DECIPHERING THE MECHANISM OF INSECTICIDE RESISTANCE IN STORED GRAIN PEST: A MOLECULAR APPROACH

Thesis Abstract

Submitted to
Department of Zoology,
Faculty of Science,
The Maharaja Sayajirao University of Baroda, Vadodara

By
Sharma Pankaj Manoharlal

Guide
Prof. Pragna Parikh

Department of Zoology, Faculty of Science,
The Maharaja Sayajirao University of Baroda,
Vadodara - 390002
Gujarat, India

Co-guide
Dr. Parth Pandya

Department of Zoology, Faculty of Science,

INTRODUCTION

By the year 2050, the world's population is predicted to reach 9.1 billion, and to feed this expanding population, food production must increase by 70% (Jiang, & Neill, 2017). In emerging nations, many of the fastest-growing populations already experience moderate to severe food insecurity and a lack of food availability. In developing nations, one in every six children experiences hunger, and the percentage of malnourishment has been continuously rising since 2015 (The State of Food Security and Nutrition in the World 2019).

In Indian scenario agriculture is the primary source of livelihood for about 58% of India's population. During 2019-20 crop year, food grain production was estimated to reach a record 295.67 million tonnes. In 2020-21, Government of India targeted food grain production of almost 300 million tonnes *(*Agricultural and Processed Food Products Export Development Authority (APEDA), Union Budget 2020-21*)*. Government of India sets a food grain production target of 328 million tonnes for the 2022-23 crop year (July-June) which is 4% more than a record foodgrain output of 315.7 million tonnes reported in the previous year.

Over 2 billion tons of grains are produced yearly for food and feed, providing two-thirds of the total direct and indirect protein intake (Erenstein et al., 2022;). A major obstacle to achieving optimal food production is the pre- and post-harvest issues, which together with insect infestation result in significant losses of grains. Before reaching the consumer, food grains must go through a number of procedures after harvesting, including threshing, cleaning, drying, storage, processing, and transportation. Food losses throughout the post-harvesting chain have been found to begin at the time of harvest and continue all the way to food marketing at the consumer's end. Technical issues include inadequate stock management facilities, poor packaging, and inadequate infrastructure resulting in grain losses (Mesterházy et al., 2020). According to UN's FAO report, globally around 1.3 billion metric tons of food, which is 33% of the total produce, is lost in the post- harvest stage and it also predicts that if the current practices continue then the loss would be around 2.1 billion metric tons by 2030 (UN FAO Report 19).

Shende, & Lifeter, (2017) that in many countries 15% of food grains are lost during or after harvest. Post-harvest grain loss in India was assessed by the FAO to be 40% and

post-harvest cereal loss to be 30% as recorded by National Academy of Agricultural Sciences in the "Saving the Harvest: Reducing the Food Loss and Waste" 2019 report. According to The monetary value of these losses amounts to more than Rs. 50,000 crores per year (Singh, 2010). As per estimates by the Associated Chambers of Commerce of India food worth 92651crore rupees is lost in post-harvest processes before it reaches the consumer (PIB, Feb 2016). A country-wide study measuring crop losses revealed that 3.9% - 6% cereals, 4.3%-6.1% pulses, 2.8%- 10.1% oilseeds, 5.8%-18.1% fruits, and 6.9%-13% vegetables were lost during harvesting, post-harvest activities, handling and storage (Jha *et al.,* 2016).

The storage losses are affected by several factors, which can be classified into two main categories: biotic factors (insect, pest, rodents, fungi) and abiotic factors (temperature, humidity, rain) (Abedin *et al.,* 2012; Lamichhane et al., 2018). Among all the biotic factors, insect pests are considered most important and cause enormous losses of around 30%–40% (Abass *et al*., 2013; Kumar and Kalita, 2017; Mesterházy et al., 2020). Insects are accountable for the deterioration of stored food/ agricultural crops, and they cause yearly losses that are estimated at about 15-25% of stored grain (Adu *et al.,* 2014; Nayak et al., 2018; Tanda et al., 2022).

Post-harvest insect pests may be primary, i.e., able to attack intact grains such as in the genus *Sitophilus*, while others are secondary pests, attacking already damaged grains or grain products such as those from the genus *Tribolium* (Adarkwah *et al.,* 2010; Tripathi, 2018). Crop damage by Lepidoptera is majorly done by the larvae (Boyer *et al.,* 2012; Ningombam *et al.,* 2017). However, in the case of Coleopterans, both larvae and adults feed on the crop/grains; and both the stages are responsible for the damage on crops (Arumugam *et al.,* 2016).

Current pest control methods are based on the use of insecticides, which are generally the most effective management tools and provide the feasible method of reducing insect pest populations to acceptable levels (Popp et al., 2013 and Dent, D., & Binks, R 2020). Studies were carried out with organochlorine and organophosphorus insecticides, which were registered for their use in the control of stored product insects. They were then replaced by pyrethroids, especially pirimiphos-methyl and deltamethrin, that were shown to be very efficient against arthropods (Chanda et al., 2013; Elzun et al., 2019). Two fumigants are currently used for the protection of stored foods: phosphine and methyl

bromide (Lee et al., 2018; Hasan et al., 2019). However, the use of methyl bromide was restricted due to its ozone depleting properties and its very high toxicity to warm-blooded animals until it was banned according to the Montreal Protocol (Alkan, 2020). Nevertheless, phosphine still remains one of the most commonly used insecticides.

In the past few years, more than 504 species of insects with insecticide resistance have been recorded, and there is still a steady increase in resistance to specific chemicals, with many species now resistant to several families of molecules for instance DDT, malathion, pirimiphos-methyl, deltamethrin and permethrin (Karaağaç, 2012; Dara, 2013 and 2016; Zhu et al., 2016; Kortbeek et al., 2019). Insects have successfully adapted to most insecticides by becoming physiologically or behaviorally resistant (Jallow et al., 2017; Nansen et al., 2016 and Dara, 2017). In postharvest ecosystems, the development of insecticide resistance is of major concern in many countries. Cases of resistance of stored products insect to grain protectants (Zhu et al., 2016; Hagstrum and Athanassiou 2019; Jian 2019) and fumigants (Bajaracharya 2013; Nguyen *et al.,* 2015; Nayak *et al.,* 2020) have been well documented.

Resistance to insecticides such as malathion in *Rhyzopertha dominica (F.) (*Ali et al 2014, Babu et al., 2017 Babu and Madhumathi 2018). Pirimiphosmethyl in *Sitophilus oryzae (F.) (*Golić*et al*., 2018) fenitrothionin, *Sitophilus zeamais (Motschulsky)* and *Tribolium castaneum* (Herbst) (Kargbo 2013; Velki et al 2014; Upadhyay et al., 2018) *S. zeamais* is resistant to DDT (Dichloro Diphenyl Trichloroethane) (Haddi et al., 2018) and deltamethrin (Guedes et al 2009 Manal et al., 2017). A few cases of organophosphate as well as pyrethroid resistance have also been reported (Muthusamy et al., 2013; Wanjala et al., 2015; Fang et al., 2019).

It has been noted that the resistance occurs in an organism when naturally occurring genetic mutations allow a small proportion of the population to resist and survive the effects of the pesticide. If this advantage is maintained by continually using the same pesticide, the resistant organisms will reproduce and the genetic changes that cause resistance will be transferred from parents to offspring (Gilleard and Beech 2007; Huijben and Paaijmans 2018). A field-based studies from Morocco have reported that the collected strains showed resistance to DDT, lindane, malathion, pirimiphos-methyl, deltamethrin and permethrin (Benhalima et al., 2014). Resistance to fumigants (phosphine, methylbromide and ethylenedibromide) has also been reported in different

parts of the world and has been well documented (Schlipalius et al., 2012; Jagadeesan et al., 2012; Kaur et al., 2012; Mau et al., 2012a & 2012b; Daglish et al., 2014; Kim et al., 2019).

At least 11 species of stored product insects are now known to have developed resistance to phosphine (Chaudhry, 2000), which has been linked to selection pressures by repeated ineffective fumigations in situations where phosphine gas was rapidly lost due to leakage (Benhalima et al., 2014). However, use of fumigants faces significant challenges, such as poor penetration through grain, significant losses to grain absorption, high concentrations of fumigant required to control insects, and flammability risks, which have limited its further development (Damalas and Koutroubas, 2016;). Moreover, there are concerns about transporting, handling, storing, and applying currently used fumigants and of insects developing resistance to them (Rajendran, 2016; Nayak et al., 2018). Insect resistance and concerns of pesticide residues in foods resulting from the use of grain protectants (Phillips and Throne, 2010; Kaushik, et al., 2018) thus demands an alternative approach for managing insect pests infesting stored products.

The work done in our lab from past few years has very well established the mechanism of insecticide toxicity on different insects as well as insect cell line (Pandya et al., 2021). Further, the successful development of primary cell line from stored grain pest (S.oryzae) for testing pesticide toxicity (Thakkar et al., 2020) has been well explored. ***However, combining the literature and lab data, there is a lacuna as far as the molecular mechanism for the pesticide resistance for the stored grain pest Callosobruchus chinensis is concerned.*** *Therefore, the present inventory was designed to understand the* ***molecular mechanism of insecticide resistance in stored grain pest*** *(C. chinensis).*

Due to resistance mechanism develop in behaviour phenotype and physiological phenotype we hypothesize that there may be alteration of genes because of continuous use of Insecticide, thus it causes behavioural and physiological resistance in stored grain pest *(C. chinensis)*.

OBJECTIVES

1. Developing the Culturing conditions of the *C. chinensis* in laboratory:
    a. Collection and identification of *C. chinensis* from ware houses of Vadodara.
    b. Studying the host preference by the insect.
2. Transgenerational effects of insecticide on the development parameters of *C. chinensis:*

a) Determine the lethal concentration of insecticide on the pest insect- Probit Analysis

b) Assessment of the transgenerational effect on the development parameters of *C. chinensis.*

c) Transgenerational effect of the insecticide on the repellency behaviour of *C. chinensis.*

3. Elucidation of Insecticide resistance using transcriptomics approach.

Material and Methods

For Objective 1:

a. Collection and identification of *C. chinensis* from ware houses of Vadodara.

The insects were collected from government ware houses located in and around Vadodara and morphological identification was done up to the species level with the help of standard taxonomic keys and by comparing with the specimens in Department Repository. Molecular identification was done using marker gene- COI, which has been found to be an important gene for species identification and has been the most widely used for DNA barcoding (Mandal et al., 2014). For the present study, genomic DNA samples were prepared from fresh insect. Total genomic DNA was extracted from the dissected femoral muscle of dung beetle using the phenol chloroform method (Huang et al., 1999) and DNA quantification was done using the nanodrop and quality was assessed by running agarose gel electrophoresis. Further, the extracted DNA was used for PCR amplification of COI gene using primers (Table 1). The amplified DNA was assessed by conducting agarose gel electrophoresis followed by Sanger sequencing and Barcoding. The obtained sequence will be further uploaded on NCBI.

Table 1: Primers of COI genes obtained

DNA marker: Cytochrome *c* oxidase subunit I primers	**Primer sequence (5' to 3')**	**Reference**
LCO-1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
HCO-2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994

Animal maintenance

Pulse Beetle, *C. chinensis* host preference on different grains was studied under laboratory conditions at the Zoology Department, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat. Initially the pulse beetle, *C. chinensis* were collected from the warehouses of Vadodara and identification of the species was confirmed using standard taxonomic keys.

After confirming the identification, *C. chinensis* were reared in laboratory conditions for at least three months before starting the final experiment, this was the stock culture. the stock, culture was reared on chick pea sees in plastic jars covered with mesh lids. The cultures were kept under 26^0-28^0C and 60-70% RH, and 12-hour photo period (Thakkar and Parikh 2018). In this way a fresh culture was developed in laboratory and this fresh culture and was used in all further experiments.

Figure 1: Collection and Maintenance of *C. chinensis*

a. Studying the host preference by *C. chinensis* on different host

Experimental regime

The grains for the final experiments were purchased from the local market of Vadodara and disinfested by fumigation. The host preference for development by *C. chinensis* on different grains *viz*. Green gram, Moth bean, Chickpea, Cowpea, Pigeon pea, Black gram and Pea was carried out under laboratory conditions. 10 pairs of freshly emerged (1-2 days old) *C. chinensis* were released in plastic jars containing 50g each of all hosts respectively. The jars were covered with muslin clothes. These jars were maintained at 26^0-28^0C, 60-70% RH and 12-hour photo period, and they were allowed to mate for ten

days, whole set up was replicated three times. Statistics of the following parameters was analysed till the termination of the experiment.

Figure 2: experimental regime for host preference

Total number of eggs: To determine the total number of eggs, the eggs laid on different host were counted using magnifying glass and recorded after 10 days and this was considered as the oviposition period of the pulse beetle.

Egg laying percentage: It was calculated by the formula (Giga and Smith 1987).

$$\text{Egg laying (\%)} = \frac{\text{No. of eggs laid in specific variety}}{\text{Total no. of eggs laid in all varieties}} \times 100$$

Hatching percentage: It was calculated by the formula

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

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

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

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

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

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

Weight loss percentage (%): It was calculated by the formula (Jaiswal et al., 2019).

$$\text{Weight loss (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Statistical Analysis

This study was in a Completely Randomized Design (CRD) by three replications. Statistical analysis was done by analysis of variance (ANOVA) with GraphPad prism 6.0v.

For Objective 2:

a. Finding out the LC_{50}

A survey was conducted in different Insecticide shops and ware houses to find the usage of different insecticides. The unworked or least explored insecticide was taken into the account. A type II semisynthetic pyrethrin insecticide, technical grade deltamethrin (98% AI, Sigma Aldrich, Saint Louis, MO) was used. To determine contact toxicity of deltamethrin against *C. chinensis*, five concentrations of deltamethrin, 6.25, 12.50, 25, 50 and 100 respectively were tested. These concentrations were obtained by dissolving 98% deltamethrin in acetone. A stock solution of 1,000 ppm was prepared from which other desired concentrations (serial dilutions) were prepared. There were three replicates for each treatment in addition to controls. One mL of each concentration was placed on the bottom of each Petri dish (9 cm diameter). After the acetone was evaporated, 20 adult of pulse beetle were placed into each dish. The same procedure was used for the control treated with acetone. Mortality percentages were recorded after 24hrs, 48hrs, 72hrs and 96 hrs of treatment. Thereafter Probit analysis was performed to obtain the LC_{50} value.

