting pulses like *C. chinensis*.

To address this, *C. chinensis* were exposed to deltamethrin, and RNA-seq analysis was carried out. This identified 330 differentially expressed genes (DEGs), offering insights into the molecular basis of insecticide-induced physiological alterations. Gene ontology (GO) analysis of DEGs revealed enrichment in metabolic processes under deltamethrin exposure. Additionally, KEGG analysis uncovered various metabolic pathways, including carbohydrate, amino acid, lipid, energy, and xenobiotic metabolism. The correlation between GO and KEGG highlighted connections between these pathways and the harmful effects of deltamethrin. Notably, carbohydrate and energy metabolism were affected, which could influence defense against deltamethrin. Previous research has shown varied effects on carbohydrate metabolism after insecticide exposure (Meng et al., 2019; Gao et al., 2020), suggesting a pivotal role for carbohydrates in stress defense.

The role of ATPase in ATP synthesis is well-established, and its expression can be influenced by insecticide treatment. Changes in ATPase expression might lead to increased resistance to insecticides. Similarly, NADPH dehydrogenase and COX, both components of the mitochondrial respiratory chain, are targeted by many insecticides. Your study found altered expression levels of ATPase, NADH dehydrogenase, and COX in *C. chinensis* exposed to deltamethrin, impacting energy metabolism. Amino acid metabolism is vital for protein synthesis and energy provision. DEG analysis showed enrichment in pathways related to amino acid metabolism, particularly glycine, serine, threonine, arginine, and proline. These pathways are connected to enzymes like dehydrogenases, serine proteases, and argininosuccinate synthase. This aligns with findings by David et al. (2010) and Wilkins (2017) and suggests a potential role in insecticide resistance. Lipid metabolism plays a crucial role in energy generation and affects insect growth, development, and reproduction. KEGG analysis revealed enrichment in pathways such as Glycerolipid metabolism, Glycerophospholipid metabolism, and Fatty acid degradation. This indicates that deltamethrin exposure significantly impacts lipid metabolism and, subsequently, the insect's growth, development, and reproduction. Further research is needed to fully understand this relationship.

Insects employ detoxification mechanisms to metabolize xenobiotics, such as insecticides. These mechanisms involve cytochrome P450 monooxygenases (P450s), esterases, glutathione S-transferases (GSTs), and ATP-binding cassette (ABC) transporters. The study identified upregulation of several P450 enzymes, GSTs, and ABC transporters in response to deltamethrin, consistent with earlier research. This suggests the importance of these genes in insecticide resistance development.

Changes in receptors and kinases associated with signal transduction were observed in *C. chinensis* exposed to deltamethrin. DEGs related to signal transduction were enriched in the "mTOR signaling pathway" and "MAPK signaling pathway-fly," suggesting their involvement in stress responses. However, further research is needed to elucidate the precise roles and mechanisms of these genes in response to deltamethrin exposure. Posttranslational modifications, protein turnover, and chaperones, including heat shock proteins (Hsps), were affected by deltamethrin exposure. Hsps are rapidly synthesized in response to various stressors, and their specific roles in deltamethrin exposure require further investigation. The study advances our understanding of gene expression's impact on organismal systems, including digestion, immune, circulatory, excretory, nervous, and sensory systems. It highlights the potential link between gene expression alterations and insecticide resistance, which could help develop effective strategies for managing insect populations and mitigating resistance. Further research is needed to advance basic biology and practical implications in fields like pest control and public health.

Chitin synthase 1 (chs1) is responsible for chitin synthesis in the cuticle and cuticular lining of various insect parts, including the foregut, hindgut, and trachea. In contrast, chitin synthase 2 (chs2) is specifically involved in chitin synthesis in the peritrophic matrix (Arakane et al., 2005 & 2008; Khajuria et al., 2010; Liu et al., 2012; Zhang 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., 2013a and 2013b; Rösner et al., 2020). 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 lac2 throughout various developmental stages. The findings in the current study are consistent with this mode of action, indicating that the upregulation of lac2 leads to changes in cuticle composition, reducing insecticide penetration, and acting as a protective mechanism against deltamethrin (Julio et al., 2017).