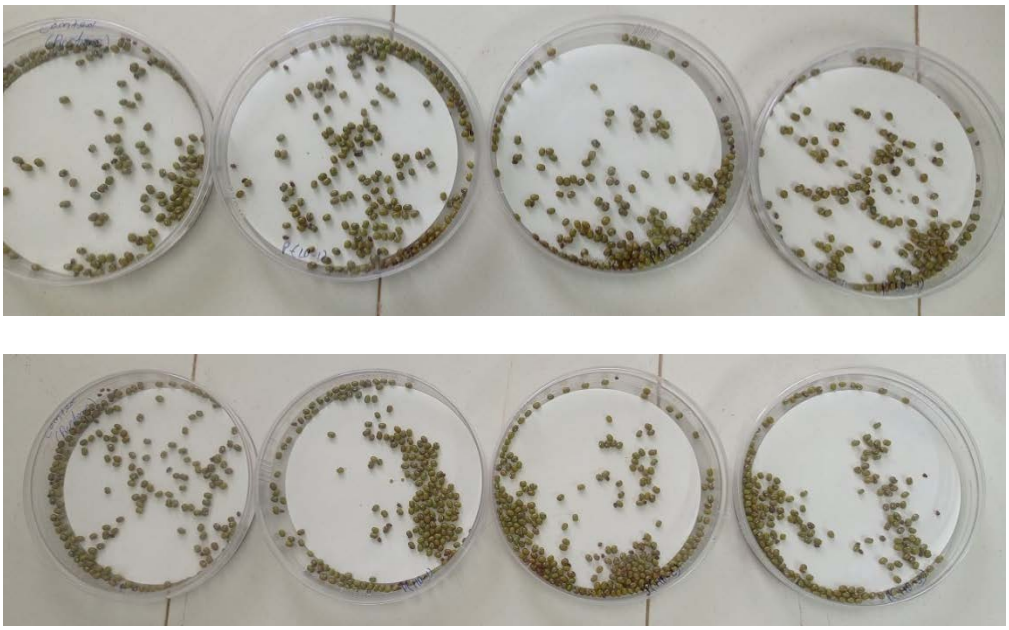

Figure 3: Experimental setup for obtaining LC_{50}

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

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

One mL of LD, HD was placed on the bottom of each Petri dish (9 cm diameter). After the acetone was evaporated, 10 pairs of freshly emerged (1-2 days old) *C. chinensis* were released in glass petri dish containing 50g host. These petri dishes were maintained at 26^0-28^0C, 60-70% RH and 12-hour photo period, and they were allowed to mate for 7 days, whole set up was replicated three times. The same procedure was used for the control treated with acetone. After the period of seven days insects were scarified for further studies.

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

Total number of eggs: To determine the total number of eggs, the eggs laid on different group were counted using magnifying glass and recorded after 7 days and this was considered as the oviposition period of the pulse beetle.

Hatching percentage: It was calculated by the formula

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

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

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

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

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

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

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

SI = LogF/DME X 100

Where,

SI: susceptibility index

Log: Logarithm

F: Total number of first-generation progenies

DME: generation development time.

The susceptibility index ranged from 0-11

0 - 3 = maximum susceptibility

4 - 7 = moderately susceptibility

8 - 10 = Less susceptibility

11 = Least susceptibility

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

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

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

calculated based on the formula:

$$PR = [(N_C - N_T) - (N_C + N_T)] \text{ X } 100$$

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

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

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

$$RI = \frac{2G}{G} + P$$

Where G = number of insects on treatment side

P = Number of insects on control side

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

For objective 3

a. Physiological resistance

The insects which were sacrificed their mid gut and fat body tissues dissected from adult and further used for RNA isolation.

RNA isolation:

Total RNA was isolated from 100 mg dissected tissues (pullin out the tissue from the insects). Tissues was homogenized using homogenizer in 500 µlTRIzol reagent (Invitrogen). The homogenate was taken into 2 ml micro centrifuge tubes (Tarsons). After successful homogenization, equal volume of Trizol reagent was added. For complete dissociation of nucleoprotein complexes, homogenized samples were incubated for 5 minutes at room temperature. The incubation was followed by the addition of 400ul chloroform and was vigorously shaken for effective mixing of both the solutions. The samples were kept at room temperature for 5 minutes till the aqueous and organic layers are distinct. Thereafter, the tubes were subjected to centrifugation at 12,000x g for 15 minutes at 4°C. The mixture was separated into a lower red phenol-chloroform phase, an interphase, and a colourless upper aqueous phase. An aliquot of upper aqueous phase will then be transferred into a new 1.5 ml micro centrifuge tube. Precipitation was done by adding 500 µl of isopropanol to the transferred supernatant. The samples were kept in -20°C for 10 minutes, centrifuged at 12,000x G for 15 minutes at 4°C. After precipitation the supernatant was discarded without disturbing the pellet and was washed in 1000 µl of 75% ethanol and then 300 µl absolute ethanol was added to the pellet. Effective mixing was done by gentle inversion and was further subjected to centrifugation at 7,500 x g for 7 minutes at 4°C. The pellets were air dried and was resuspended by adding 50 µl of DEPC water (Diethylpyrocarbonate) and was incubated at room temperature for 10mins.

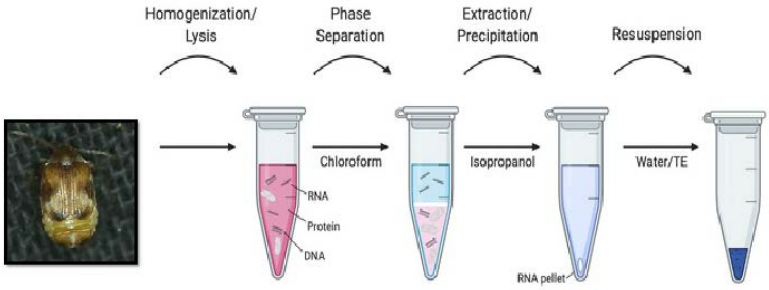

Figure 4: Steps in RNA isolation

Quantification of RNA:

Prior to quantification, DNAase (Thermo Scientific) treatment was performed. 1 µg of 10X RNA reaction buffer with MgCl2 was added to the tube and incubated at 37 °C for 30 min. Then after 20 µl chelating agent EDTA added in 50 mM concentration and will incubate at 65°Cfor 10 min in water bath as RNA get hydrolyzed during heating with divalent cations in the absence of a chelating agent. RNA quantification was done by taking A260/A280 ratio using Perkin Elmer spectrophotometer. This ratio reveals the presence of contaminants and give evidence of possible degradation. An A260/A280 ratio of 1.8 is considered acceptable for RNA. 5µl of template RNA was aliquoted and added to 1.5ml micro centrifuge tube. To the aliquot, 995µl of nuclease free water was added and absorbance was measured at the mentioned ratio against the blank having1000µl of nuclease free water.

The concentration of RNA was done using following standard formula:

1 OD260 corresponds to 40 µg/mL of RNA.

Amount of DNA (µg/mL) (ng/µL) =OD at 260nm × 40 × dilution factor

Dilution Factor generally taken is 200.

cDNA Synthesis:

After the purity check of RNA was validated using spectrophotometer, the RNA template then reversed transcribe to form cDNA from tissue. A cDNA kit employed from Thermo scientific- AB-1453/A. Briefly, fresh nuclease free PCR tubes (Tarsons) was taken, in which 4µl of 5X cDNA synthesis buffer, 2µl dNTP mix, 1µl of RNA primer (oligonucleotides),1µl of RT enhancer, 1µl of verso enzyme mix, 1-2µl of RNA template (according to the spectroscopic quantification i.e., 1 ng) and the final assay volume was made to 20µl using nuclease free water. The tubes effectively mix by giving a short

centrifuge spin for 30s at around 2000 xg. The tubes containing the kit mixture was PCR amplified by 2 step reaction process. Firstly, the 1 cycle of cDNA synthesis was carried out at 42°C for 30 mins followed by 1 cycle of inactivation at 95°C for 2mins.

Rna-seq:

The synthesized cDNA was subjected to end repair and a-Tailing processes before ligation of the adaptors. The end products were purified using a 2 % TAE-agarose gel and enriched by PCR to create the final cDNA library with sequences of approximately 300 bp. After detection using an Agilent 2100 Bioanalyzer, the cDNA library clusters were generated by cBot machine (Illumina, San Diego, CA, USA) and then sequenced using an Illumina miSeq^{TM} 2000 (Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

Prior to assembly, the raw reads were cleaned by removing adapter sequences through the standard Illumina pipeline including the CASSAVA program (http://support.illumina.com/sequencing/sequencing_software/casava.ilmn). Low quality reads (those with quality value less than 20) and reads containing N (N represents ambiguous bases in reads), length less than 35 bp were filtered by a sliding window approach, the window size is 5 bp.

Bioinformatic analysis

The assembled unigenes were searched against the NCBI nr sequence database (ftp://ncbi.nih.gov), the Swiss-Prot database (http://web.expasy.org/docs/swiss-prot_guideline.html), kyoto encyclopedia of genes and genome (KEGG, http://www.genome.jp/kegg/), cluster of orthologous groups (COG) and eukaryotic orthologous groups (KOG) (ftp://ncbi.nih.gov/pub/COG/COG) with the BLASTX algorithm (accessed in Sept 2012). The E-value cut-off was set at 10^{-5}. Genes were tentatively identified based on the best hits against known sequences. Blast2GO was used to predict the functions of the sequences, to assign gene ontology (GO) terms (http://www.geneontology.org/), and to predict the metabolic pathways in COG and KEGG databases. Amino acid sequences were deduced by using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and GENSCAN (http://genes.mit.edu/GENSCAN.html). The putative protein sequences were used for alignment by ClustalX (v1.83) program.

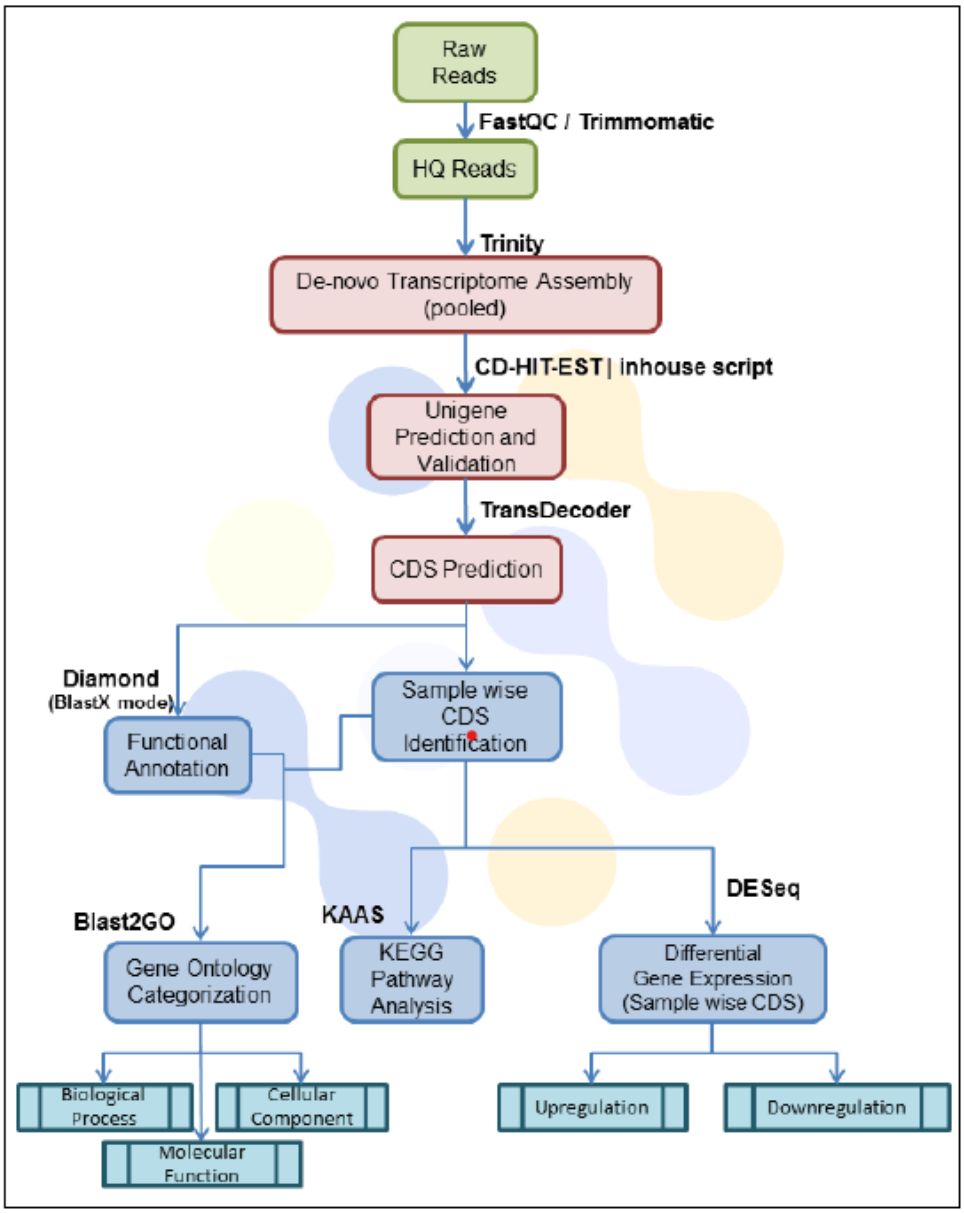

Figure 4: Bioinformatics workflow

RESULTS

Objective 1: Morphological Identification:

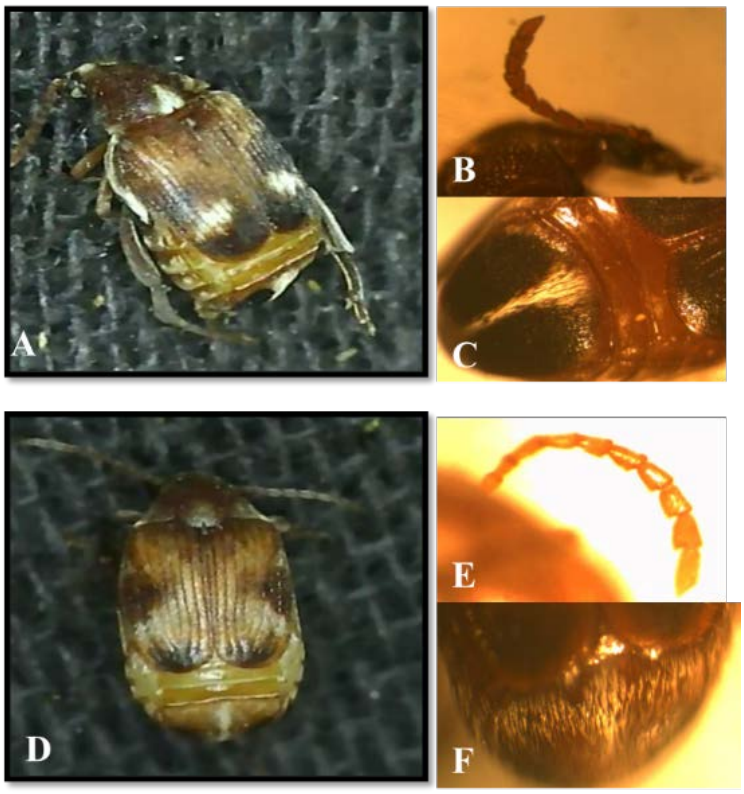

Figure 5: Morphology of *C. chinensis*

Morphological identification (Fig. 5) was done using standard references and the characteristic features of *C. chinensis* are as follow:

- Female antennae are of serrate type; antennae usually with segments 4-11while Male antennae pectinate segments 4-10.
- Female genitalia: median lobe less elongate, apex valve spearhead-shaped, and base with two sclerotized plates;
- Male genitalia: median lobe more elongate, apex with exophallic valve spearhead-shaped, and base with two sclerotized plates.

Molecular Identification:

DNA quantification

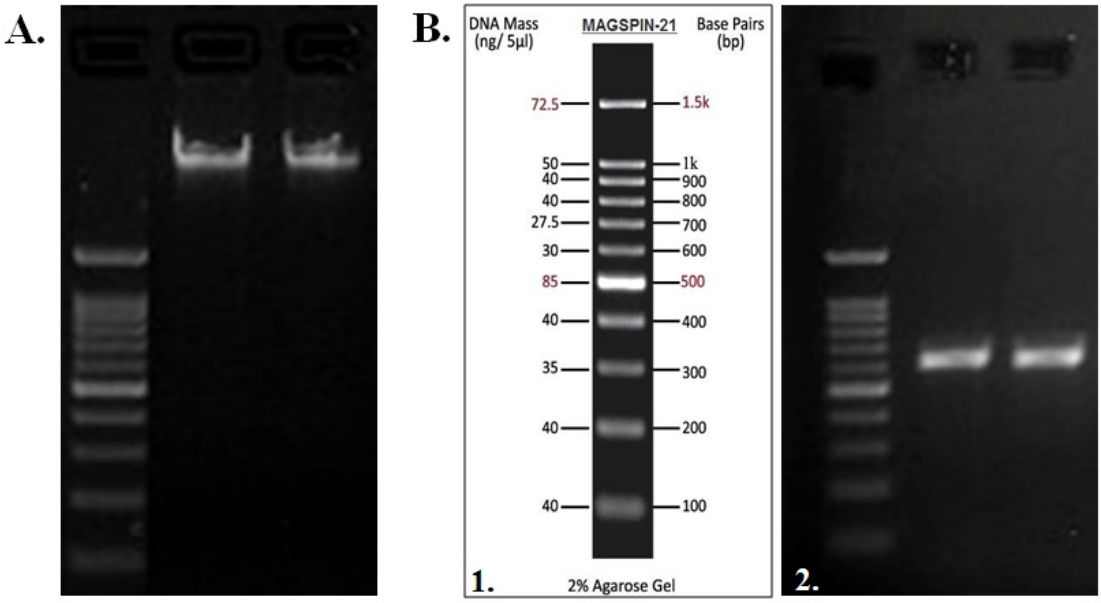

Figure 6: Agarose Gel Electrophoresis. A. Genomic DNA B. PCR Product 1. Base pair size 2. PCR product of COI

Bands of Genomic DNA are shown in Fig. 6A and that for the COI gene in Fig. 6B. The COI gene consisted of 85ng/5µl DNA mass with a 500bp length, when run on 2% Agarose gel. Further, the barcode (Fig. 7) and sequence of amplified COI gene was obtained which showed similarity with the pulse beetle species, *C. chinensis*, on NCBI Blast, confirming its identity.

Barcode and sequence of COI gene

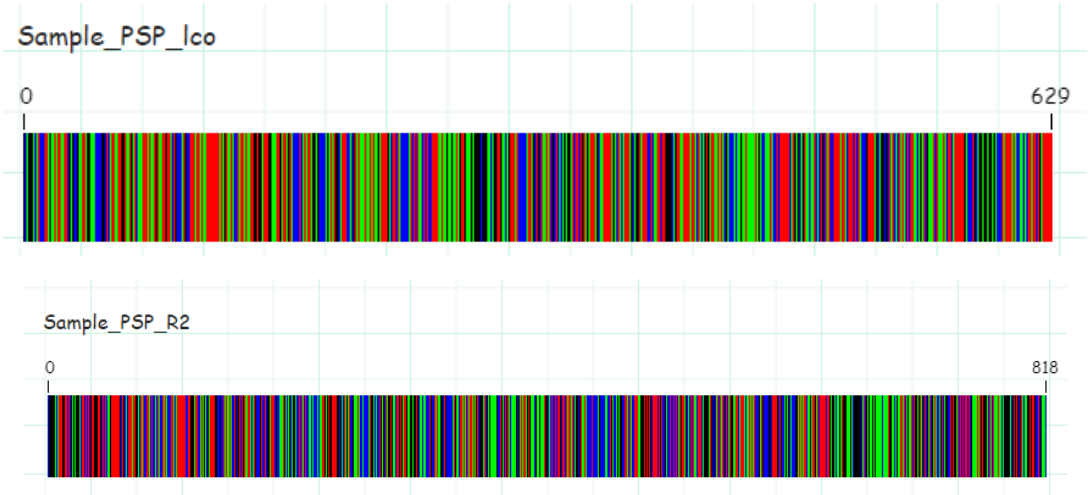

Figure 7: DNA barcode of *C. chinensis*

0722_276_003_PCR_PSP_lco

GCAGGGACATCCCTTAGAATATTAATTCGAGCCGAATTAGGAAACCCCGGCTCATTAATTGGT
AATGATCAAATTTATAATGTAATTGTTACTGCCCATGCCTTTATTATAATTTTTTTTATAGTAAT
ACCTATTATAATTGGTGGATTTGGAAATTGATTAGTACCCCTCATATTAGGAGCCCCAGATATG
GCATTTCCACGTATAAATAATATAAGATTTTGACTTCTACCCCCATCTCTAACTCTCCTTTTAAT
AAGAAGAATAGTTGAAAGAGGGGCGGGAACAGGTTGAACAGTTTATCCCCCATTAGCTGCTA
ATATCGCTCACAGAGGATCTTCTGTAGATTTAGCAATTTTTAGACTACATTTAGCAGGTATTTC
CTCAATTCTTGGGGCAGTTAATTTTATTACTACAGTGATTAATATACGACCCACAAGAATAAA
AATAGACCAAATACCTCTTTTTTCATGAGCAGTAACAATCACTGCTATCCTTTTATTACTTTCTC
TACCCGTTTTAGCAGGCGCTATCACTATACTCTTAACAGATCGAAATCTAAATACTTCATTTTT
TGACCCTGCAGGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTTTTT

0722_276_004_PCR_PSP_R2

TGCGAAAAATATCGGATCTCTTCCTCCTGCTCGGTCTGCAAGGACGTATTTATATTTTTTTCTGT
TCCGAGTTAGAGATAGTGCCTGCTACTCCGGCTAGAGAAAGTAATAAATGGATAACAGGGAT
AGCTACTGCTGTTGCTGAAAAAGAAATAGAGGTATTTGTATTATTGTGGTTCGTGTGTTACTCA
CAGTAATCACAGAATTAATTGCCCCAACAGTCCCGGAAATTGAGGATAAACGTGCTATATGTA
TTGTTAAATTTACTAAATATCCTCAGGATCCTCTGTGAGCGATATTAGCAGGTAATGGGGGAC
AACCTGTTCCCCCCCCTCCCTCCCCTCTTCCTCCTATTCATCGGATAAAAAGAAAAGTTGGAAA
AGGGCAAAAAATTATAAATCTTATATTAGTAATTGCCGTATCTGCCATACCTGGGGTTACGAA
TATGAGGCAAACTACTCACTCACCAATTCCACCAGGTATTATAAGAATTACTATTATAATAATT
ATAAGGACGGCATCGACTACATCATTTATTTGATCATTTCCATCATTAGCGACGGGGGAGCCG
AGTTCTCCTAAATCGAATCAAATTAAGAATCTAACTGATGTCCCTGCTCTTGCGGCTCAAGCA
AAAAATAAAGAGCATATAGCTTCATTATCTTTATGATATGCTGACCAAAAAAGGGCACGCCTG
AGCACGACAACAAAATCGACAAGGTTATCGTGATATTTCCTGATACCTTGGAGATCAGAA

>***C. Chinensis*** **contig**

AAAAAATCAGAATAAGTGTTGCGTATAAAATAGCGGATCTCCTCCTCCTGCACGGTCAAAAAA
TGAAGTATTTAGATTTCGATCTGTTAAGAGTATAGAGATAGCGCCTGCTAAAACGGCTAGAGA
AAGTAATAAAAGGATAACAGGGATAGCTACTGCTGTTGCATGAAAAAAGAAATAGATGGTCT
ATTTGTATTATTGTGGGTCGTATATTAATCACTGTAGTAATCACAGAATTAACTGCCCCAACAG
TCCCGGAAATTGAGGATAATACCTGCTAAATGTATTGTCTAAAAATTACTAAATATCCTACAG
AAGATCCTCTGTGAGCGATATTAGCAGCTAATGGGGGACAAACTGTTCAACCCCCTCCCGCCC
CTCTTCCTCAACTATTCATCGGATAAAAAGAAAAGTTAGAAAAGGGCAAAGAAATCATAAAT
CTTATATTAGTAATAGCCGTGGAAATGCCATACCTGGGGCTACGAATATGAGGCAAACTAATC
AATCACCAAATCCACCAGGTATTATAATAGGAATTACTATAAAAAAAATTATAAGAAAGGCA
TCGACAGTAACAATTACATTATAAATTTGATCATTACCATCATTAGCGACGGGGGAGCCGAGG
TTCTCCTAAATCGAATCAAATTAAGAATCTAACGGATGTCCCTGCNTCTTGCGGCTCAAGCAA
AAAATAAAGAGCATATAGCTTCATTATCTTTATGATATGCTGACCAAAAAAGGGCACGCCTGA
GCACGACAACAAAATCGACAAGGTTATCGTGATATTTCCTGATACCTTGGAGATCAGAA

Host Preference

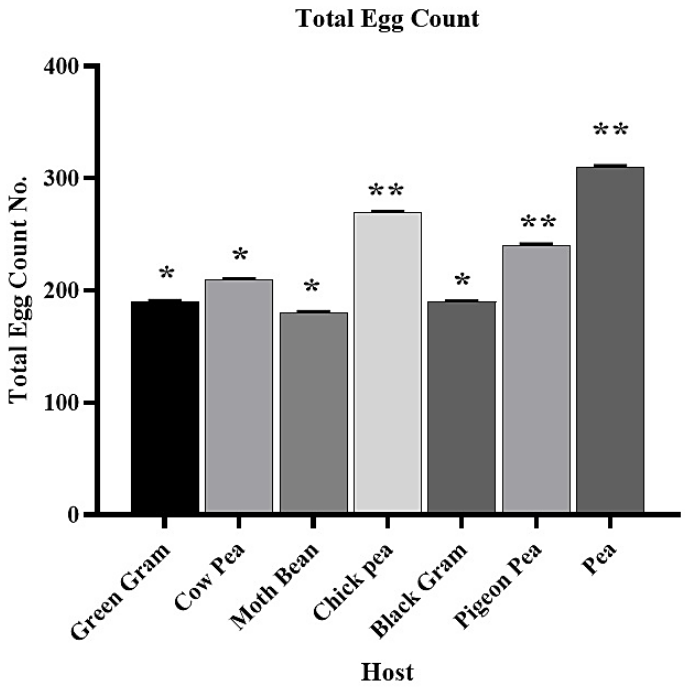

Figure 8: Total egg count of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

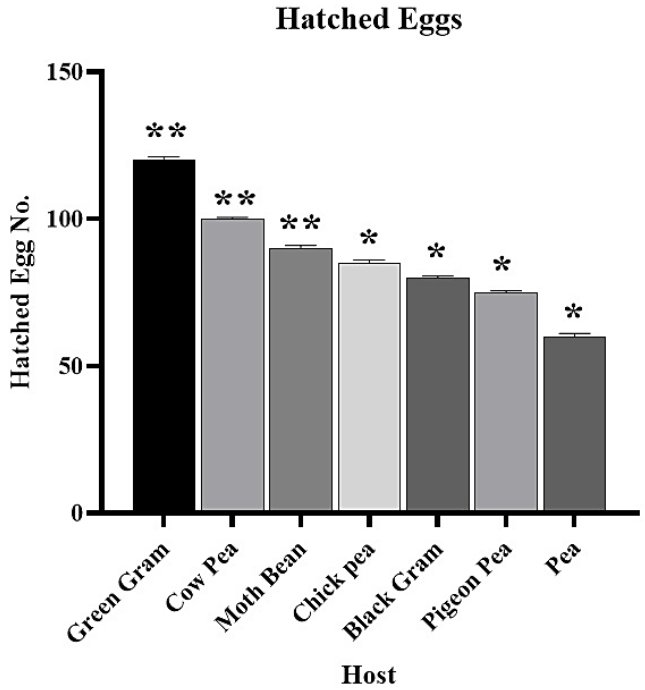

Figure 9: Total hatched eggs of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

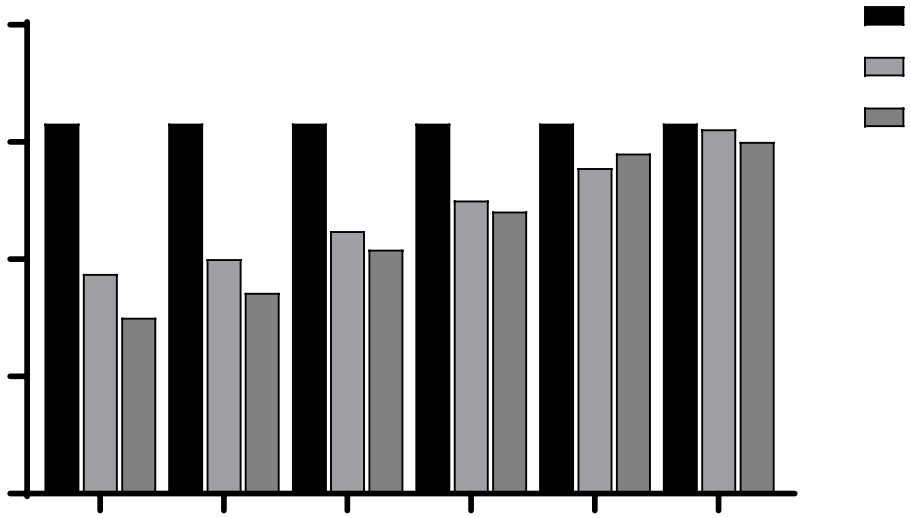

Figure 10: Egg laying % and hatching % of *C. chinensis* on different host.

Total number of eggs vary on different hosts (Fig. 8) maximum number of eggs were on Pea 310±1.15 followed by chick pea 270±0.58 and least number of eggs were counted on moth beans 180±1.15. Not much difference was observed on green gram, black gram and cow pea they were 190±1.10, 190±0.60 and 210±0.60 respectively. Fig. 9 represented that pea (19.49%) were highly preferred for egg laying followed by chick pea (16.98%) and pigeon pea (15.09%). The difference in egg laying preference in Moth Bean (11.32%), green grams (11.94%), Black Gram (11.94%), and Cow Pea (13.20%) were in a narrow range. Significant variation in hatching percentage on different host was recorded (Fig. 10), maximum hatching preference was recorded on green gram (63.16%) and least hatching preference was recorded pea (19.35%).

Table 2: Egg incubation, Larval + pupa period and total development period of *C. chinensis* on different host.