The present work identifies key genes encoding detoxifying enzymes and cuticle penetration genes in *C. chinensis*, including cyp450s, gst, and cares. The study reveals that differentially expressed genes (DEGs) are enriched in metabolism and information processing pathways, potentially involved in toxic mechanisms. Sublethal exposure to deltamethrin can upregulate or downregulate p450s, cests, gsts, and abc transporters. These findings are crucial for understanding detoxification-related genes in *C. chinensis* and elucidate the systematic toxicity processes triggered by deltamethrin, affecting environmental management and hazards. However, most genes' contributions require further analysis. Rna-seq technology can investigate molecular pathways behind adverse effects of pesticides. The adaptation of insects to pesticides involves a complex interplay of detoxification enzymes, transcription factors, and transporters, and understanding these mechanisms is crucial for effective pest management and the development of more sustainable pest control strategies.

In summary the current work sheds light on the molecular mechanisms underlying the toxic effects of deltamethrin on C. chinensis. It highlights the significance of various metabolic pathways, including carbohydrate, amino acid, lipid, energy, and xenobiotic metabolism, in the insect's response to deltamethrin, as well as the involvement of detoxification mechanisms and signal transduction pathways. Further research is necessary to fully understand these specific processes and their roles in insecticide resistance.

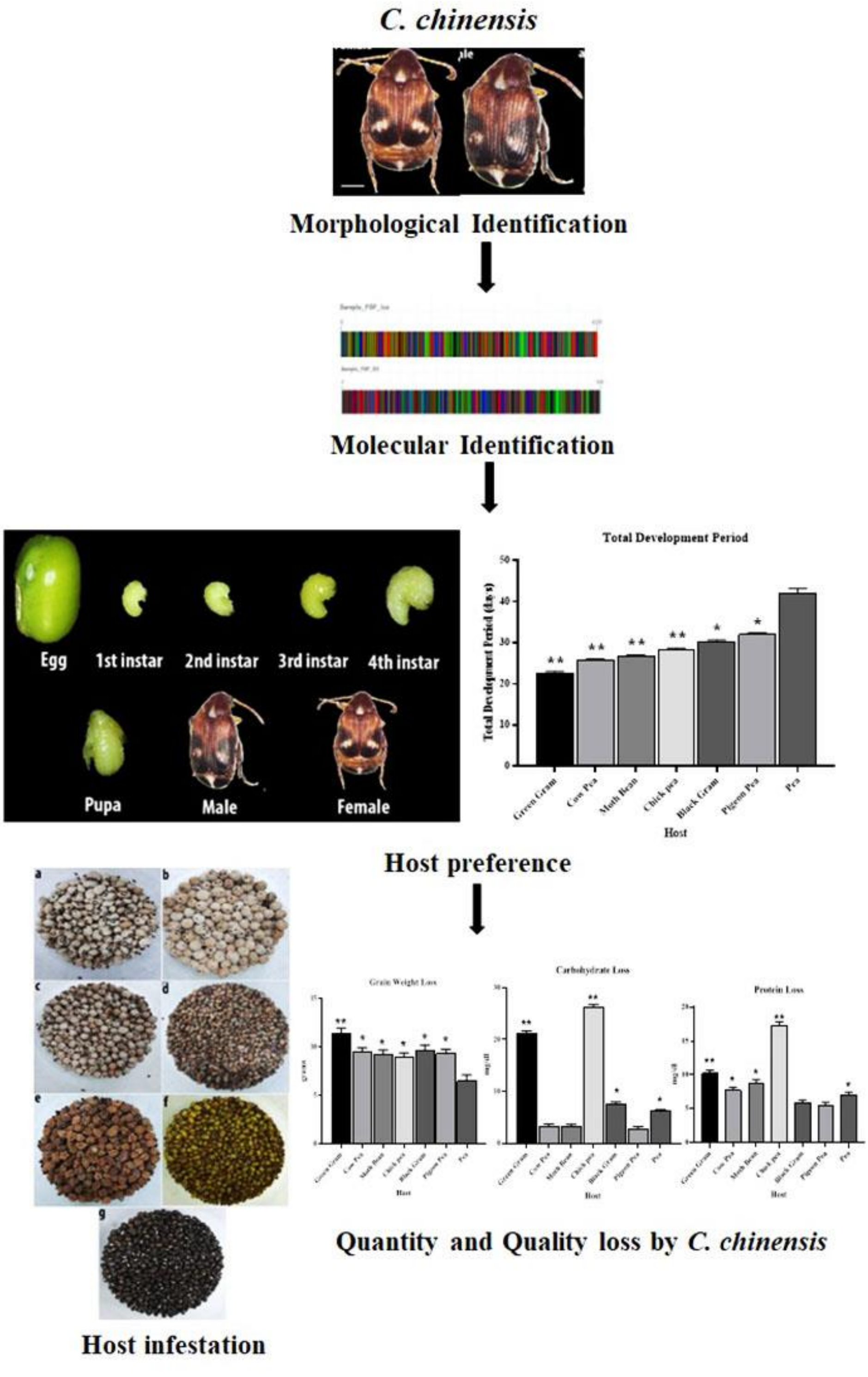

Figure GC I: The graphical for chapter 1 depicts the host preference of *C. chinensis*. Green gram was found to be most preferred host with lowest total development period and giving highest number of eggs, adult emergence, adult longevity along with the second highest in quantity and quality losses.

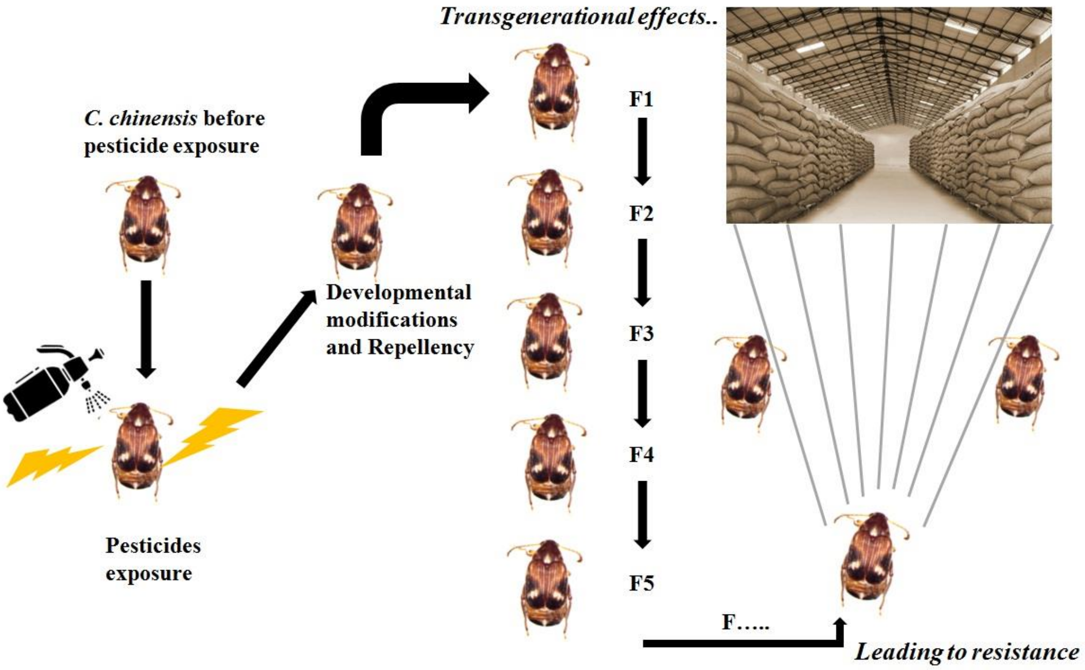

Figure GC II: The graphical for chapter 2 depicts the toxic transgenerational effects of deltamethrin on the developmental processes and repellency behaviour of *C. chinensis*. In the early generations (F1 and F2), the effects were most pronounced. As subsequent generations progressed, an increasing level of tolerance to deltamethrin was seen. This observation indicates that *C. chinensis* have the ability to adapt and develop resistance to deltamethrin.