Host	Egg Incubation Period (Days)	Larval + Pupa Period (Days)	Total Development Days
Green Gram	3	20	22.67±0.33
Cow Pea	4	21	25.67±0.33
Moth Bean	4	22	26.66±0.34
Chick pea	5	23	28.33±0.35
Black Gram	5	25	30.30±0.35
Pigeon Pea	6	26	32±0.54
Pea	10	32	42±1.15

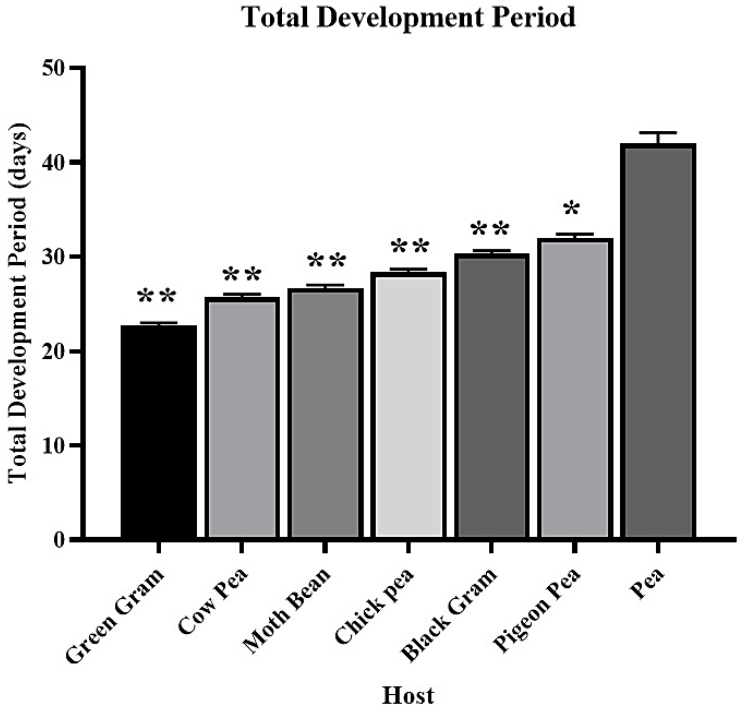

Figure 11: Total development period of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

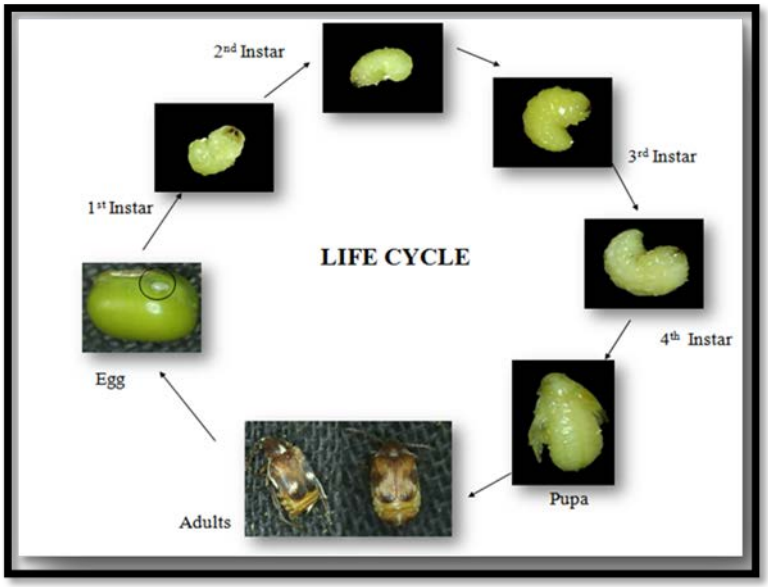

Figure 12: Stages in the life cycle of *C. chinensis*

Figure 12 depicts the lifecycle of *C. chinensis*, it was found to vary according to the hosts, it was found to be in the range of 23-42 days. After copulation, adult female was found to deposit eggs on the grains. The eggs were oval in shape, each egg was found to be tiny and white in colour. The tiny, white, fleshy, legless pest with biting jaws develops inside the grains and the fully developed adult emerged out from the seeds of grains through emergence window.

Table 2 shows that maximum period for incubation was recorded on pea (10 days) and least on green grams (3 days), incubation period on moth bean and cow pea was (4 days) while on chick pea and black gram it was (5 days). Larval + Pupal period in Table 2 represents that maximum larval pupal period was recorded on pea (32 days) followed by pigeon pea (26 days) while least was recorded on green gram (20 days) followed by cow pea (21 days). The longest total development period was observed on pea (42 ± 1.15 days) followed by pigeon pea (32 ± 0.54 days) and shortest total development period was observed on green gram (22.67 ± 0.33 days) followed by cow pea (25.67 ± 0.54 days).

Table 3: Adult longevity of *C. chinensis* on different host.

Host	Male Count	Female Count	Adult Emergence	Adult longevity
Green Gram	58	62	120±1.15	16
Cow Pea	46	54	100±0.58	14
Moth Bean	44	46	90±1.05	14
Chick pea	40	45	85±1.10	12

Black Gram	37	43	80±0.55	13
Pigeon Pea	35	40	75±0.60	11
Pea	28	32	60±1.12	09

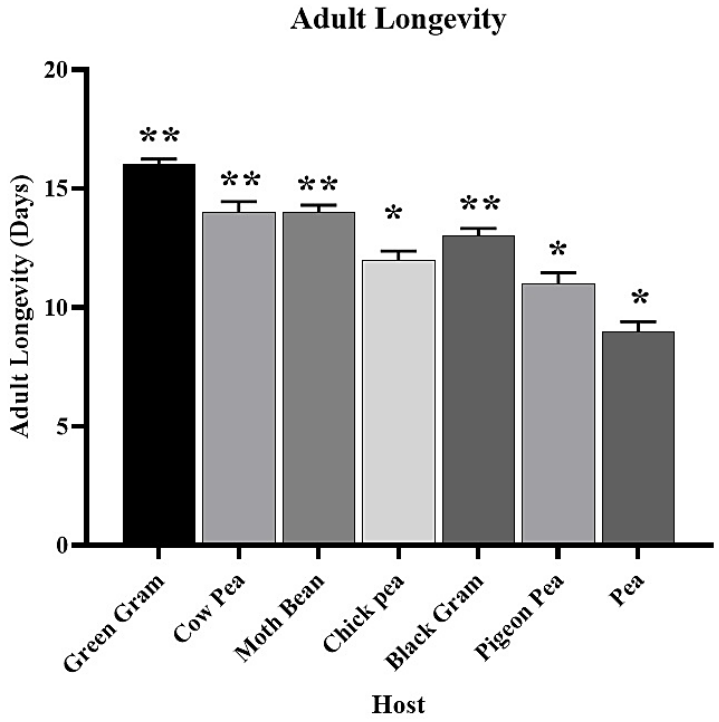

Figure 13: Adult longevity of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

Total adult emergence and adult longevity is represented in Table 4, where the maximum adult emergence was recorded on green gram (120±1.15) which consist male (58) and female (62) least emergence was recorded on pea (60±1.12) which consist females (32) males (28). The number of days that the emerged adult survive were recorded maximum on green grams (16 days) followed by cow pea and moth bean (14 days) and it was least on pea (09 days) followed by pigeon pea (11 days).

Table 4: Quantity (weight loss) and Quality losses (protein and carbohydrate loss) by *C. chinensis* on different hosts.

Host	**Weight loss (grams)**	**Weight Loss%**	**Protein Loss**	**Carbohydrate Loss**
Green Gram	11.4±0.58	27.22	10.2±0.47	21.2±0.45
Cow Pea	9.5±0.46	20.40	7.7±0.45	3.25±0.48
Moth Bean	9.2±0.50	19.48	8.8±0.46	3.20±0.47

Chick pea	9±0.42	17.12	17.34±0.49	26.30±0.51
Black Gram	9.7±0.51	18.23	5.85±0.40	7.50±0.42
Pigeon Pea	9.3±0.48	16.66	5.5±0.44	2.80±0.40
Pea	6.5±0.64	12.58	7±0.42	6.10±0.43

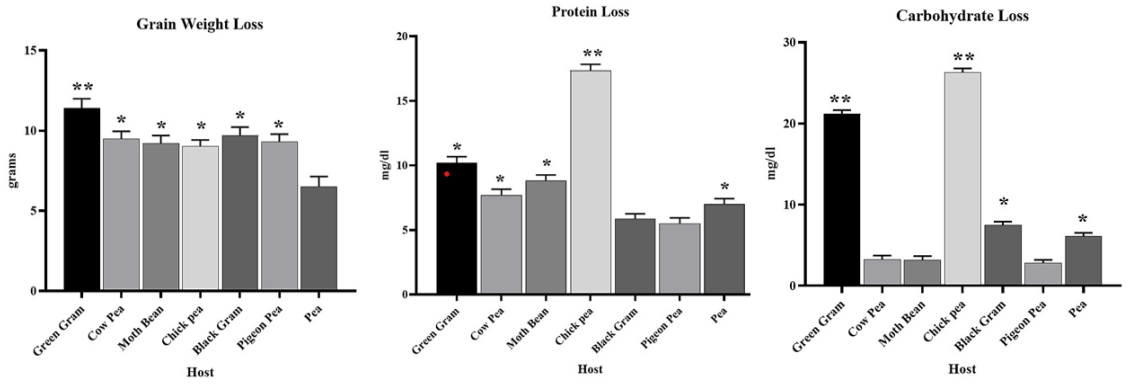

Figure 14: Quantity and Quality losses by *C. chinensis* on different host (Grain Weight loss, Carbohydrate loss, Protein loss). Significant level *($p<0.05$); **($p<0.01$)

A significant weight loss was recorded among all the hosts (Fig. 14), maximum weight loss was recorded in green gram (11.4±0.58g) (27.22%) followed by cow pea (9.5±0.46g) (20.40%) and least weight loss was recorded in pea (6.5±0.64g) (12.58%) followed by pigeon pea (9.3±0.48g) (16.66%). A significant loss in nutritional content (protein and carbohydrates) was recorded among all the hosts (Table 4), maximum protein loss was recorded in chick pea (17.34±0.49 mg/dl) followed by green gram (10.2±0.47 mg/dl) and least protein loss was observed in pigeon pea (5.5±0.44 mg/dl) similar trend was observed in carbohydrate loss which was also shows maximum loss in chick pea (26.30±0.51mg/dl) followed by green gram (21.2±0.45 mg/dl) and minimum in pigeon pea (2.8±0.40 mg/dl).

Objective 2: Transgenerational effects of deltamethrin

a) Determining LC_{50} value of Deltamethrin

LC_{50} value of Deltamethrin was determined using Probit Analysis after 96 hours of exposure of pulse beetles. The 50% probit mortality ranged between 6.25 to 100 ppm concentration (**Table 5**). LC_{50} value was obtained as 22.93 ppm from the dose response curve (**Fig. 15**). Further, the sub-lethal concentrations: Low dose (LD)-1/20th of LC_{50}, and High dose (HD)-1/5th of LC_{50} (**Table 6)** were used to understand the effects of Deltamethrin on pulse beetles.

Table 5: Probit Mortality obtained after 96 hours of exposure to Deltamethrin

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

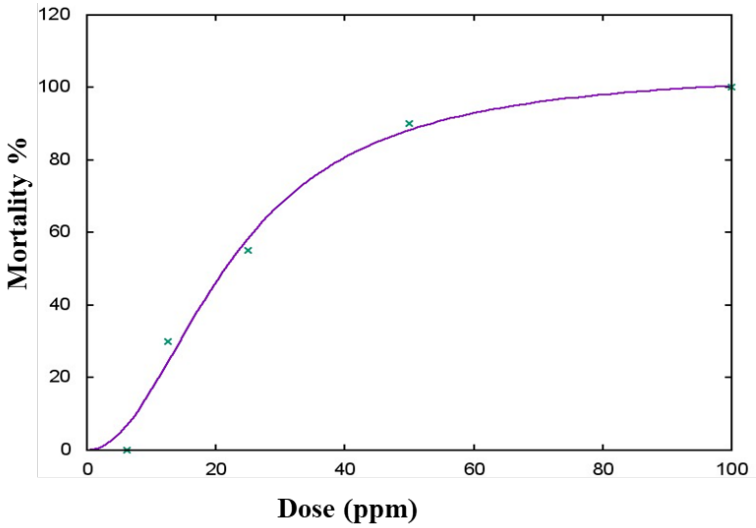

Figure 15: Dose response curve for the LC_{50}

Table 6: LC_{50} value obtained and the sub-lethal doses selected for further studies

LC_{50}	**22.93 ppm**
Low Dose (LD)	**1.15 ppm**
High Dose (HD)	**4.59 ppm**

The effect of deltamethrin on the *C. chinensis* were tested after 96hrs and as shown in Table 5. 100% mortality at higher concentration (50 ppm and 100ppm) and 0% mortality at (6.25ppm). The mortality was concentration dependent as the concentration increases it increases the mortality.

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

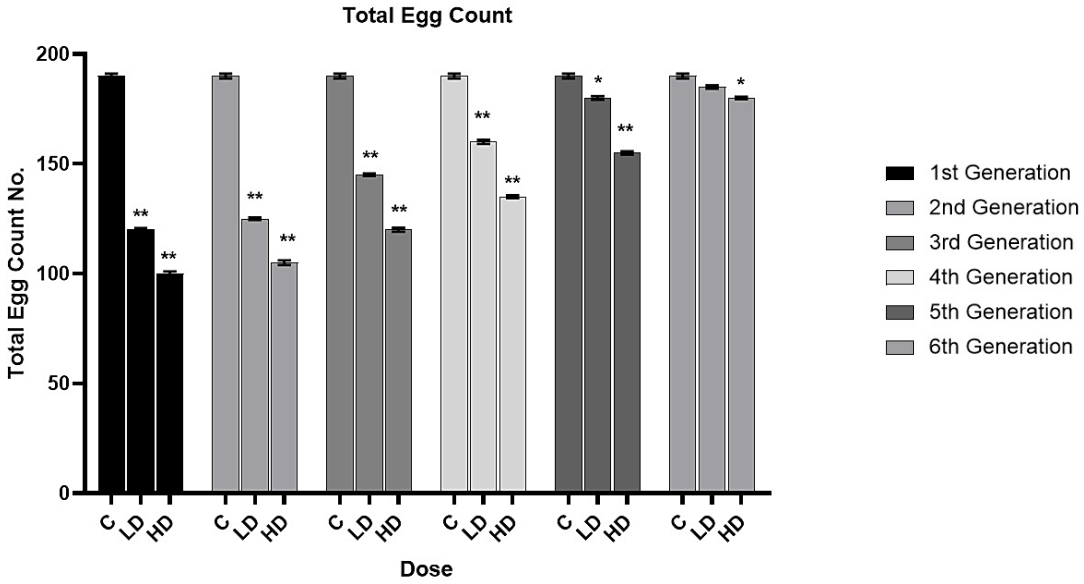

Figure 16: Transgenerational effect of deltamethrin on the total egg count of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

Total egg count significantly ($p<0.01$) varies on different group at different generations (Fig. 16). In the 1st generation effect of deltamethrin is clearly seen as the total egg count drastically decreases compared to control (190±1.10), in low dose it was 120 and high dose 100. In successive generations the total egg count slowly starts increasing compare to 1st generation and at the 6th generation the total egg count of control (190±1.10), low dose (185) and high dose (180) was almost same, which shows that the pulse beetle at 6th generation has overcome from the effect of the deltamethrin.

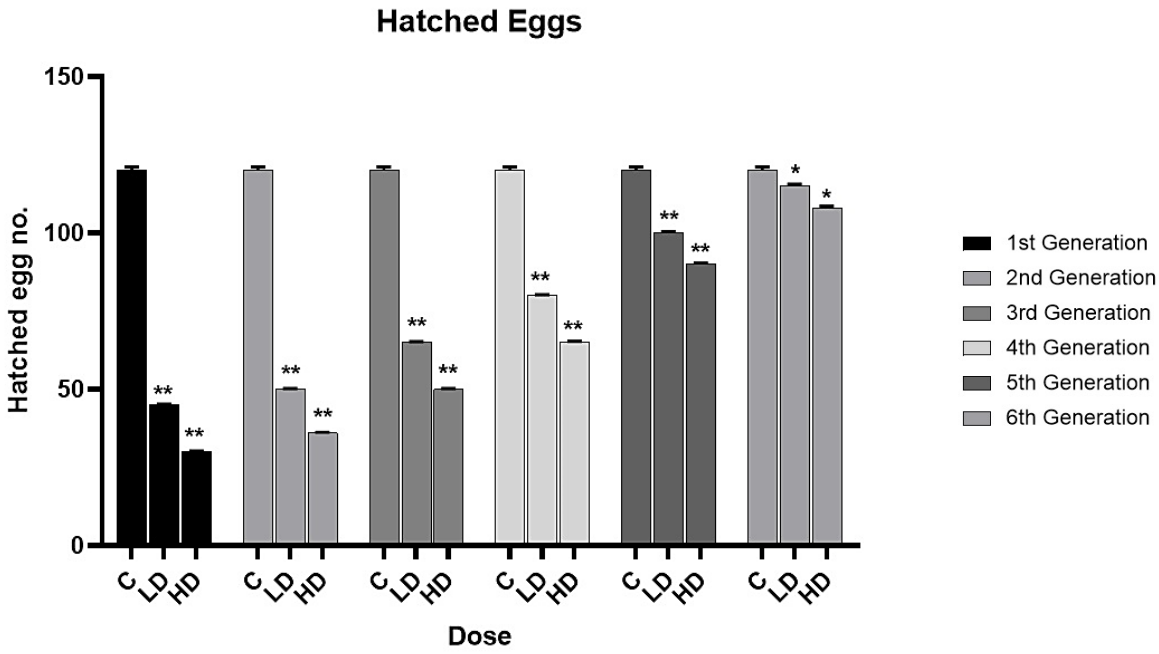

Figure 17: Transgenerational effect of deltamethrin on the total hatching of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

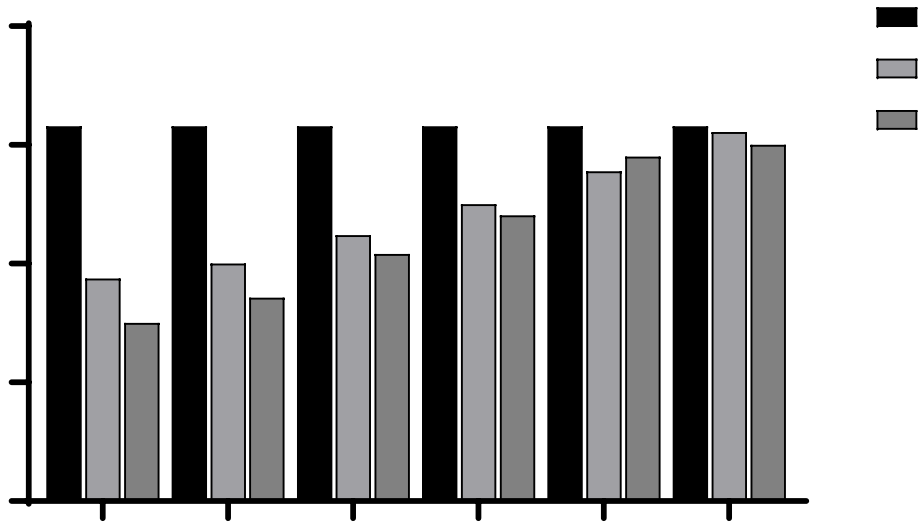

Figure 18: Transgenerational effect of deltamethrin on the hatching% of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

Total hatching show similar trend like the total egg count it also significantly ($p<0.01$) vary on different group at different generations (Fig. 17). In the 1st generation effect of deltamethrin is clearly visible as the total hatching compared to control (120±1.15), in low dose 45 and high dose 30 significantly reduced. In successive generations the total hatching slowly starts increasing compare to 1st generation and at the 6th generation the total hatching of control (120±1.15), low dose (115) and high dose (105) was very close. This trend was also seen in hatching percentage (Fig. 18) were significant ($p<0.01$) variation in hatching percentage on different group at different generation was recorded. As in successive generations the total egg count and hatching increases hatching percentage was also increased from 37.5% in 1st generation to 62.16 in 6th generation in low dose and 30% in 1st generation to 60% in 6th generation.

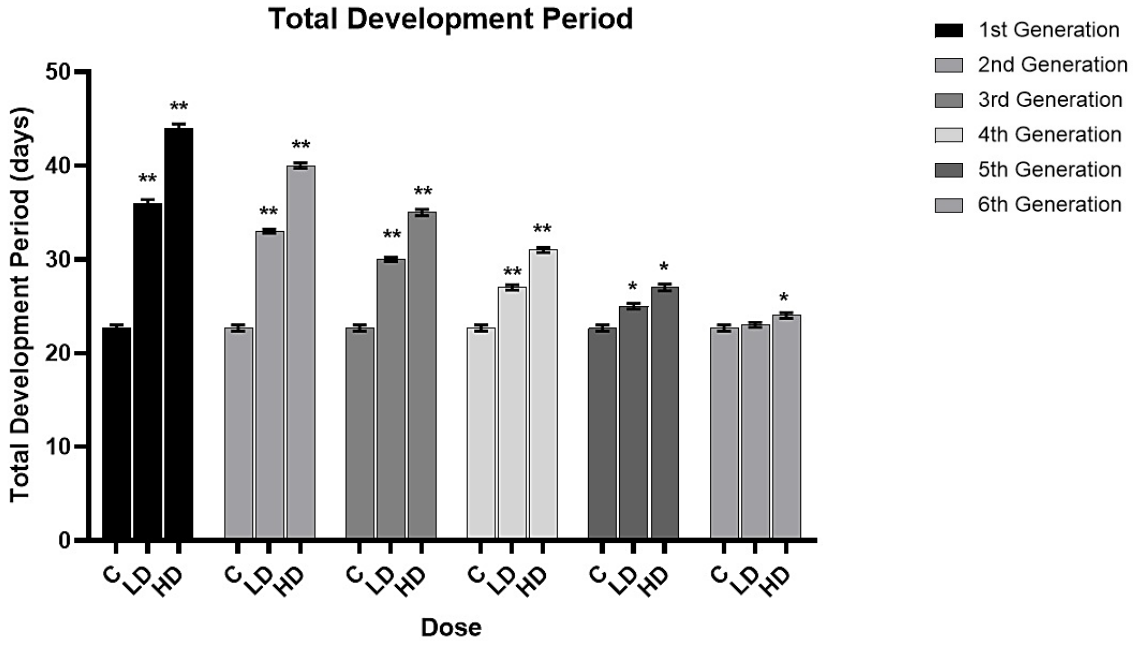

Figure 19: Transgenerational effect of deltamethrin on the total development period of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

In 1st generation the total development period (Fig. 19) was observed to be (46 days) in high dose and (40 days) in low dose which was significantly ($p<0.01$) very long compared to control (22.670.33 days). In successive generations the trend was changed and the duration of total development period shows a reducing trend in low dose and high dose at the 6th generation low dose (23 days) and high dose (24.5 days) which was almost at par with the control.

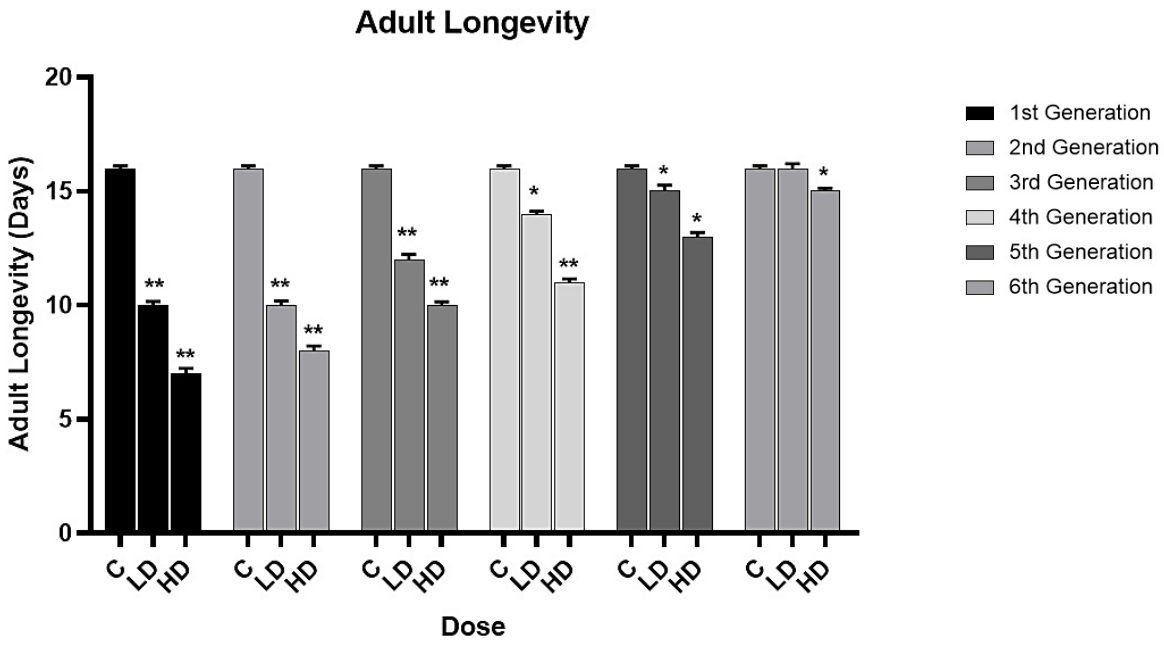

Figure 20: Transgenerational effect of deltamethrin on the adult longevity of *C. chinensis.* Significant level *($p<0.05$); **($p<0.01$)

The number of days that the hatched adult survive shows a significant ($p<0.01$) variation it was (16 days) on control, (10 days) on low dose and (7 days) at the 1st generation. In successive generations the over days of survival increases at the 6th generations it was (16 days) in low dose and (15 days) in high dose which was at par with the control (Fig.20).

Table 6: Transgenerational alteration in the Susceptibility index

Susceptibility Index			
Generations	**Control**	**Low Dose**	**High Dose**
1st Generation	9.16	4.58	3.36
2nd Generation	9.16	5.15	3.89
3rd Generation	9.16	6.03	4.86
4th Generation	9.16	7.03	5.84
5th Generation	9.16	8	7.22
6th Generation	9.16	8.96	8.25

Susceptibility index confirms that at initial generation pulse beetle were more susceptible specially at high dose of the insecticide but later the susceptibility shows a decreasing trend and at the 6th generation it shows very less susceptibility (Table 6).

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

Table 7: Percent repellency of deltamethrin against *C. chinensis* on LD exposure.

Duration of Exposure in hours							
	0 hr	**1 hr**	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**
	LD Mean Repellency %						
1st Generation	100	90	85	75	70	55	40
2nd Generation	100	85	75	68	60	45	35
3rd Generation	95	70	65	55	45	30	20
4th Generation	90	55	50	40	35	25	5
5th Generation	86	45	40	30	22	15	-10
6th Generation	80	35	25	20	15	5	-20

Table 8: Percent repellency of deltamethrin against *C. chinensis* on HD exposure.

Duration of Exposure in hours							
	0 hr	**1 hr**	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**
	HD Mean Repellency %						
1st Generation	100	92	90	82	75	60	50
2nd Generation	100	90	84	80	66	52	40
3rd Generation	100	75	72	62	50	40	30
4th Generation	94	65	55	50	40	28	15
5th Generation	90	60	50	34	30	20	10
6th Generation	85	50	35	25	20	14	-10

Table 9: Repellent Index (RI) of deltamethrin against *C. chinensis* on LD exposure.

	Duration of Exposure in hours (LD)						
	0 hr	**1 hr**	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**
1st Generation	--	0.10±0.06	0.15±0.02	0.25±0.15	0.30±0.10	0.45±0.04	0.60±0.10
2nd Generation	--	0.15±0.10	0.25±0.06	0.32±0.10	0.40±0.06	0.55±0.10	0.65±0.16
3rd Generation	--	0.30±0.08	0.35±0.12	0.45±0.10	0.55±0.18	0.70±0.08	0.80±0.22
4th Generation	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
5th Generation	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
6th Generation	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 10: Repellent Index (RI) of deltamethrin against *C. chinensis* on HD exposure.

	Duration of Exposure in hours (HD)						
	0hr	**1 hr**	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**
1st Generation	--	0.08±0.02	0.10±0.04	0.18±0.12	0.25±0.14	0.40±0.2	0.50±0.12
2nd Generation	--	0.10±0.04	0.16±0.06	0.20±0.08	0.34±0.05	0.48±0.12	0.60±0.16
3rd Generation	--	0.25±0.1	0.28±0.1	0.38±0.12	0.50±0.08	0.60±0.06	0.70±0.04
4th Generation	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
5th Generation	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
6th Generation	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

Table 7 and 8 describes the repellent percentage and Table 9 and 10 shows the repellent index of deltamethrin which shows that *C. chinensis*, maximum repellency was observed in the 1st generation and the repellency was decreasing in successive generations. Similar trend observed in the repellent index where initially it was repellent but in later on generations it become neutral to attractant. The repellent effect of deltamethrin was time and generation dependent. The repellency decreases with the exposure time and following generations.

Objective 3: Understanding the Insecticide resistance using transcriptomics approach

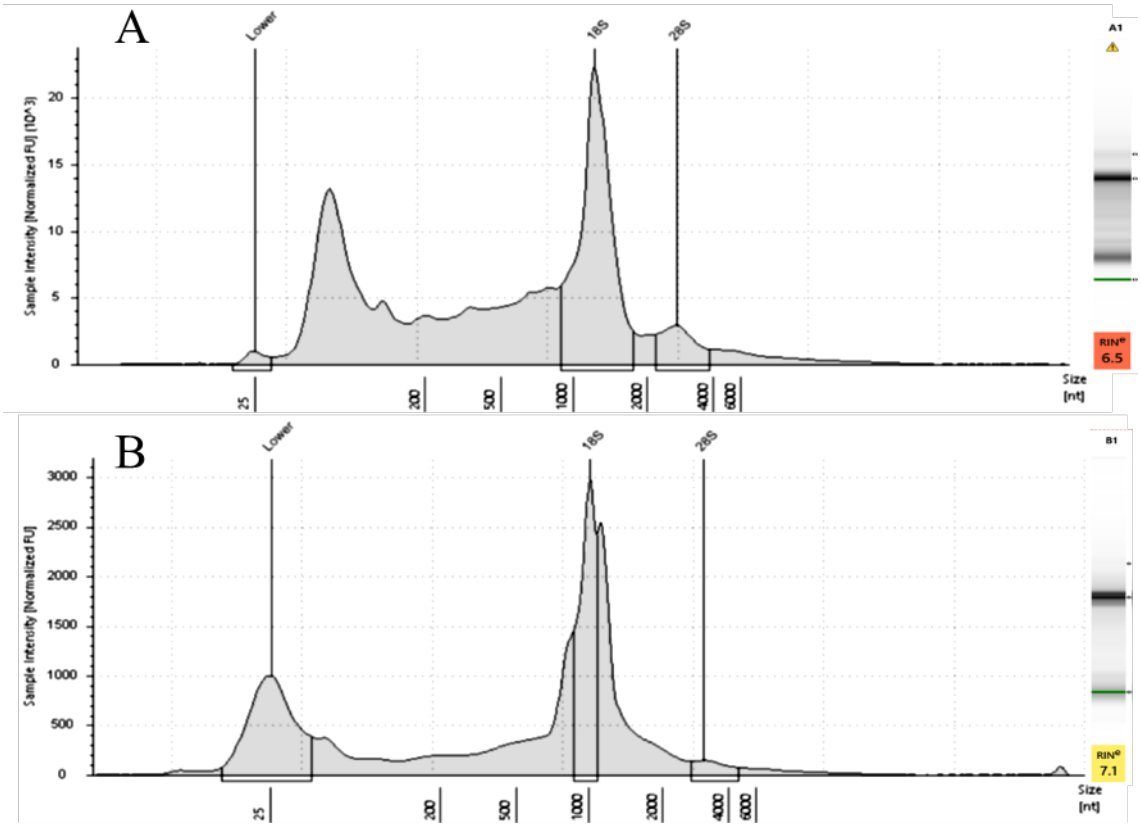

Figure 21: QC of Extracted RNA Samples on Agilent TapeStation

The quality and quantity of the extracted RNA samples were checked on NanoDrop followed by Agilent Tape station using High Sensitivity RNA ScreenTape. A single distinct 18S peak was observed for both the extracted RNA samples confirm the quality of RNA, thus graded as 'PASS' in initial QC step and can be processed for library in regular process.