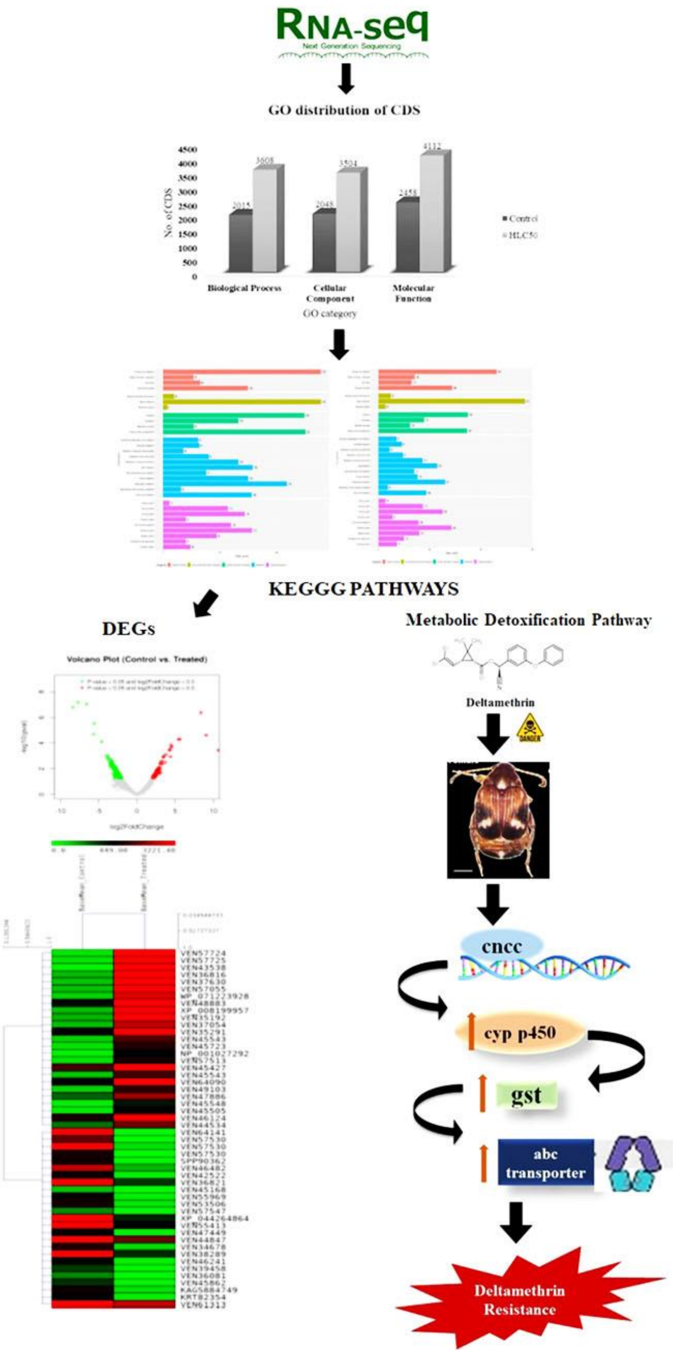

Figure GC III: The graphical for chapter 3; The *de novo* transcriptomic study of *C. chinensis* detected over 13,000 genes involved in biological, cellular, and molecular processes, with more gene counts in the insecticide-treated group. The KEGG pathway identified 31 pathways, including metabolism, genetic information processing, cellular processes, environment information processing, and organismal system pathways, potentially involved in insecticide resistance mechanisms. Significant differentially expressed genes (DEGs) in these pathways were further validated by RT-PCR and similar trend was confirmed.

Future prospects and Recommendations:

1. The present study has identified the host preference by *C. chinensis* by analysing developmental parameters and loss in nutritional content. To validate these findings, studying insect pest-host **co-evolution** will help in understanding the long-term dynamics between pests and host.
2. The current research which focuses on the deltamethrin induced transgenerational alteration repellency behaviour. The detailed **behaviour alterations** such as avoidance, foraging, or reproductive patterns, which are important parameters influenced across generations. Similarly, **Multiple generational** studies can be done to have a deeper understanding of how insecticides impact insect pest populations in the short term (**intragenerational**) and over successive generations (**intergenerational**).
3. **Scanning Electron Microscopy** (SEM) and Transmission Electron Microscopy (TEM) offer high-resolution imaging of insect anatomy and cellular structures, aiding in the examination of physical adaptations and ultrastructural changes associated with resistance and **Liquid Chromatography-Mass Spectrometry** (LC-MS) will help us to identify and quantify insecticide and metabolite levels in resistant insects this will enhance the understanding of the detoxification mechanisms.
4. Current work has focused on the **differentially expressed genes** through transcriptomic approach, to further validate the precise detection and quantification of target proteins, by western blot will help us in the characterization and monitoring of resistance traits. Further, Recent advancements in technology like (**RNAi, gene knock in/knockdown, CRISPER**) will enable researchers to understand the genetic underpinnings of resistance and develop innovative strategies to counter it.
5. **Epigenetics modifications** like histone, methylation, acetylation will provide deeper understanding of the insecticide resistance on *C. chinensis*, that can help to uncover the potential impacts and provide insights into resistance mechanisms.

Overall, the potential for further advancements in the field indicates that next research endeavours can expand upon the current findings and explore more comprehensive research pertaining to insecticide resistance in *C. chinensis,* as well as other significant storage insect pests.