Illumina PE library preparation

The RNA-Seq paired end sequencing libraries were prepared from the QC passed RNA samples using illumina TruSeq Stranded mRNA sample Prep kit. Briefly, mRNA was enriched from the total RNA using Poly-T attached magnetic beads, followed by enzymatic fragmentation, 1st strand cDNA conversion using SuperScript II and Act-D mix to facilitate RNA dependent synthesis. The 1st strand cDNA was then synthesized to second strand using second strand mix. The dscDNA was then purified using AMPureXP

beads followed by A-tailing, adapter ligation and then enriched by limited no of PCR cycles.

Quantity and quality check (QC) of library on Agilent 4200 Tape Station

The PCR enriched libraries were purified using AMPureXP beads and analyzed on 4200 Tape Station system (Agilent Technologies) using high sensitivity D1000 Screen tape as per manufacturer instructions.

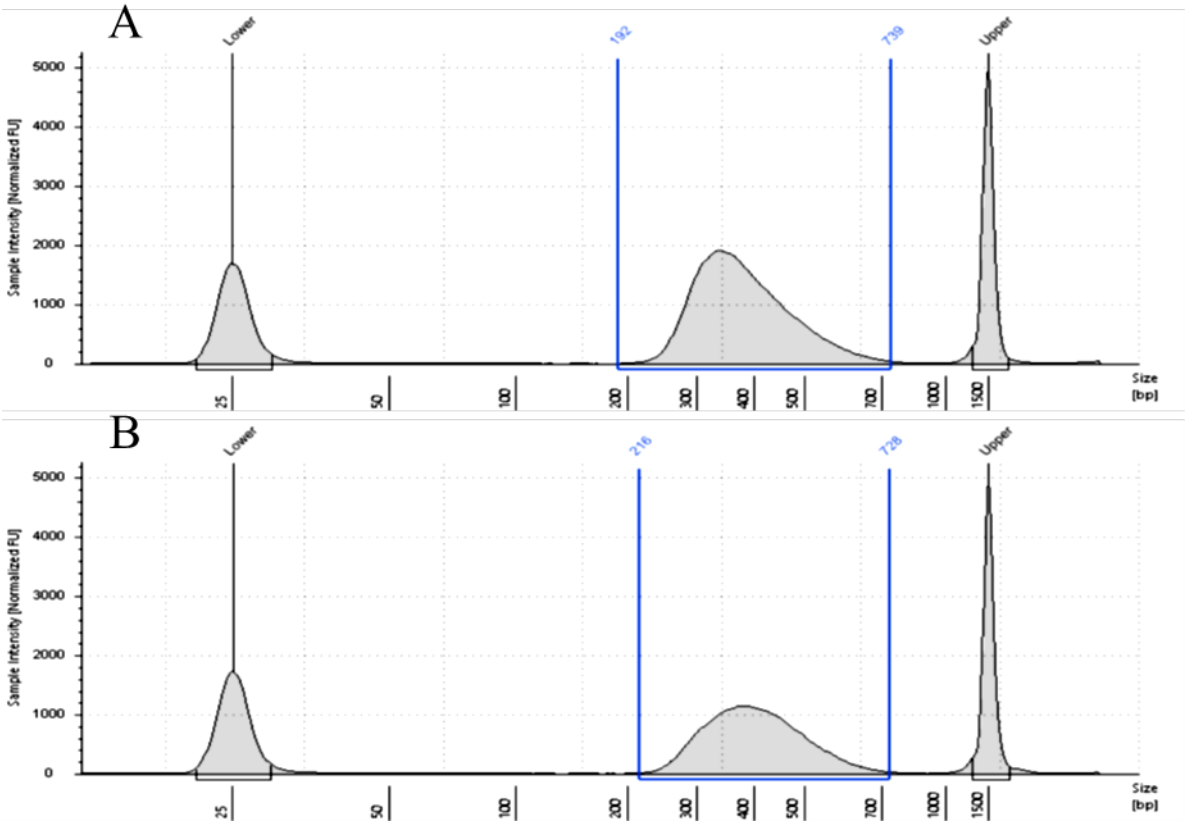

Figure 22: Library Profile of Samples: Control and Treated on Agilent TapeStation

Total RNA was extracted from the received insect tissue using conventional TRIzol method followed by Column purification using Quick RNA Miniprep Plus Kit (Zymo Research). The quality and quantity of the extracted RNA samples were checked on NanoDrop followed by Agilent Tape station using High Sensitivity RNA ScreenTape. The QC passed RNA samples were processed for PE library preparation using TruSeq stranded mRNA Library Prep Kit as per the kit protocol. The quality of both the prepared libraries passed in our QC step and can be processed for sequencing on illumina platform using 2 x 150bp chemistry.

De novo Transcriptome Assembly

The high-quality reads were assembled together into transcripts using Trinity de novo assembler (version 2.1.1) with a kmer of 25.

Table 11: 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

The assembled transcripts were then further clustered together using CD-HIT-EST-4.8.1to remove the isoforms produced during assembly. This resulted in sequences that can no longer be extended. Such sequences are defined as Unigenes. Only those unigenes which were found to have >85% coverage at 3X read depth were considered for downstream analysis. The statistics of the validated unigenes are summarized in the following tables.

Table 12: Validated unigene (Pooled) summary

Description	Unigenes
No. of Unigenes	25,343
Total unigene length (bases)	36,367,379
N50	2,045
Length of the longest unigene (bp)	20,096
Length of the shortest unigene (bp)	301
Mean unigene length (bp)	1,435

Coding sequence (CDS) Prediction

TransDecoder-v5.3.0 was used to predict coding sequences from the above mentioned unigenes. TransDecoder identifies candidate coding regions within unigene sequences. TransDecoder identifies likely coding sequences based on the following criteria:

- A minimum length open reading frame (ORF) is found in a unigene sequence
- A log-likelihood score similar to what is computed by the GeneID software is > 0.
- The above coding score is greatest when the ORF is scored in the 1st reading frame as compared to scores in the other 5 reading frames.

If a candidate ORF is found fully encapsulated by the coordinates of another candidate ORF, the longer one is reported. However, a single unigene can report multiple ORFs (allowing for operons, chimeras, etc.).

Table 13: 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

Functional Annotation

Functional annotation of the pooled CDS was performed using DIAMOND program, which is a BLAST-compatible local aligner for mapping translated DNA query sequences against a protein reference database. DIAMOND (BLASTX alignment mode) finds the homologous sequences for the genes against NR (non-redundant protein database) from NCBI. Majority of the blast hits were found to be against *Callosobruchus maculatus.*

Table 14: 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

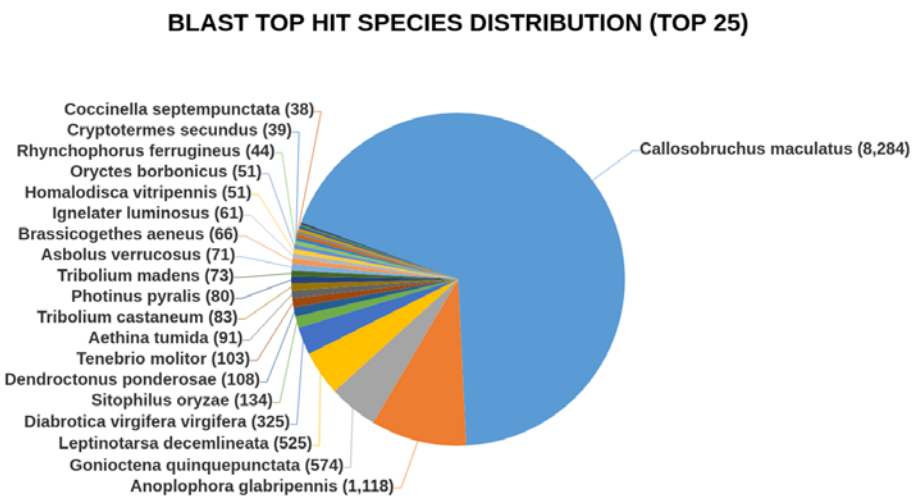

Figure 23: Top Blast Hit Species Distribution of pooled CDS.

Sample-wise CDS identification

To identify sample wise CDS from above mentioned pooled set of CDS, reads from each sample were mapped on the final set of pooled CDS. The read count (RC) values were calculated from the resulting mapping and those CDS having 85% coverage and 3X read depth were considered for downstream analysis for each of the samples. Sample wise CDS statistics have been summarized in the following table.

Table 12: Sample-wise CDS summary

SL. No.	Sample Name	No. of CDS	Total CDS length (bp)	Length of the longest CDS (bp)	Length of the Shortest CDS (bp)	Mean CDS length (bp)
1	Control	6,596	6,875,946	17,022	276	1,042
2	Treated	11,622	14,611,353	17,022	276	1,257

Gene Ontology Analysis (GO)

Gene ontology (GO) analysis of the CDS identified for all samples were carried out using Blast2GO program. GO assignments were used to classify the functions of the predicted CDS. The GO mapping also provides ontologyof defined terms representing gene product properties which are grouped into three main domains: Biological Process (BP), Molecular Function (MF) and Cellular Component (CC). GO mapping was carried out in order to retrieve GO terms for all the functionally annotated CDS. Molecular Function was found to have the highest number of CDS associated with it for all the samples.

Table 13: GO category distribution of CDS

SL. 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	Treated	11,622	5,654	3,608	3,504	4,112

Biological Process		
	Control	**Treated**
Regulation of cellular process	213	504
Establishment of localization	387	706
Primary metabolic process	1264	2075
Organic substance metabolic process	1314	2154
Biogenesis	269	489
Biosynthetic process	518	387
Cellular metabolic process	1020	1635
Small molecule metabolic process	306	409
Nitrogen compound metabolic process	1104	1819
Molecular Functions		
Catalytic activity	314	560
Heterocyclic compound binding	849	1484
Hydrolase activity	516	866
Carbohydrate derivative binding	365	685
Small molecule binding	456	822
Organic cyclic compound binding	849	1485
Ion binding	883	1516
Transferase activity	403	749
Cellular Component		
Organelle	1018	1671
Intracellular anatomical Structure	1215	1973
Cytoplasm	707	1050
Membrane	1009	1771

Functional Annotation of KEGG Pathway

To identify the potential involvement of the predicted CDS in biological pathways, all the identified CDS of 2 samples were mapped to reference canonical pathways in KEGG (*Tribolium castaneum*(tca), *Dendrotonus ponderosae*(dpa), *Aethina tumida*(atd), *Nicrophorus vespilloides*(nvl), and *Drosophila melanogaster*(dme)) database. The identified CDS for all 2samples were found to be categorized into 31KEGG pathways under five main categories: Metabolism, Genetic information processing, Environmental information processing, Cellular processes and Organismal systems. The output of KEGG analysis includes KEGG Orthology (KO) assignments and corresponding Enzyme Commission (EC) numbers and metabolic pathways of predicted CDS using KEGG automated annotation server, KAAS.

Table 14: 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
Treated	11,622	3,037	31

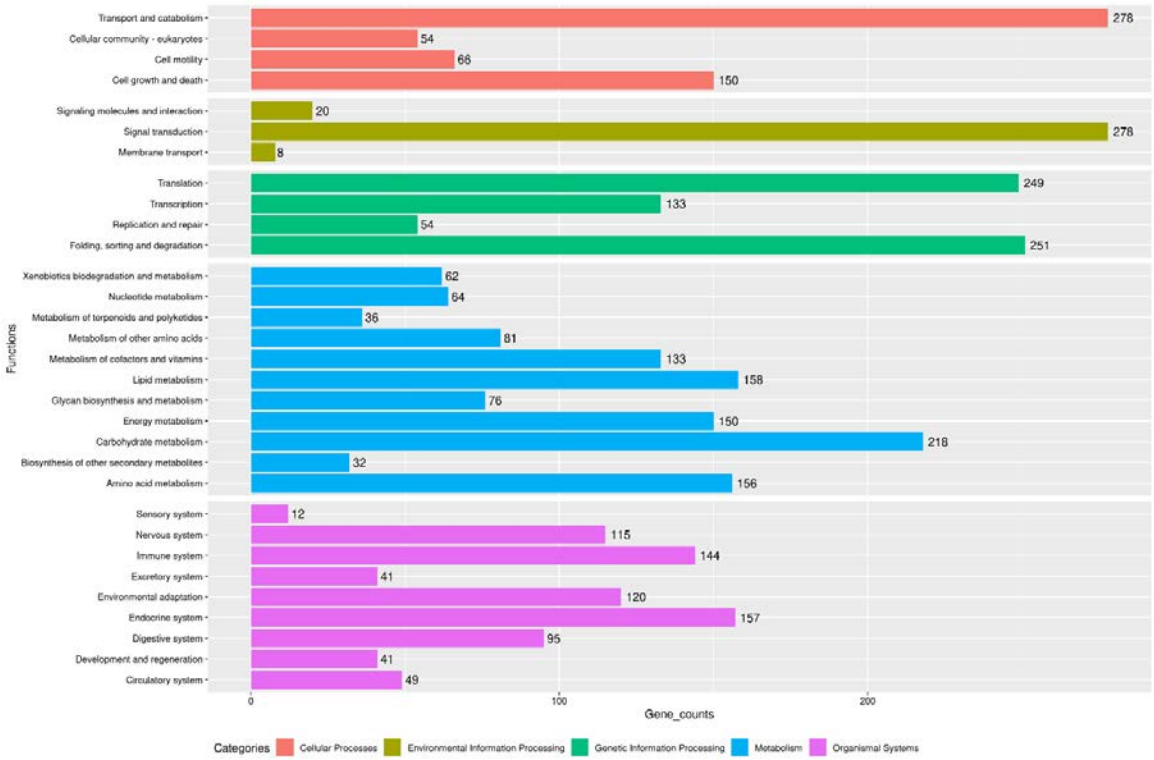

Figure 24: KAAS summary for Control

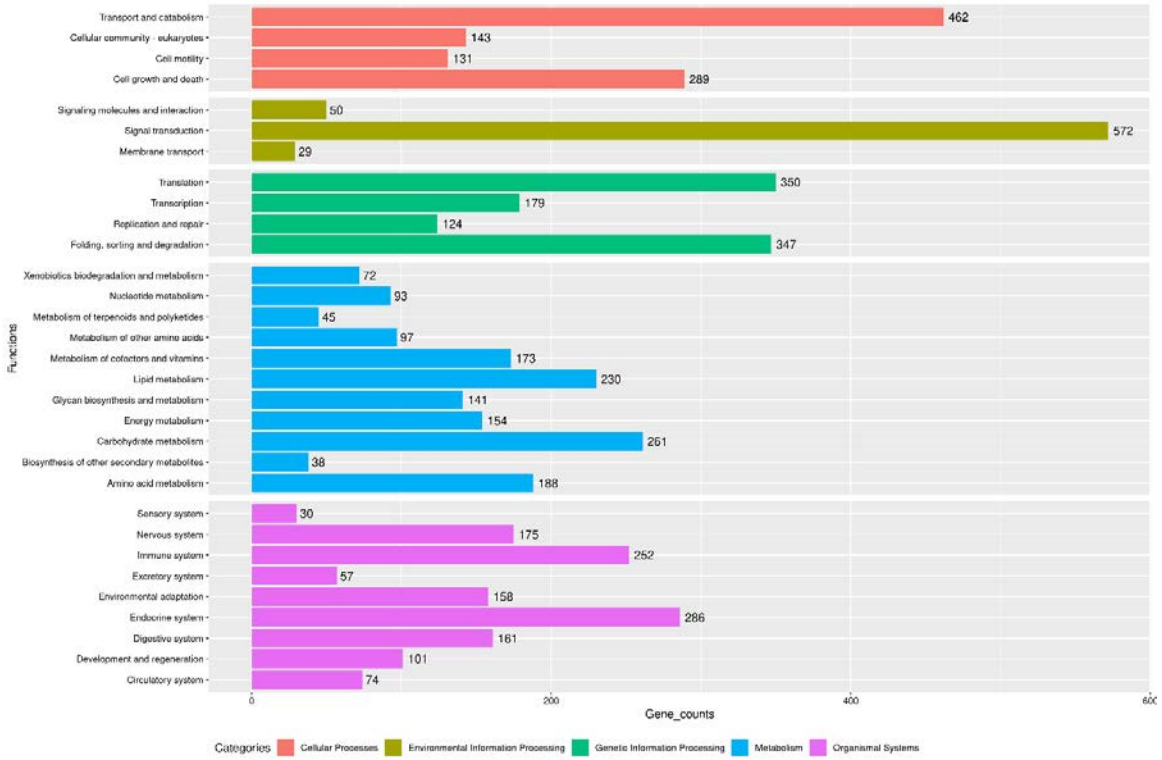

Figure 25: KAAS summary for Treated

Differential Gene Expression Analysis

Differential expression analysis was performed on the CDS between control and treated samples by employing a negative binomial distribution model in DESeqpackage (version1.22.1 -http://www.huber.embl.de/users/anders/DESeq/) as mentioned below.

The CDSs having log2foldchange value greater than zero were considered as up-regulated whereas less than zero as down-regulated. P-value threshold of 0.05 was used to filter statistically significant results.

Table 15: Statistics of differentially expressed Genes

#Commonly Expressed	#Upregulated (Significant)	#Downregulated (Significant)
6,282	50	280

Heatmap

An average linkage hierarchical cluster analysis was performed on top 50 differentially expressed genes using multiple experiments viewer (MeV v4.9.0). The heatmap shows level of gene abundance. Levels of expression are represented as log2 ratio of gene abundance between control and treated samples. Differentially expressed genes were

analyzed by hierarchical clustering. A heat map was constructed using the log-transformed and normalized value of genes based on Pearson uncentered distance and average linkage method. In heatmap, each horizontal line refers to a gene. The color represents the logarithmic intensity of the expressed genes. Relatively high expression values are shown in red.

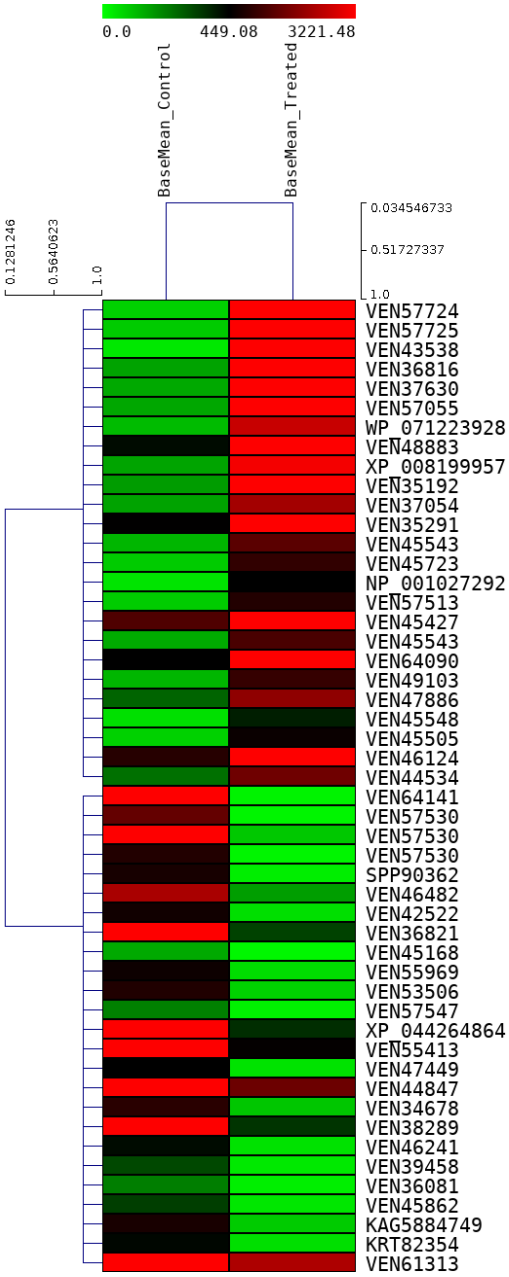

Figure 26: Heat map depicting the top 50 differentially expressed genes (significant); Basemean_Control represents the normalized expression values for Control sample and Basemean_Treated represents the normalized expression values for Treated sample for DGE Combination 1.

Scatter Plot

The Scatter Plot is useful for representing the expression of genes in two distinct conditions of each sample combinations. It helps to identify genes that are differentially expressed in one sample with respect to another and also allows comparing two values associated with genes. In scatter plot, each dot represents a gene. The vertical position of each gene represents its expression level in the Control samples while the horizontal position represents its expression level in the Treated samples. Thus, genes that fall above the diagonal are over-expressed and genes that fall below the diagonal are under-expressed as compared to their median expression level in experimental grouping of the experiment.

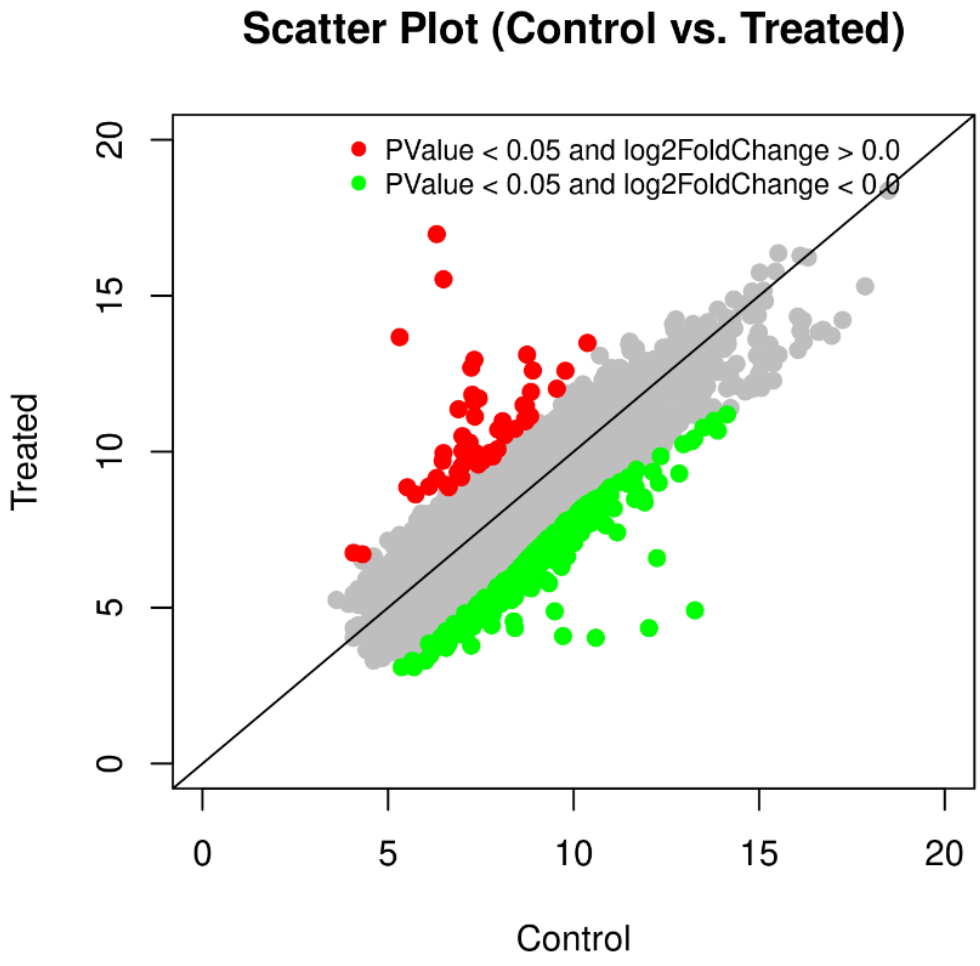

Figure 27: Scatterplot of differentially expressed genes; green dots represent the downregulated (significant) and red dots represent the upregulated (significant) genes in DGE Combination 1.

Volcano Plot

The Eurofins proprietary R script was used to depict the graphical representation and distribution of differentially expressed genes which were found in Control as well as Treated samples. The 'volcano plot' arranges expressed genes along dimensions of biological as well as statistical significance. The red block on the right side of zero represents the up regulated genes whereas green block on the left side of zero represents significant down regulated genes. While Y-axis represents the negative log of p-value (p value <0.05) of the performed statistical test, the data points with low p-values (highly significant) appears towards the top of the plot. Grey block shows the non-differentially expressed genes.

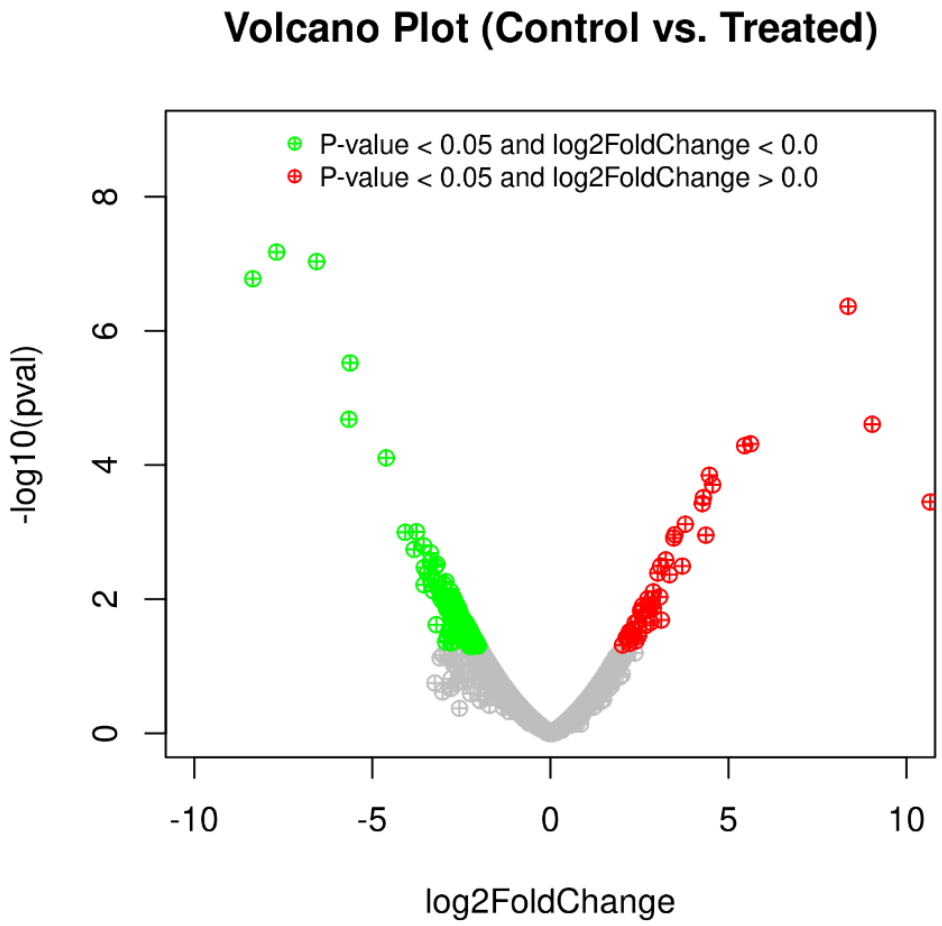

Figure 28: Volcano plot of differentially expressed genes; green dots represent the downregulated (significant) and red dots represent the upregulated (significant) genes for DGE Combination 1.

Discussion

Development of the insect pest depends on physical characteristics of host like texture, shape, size, hardness or softness (Singh et al., 2013; Mason et al., 2016; Sewsaran et al., 2019). Padmasri et al. (2017) had reported that oviposition depends on softness or hardness of the grain and Adebayo and Ogunleke (2016) reported that increase in the length and width leads to high oviposition activity, these was observed in the present study where the maximum oviposition activity was observed on pea which have more hardness as well as surface area. The minimum oviposition activity was recorded on Moth bean followed by green gram, whose seeds have less surface area and softness is more as compared to pea. Highest hatching percentage was recorded on green grams followed by cow pea and minimum hatching was recoded on pea which was also reported by Sharma et al. (2016) and Nisar et al. (2021) while working on *Callosobruchus maculatus*.

In the present study the range of the incubation period was 3 to 6 days where pea with 10 days was out from this range, these observations were supported by Hosamani et al. (2018), Jaiswal et al. (2019) and Sekender et al. (2020) who recorded 3-6 days of incubation by *C. chinensis* on different hosts. Kumar and Kumar (2018) and Augustine and Balikai (2018) recorded similar incubation range in chick pea and cow pea whereas Satish et al. (2020) and Dalal et al. (2020) reported on green gram and black gram. All these studies confirm the present findings.

The maximum larval and pupal period was recorded in pea followed by pigeon pea and shortest in green gram followed by cow pea. Hosamani et al. (2018) while working on different host reported shortest larval and pupal period in green gram and cow pea, Jaiswal et al. (2019) also reported similar findings. Kumar and Kumar (2018) reported a range of 30-35 days in green gram, and Augustine and Balikai (2018) reported 20-31 days of range on cow pea while Satish et al. (2020) and Dalal et al. (2020) states a range of 24-29 days on chick pea and 24-28 days on black gram respectively. The difference of the range may be because of the different variety, temperature, humidity during the respective studies.

Total development period in our finding was in the range of 23 to 32 days where pea was not in that range, minimum development period was reported in green gram followed by cow pea. Similar finding was reported by Radha and Sushila (2014) and it was confirmed again by Hosamani et al., (2018) and Jaiswal et al. (2019). In our finding development

period in chick pea was in a range 28±0.35 days this was almost in the range given by Swella and Mushobozy (2009) Kamble et al. (2016) and Ahmad et al. (2017).

In the current work maximum adult emergence was recorded in green gram followed by cow pea and least in pea, these finding are in accordance with the findings of Chandel and Bhaudaria (2015) and Jaiswal et al., (2020), who reported maximum adult emergence in green gram. The drop in adult emergence in pea could be because of the low hatchability of eggs due to hard seed coat as reported by Padmasri et al. (2017).

The significant difference was recorded in the longevity of adult *C. chinensis* reared on different host where maximum longevity was recorded in green grams and least on the pea. The present findings are in agreement with Hosamani, et al., (2018) and Mehta and Negi (2020), who found significant alteration in the longevity of adult of *C. chinensis* in different varieties of stored grain.

In the present study, maximum weight loss by *C. chinensis* was observed in green gram followed by cow pea and least weight loss was recorded in pea followed by pigeon pea. Similarly, Gupta and Apte (2016) and Bharathi et al. (2017) reported maximum weight loss percentage due to *C. maculatus* also Jaiswal et al. (2019) reported maximum loss percentage in chick pea and green gram by *C. chinensis.* Losses in nutritional values are mainly attributed to storage insect pests, which preferentially feed on grain embryos (Taddese et al., 2020). These observations are in agreement of previous studies by Thakkar and Parikh (2018) who reported nutritional loss by *Sitophilus oryzae* when exposed to different stored grains.

Deltamethrin is a broad spectrum synthetic pyrethroid insecticide that is widely used to control stored-product insect pests (Trostanetsky et al., 2023). It is registered in many parts of the world for stored grain protection (Vayias et al. 2010). Deltamethrin is a synthetic version of naturally occurring pyrethrins extracted from pyrethrum of dried Chrysanthemum flowers (Shrivastava et al., 2011; Bhanu et al. 2011). It kills insects by paralyzing their nervous system, giving a quick knockdown effect, loss of co-ordination, and eventually death (Velki et al. 2014).

In insects, neurotoxic activity of deltamethrin is caused by disruption of axonal transmission of nerve impulses as a result of altering ion permeability of nerve membranes (Paudiyal et al., 2016 & 2017). Deltamethrin is known for its rapid knockdown activity on various insects, including coleopterans (Velki et al. 2014), so the effectiveness of higher concentrations of deltamethrin to adults of *C. chinensis* observed

in this study was expected. Jacob et al. (2014) found that 98% of *S. zeamais* adults were knocked down after of exposure to a deltamethrin concentration of 1,000 ppm whereas mortalities of 76.7, 53.3, 30, 28, 21.7, 23.3, and 0 % were recorded for 500, 250, 100, 50, 25, 12.5, and 0.0 ppm, respectively these findings are in accordance with our present findings where mortalities 0, 33, 70, 100 and 100 % were recorded for 6.25, 12.5, 25, 50 and100 ppm. Paudiyal et al., 2016 similarly reported that high mortalities were recorded on *Tribolium castaneum*, *Sitophilus oryzae* and *Rhyzopertha dominica* adults when they were exposed to high concentration of deltamethrin.

The persistence of insecticide effects from one generation to the next are unknown but have important consequences. Studies typically focus on intragenerational and intergenerational rather than transgenerational effects (Margus et al. 2019). Whether, and how, insecticides exposure leads to transgenerational effects remains poorly understood. In the current study we explore the transgenerational effect of sub lethal dose of the deltamethrin on the developmental parameters *viz.* total egg count, total hatching, hatching %, total development period and adult longevity. The present study demonstrated that deltamethrin was toxic to *C. chinensis* with an LC_{50} value of 22.93ppm when adult of *C. chinensis* were exposed with deltamethrin. Deltamethrin shows a negative effect on the development parameters of all the generations with respect to the control. When a comparison between all the generations was made it shows that the negative effect of the insecticide on the development parameters was significantly decreasing with successive generations which predicts that the effect of deltamethrin on the transgenerations shows a trend where the insects were able to tolerate it.

Many reports indicated that the egg laying and hatching of insects influenced by the sublethal concentration of insecticides (Qu et al. 2017; Xu et al. 2019; Tamilselvan et al., 2021). In this study, the sublethal concentration of deltamethrin significantly reduced the total egg count and hatching of *C. chinensis* in the F1generation with respect to control but in successive generation the effect was almost nil. In *Rhyzopertha dominica* (Fabricius), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus granaries* (Linnaeus) (Coleoptera: Curculionidae), insecticide at lower concentration significantly suppressed the progeny production (Vassilakos et al. 2012 & 2015; Rumbos et al. 2018). (Ali et al. 2017) reported that fecundity and hatchability are significantly decreased for generations of *Sogatella furcifera* after LC_{30} buprofezin treatment. The

negative impact of sublethal concentration of methyl-thiodiafenthiuron on the fecundity of *P. xylostella* F1 progeny have also been reported (Su and Xia 2020).

The total developmental period of F1 progeny of *C. chinensis* were prolonged significantly after exposure of F1 generation to sublethal doses of deltamethrin. Similarly, lower concentration of insecticides increases the development period of insects (Zhu et al. 2012; Guo et al. 2013; Xu et al. 2016), e.g., sublethal concentration of Spinosad and chlorantraniliprole significantly increased the duration of *P. xylostella* and *H. armigera* F1 progeny, respectively (Yin et al. 2008; Zhang et al. 2013). The adult longevity of the F1 *C. chinensis* was significantly reduced compared to control after exposure of the sublethal doses of the deltamethrin, (Ali et al. 2017) reported that adult longevity significantly decreased for generations of *S. furcifera* after buprofezin exposure. Similarly, Deng et al., 2019 reported that the administration of sub lethal doses of dinotefuran on *R. padi* adults of the F1 generation reduced adult longevity.

The transgenerational effects of insecticide on the repellency behaviour of *C. chinensis* shows that the repellent effect of deltamethrin was time and generation dependent. The repellency decreases with the exposure time and following generations. Muntaha et al., 2017 studied the repellent effect of pyrethroids on *C. chinensis*, and highest repellency 92% was recorded with deltamethrin which was followed by cypermethrin (90%) and bifenthrin (89%).In the present study at 1st generation repellency was 85% even at 24 h duration this suggests that the insects may not be able to tolerate the insecticide but in successive generation a decreasing trend in the repellent effect was observed which shows 35% repellency at the sixth generation which suggests that over the period of time the insects were able to tolerate the insecticide.

Although deltamethrin is effective against *C. chinensis* at low or sublethal concentration. It was recorded that when insects were subjected to multiple generations of exposure to sublethal concentration of deltamethrin the effect was showing a decreasing trend this suggests that the insects may eventually lead to resistance (Deng et al. 2019).

Conclusion

The study on the host preference by *C. chinensis* reports that egg count, total development period, adult emergence, adult longevity, weight loss was maximum in green gram also a good amount of nutritional loss was recorded in green grams. Thus, it

can be concluded that for laboratory work green grams are the suitable host for mass rearing of pulses beetle.

The transgenerational effect of deltamethrin suggest that not only the lethal effects, but also the sublethal effects of deltamethrin could influence the development of *C. chinensis*. Sub lethal concentration of deltamethrin can reduce the total egg count, hatching, the delayed development period, reduced adult longevity at first generation but in successive generation the insect was able to overcome of the changes. Similarly, sub lethal concentration of deltamethrin leads to high repellent effect at first generation but the repellent effect was time and generation dependent as the time and generation are increases the repellency is also decreases. This indicates that the transgenerational effect of deltamethrin on *C. chinensis* was high at first generation but in successive generation it was able overcome these effects which suggests that the *C. chinensis* may eventually become to resistance to deltamethrin.

Whole transcriptome analysis was performed on 2 samples, a total of 25,343 non-redundant validated unigenes were further taken for downstream analysis, from which TransDecoder resulted in a total of 13,614 CDS. A total of 12,629 CDS were functionally annotated. The majority of the CDS were found to be homologous with *Callosobruchus maculatus.* Further, Gene ontology (GO) analysis revealed that maximum number of CDS were involved in Molecular Function in addition to cellular and biological process. KEGG pathway analysis for all the individual set of CDS were found to be categorized into 31KEGG pathways under five main categories: Metabolism, Genetic information processing, Environmental information processing, Cellular processes and Organismal systems. Differential gene expression analysis further depicted that 6282 CDS were commonly expressed, of which 50 CDS were significantly upregulated and 280 CDS significantly downregulated. Our studies are parallel to the work of Sayadi, et al., 2016 and Brar et a., 2020 were transcriptomic analysis of *C. maculatus* thoroughly done.

In conclusion this study provides a comprehensive assembly of the *C. chinensis* transcriptome which covers a large number of genes expressed in both control and treated adults, however a greater number of CDS were expressed in treated group. Thus, we believe that this data will provide a valuable resource for future studies of the seed beetle *C. chinensis* as well as for comparative gene expression and genomic analyses of beetles more generally.

REFERENCES

Abass, A. B., Ndunguru, G., Mamiro, P., Alenkhe, B., Mlingi, N., &Bekunda, M. (2014). Post-harvest food losses in a maize-based farming system of semi-arid savannah area of Tanzania. *Journal of stored products research*, *57*, 49-57.

Abedin, M. Z., Rahman, M. Z., Mia, M. I. A., &Rahman, K. M. M. (2012). In-store losses of rice and ways of reducing such losses at farmers' level: An assessment in selected regions of Bangladesh. *Journal of the Bangladesh Agricultural University*, *10*(452-2016-35548), 133-144.

Adarkwah, C., Obeng-Ofori, D., Büttner, C., Reichmuth, C., &Schöller, M. (2010). Bio-rational control of red flour beetle *Triboliumcastaneum* (Herbst)(Coleoptera: Tenebrionidae) in stored wheat with Calneem® oil derived from neem seeds. *Journal of Pest Science*, *83*(4), 471-479.

Adebayo, R. A., Ogunleke, O. M. (2016). Influence of seed size on the performance of *Callosobruchus maculatus* in four cowpea varieties. Journal of Agricultural Science and Food Technology, 2(10), 164-168.

Adelman, Z. N., Kilcullen, K. A., Koganemaru, R., Anderson, M. A., Anderson, T. D., & Miller, D. M. (2011). Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. *PLoS One*, *6*(10), e26228.

Adu, G. B., Abdulai, M. S., Alidu, H., Nustugah, S. K., Buah, S. S., Kombiok, J. M., &Etwire, P. M. (2014). Recommended production practices for maize in Ghana. *CSIR-AGRA Maize production guide*, 1-18.

Agricultural and Processed Food Products Export Development Authority (APEDA), Department of Commerce and Industry, Union Budget 2020-21, *Press Information Bureau, Ministry of Statistics and Programme Implementation*, Press Releases, Media Reports, Ministry of Agriculture and Farmers Welfare, Crisil

Ahmad, M. A., Khan, M. S., Agnihotri, M. (2017). Evaluation of resistance in different chickpea varieties to *Callosobruchus chinensis* Linnaeus (Coleoptera: Bruchidae) under Linnaeus conditions. The Bioscan, 12(4), 1897-1901.

Ahmad, R., Hassan, S., Ahmad, S., Nighat, S., Devi, Y. K., Javeed, K., & Hussain, B. (2021). Stored grain pests and current advances for their management. Postharvest technology-recent advances, new perspectives and applications.

Ali, E., Liao, X., Yang, P., Mao, K., Zhang, X., Shakeel, M., ... & Li, J. (2017). Sublethal effects of buprofezin on development and reproduction in the white-backed planthopper, Sogatella furcifera (Hemiptera: Delphacidae). *Scientific reports*, *7*(1), 16913.

Ali, N. S., Ali, S. S., & Shakoori, A. R. (2014). Biochemical response of malathion-resistant and-susceptible adults of *Rhyzopertha dominica* to the sublethal doses of deltamethrin. *Pakistan Journal of Zoology*, *46*(3).

Alkan, M. (2020). Chemical composition and insecticidal potential of different Origanum spp.(Lamiaceae) essential oils against four stored product pests. *Turkish Journal of Entomology*, *44*(2), 149-163.

Araújo, R. A., Williamson, M. S., Bass, C., Field, L. M., &Duce, I. R. (2011). Pyrethroid resistance in *Sitophiluszeamais* is associated with a mutation (T929I) in the voltage-gated sodium channel. *Insect Molecular Biology*, *20*(4), 437-445.

Arumugam, G., Velayutham, V., Shanmugavel, S., &Sundaram, J. (2016). Efficacy of nanostructured silica as a stored pulse protector against the infestation of bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Applied nanoscience*, *6*(3), 445-450.

Augustine, N., Balikai, R. A., Deshpande, S. K. (2018). Varietal screening and bio chemical basis of resistance in cowpea against pulse beetle, *Callosobruchus chinensis* (L.) in storage. Journal of Experimental Zoology India, 21(2), 1151-1154.

Babu, S. R., Kumar, D. S. R., Sri, C. N. S., & Madhumathi, T. (2017). Evaluation of spinosad against malathion and deltamethrin resistant population of lesser grain borer, *Rhyzopertha dominica* in Andhra Pradesh, India. *Int. J. Curr. Microbiol. App. Sci*, *6*(6), 165-171.

Bajracharya, N. S., Opit, G. P., Talley, J., & Jones, C. L. (2013). Efficacies of spinosad and a combination of chlorpyrifos-methyl and deltamethrin against phosphine-resistant *Rhyzoperthadominica* (Coleoptera: Bostrichidae) and *Triboliumcastaneum* (Coleoptera: Tenebrionidae) on wheat. *Journal of economic entomology*, *106*(5), 2208-2215.

Beckett, S. J. (2011). Insect and mite control by manipulating temperature and moisture before and during chemical-free storage. *Journal of stored products research*, *47*(4), 284-292.

Bell, C. H. (2000). Fumigation in the 21st century. *Crop protection*, *19*(8-10), 563-569.

Benhalima, H., Chaudhry, M. Q., Mills, K. A., & Price, N. R. (2004). Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *Journal of Stored Products Research*, *40*(3), 241-249.

Beuge, J. A., & Aust, S. D. (1978). The thiobarbituric acid assay.

Beutter, F. (1963). ZursittlichenBeurteilung von Inflationen: Grundsätze und Mass-Stäbe.

Bhanu, S., S. Archana, K. Ajay, J. L. Bhatt, S. P. Bajpai, P. S. Singh, & V. Bhatnagar. (2011). Impact of deltamethrin on environment, use as an insecticide and its bacterial degradation- a preliminary study. *International Journal Environment Science*. 1: 977–985.

Bharathi, T. D., Krishnayya, P. V., Madhumathi, T. (2017). Comparative studies on the susceptibility of different grains to pulse beetle, *Callosobruchus maculatus*. International Journal of Science, Environment and Technology, 6, 308-318.

Bonjour, E. L., Opit, G. P., Hardin, J., Jones, C. L., Payton, M. E., &Beeby, R. L. (2011). Efficacy of ozone fumigation against the major grain pests in stored wheat. *Journal of Economic Entomology*, *104*(1), 308-316.

Boxall, R. A. (2002). Damage and loss caused by the larger grain borer *Prostephanustruncatus*. *Integrated Pest Management Reviews*, *7*(2), 105-121.

Boyer, S., Zhang, H., &Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. *Bulletin of entomological research*, *102*(2), 213.

Brar, G. S., Kaur, G., Singh, S., Shukla, J. N., & Pandher, S. (2022). Identification and validation of stage-specific reference genes for gene expression analysis in Callosobruchus maculatus (Coleoptera: Bruchidae). *Gene Expression Patterns*, *43*, 119233.

Chakraborty, S., Mondal, P., Senapati, S. K. (2015). Evaluation of relative susceptibility of *Callosobruchus chinensis* Linn. on five different stored pulse seeds. Asian Journal of Plant Science and Research, 5(10), 9-15.

Chanda, E., Chanda, J., Kandyata, A., Phiri, F. N., Muzia, L., Haque, U., &Baboo, K. S. (2013). Efficacy of ACTELLIC 300 CS, pirimiphos methyl, for indoor residual spraying in areas of

high vector resistance to pyrethroids and carbamates in Zambia. *Journal of medical entomology*, *50*(6), 1275-1281.

Chandel, B. S., Bhadauria, D. S. (2015). Assessment of resistance to the attack of pulse beetle, *Callosobruchus chinensis* (Fabricius) in chickpea genotypes on the basis of various physical parameters during storage. Journal of Entomology and zoology studies, 3(2), 160-165.

Chaudhry, M. Q. (2000). Phosphine resistance. *Pesticide Outlook*, *11*(3), 88-91.

Collins, P. J. (2006, October). Resistance to chemical treatments in insect pests of stored grain and its management. In *Proceedings of the 9th International Working Conference on Stored Product Protection* (Vol. 15).

Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The use of push-pull strategies in integrated pest management. *Annual review of entomology*, *52*.

Daglish, G. J., Nayak, M. K., Pavic, H. (2014). Phosphine resistance in *Sitophilus oryzae* (L.) from eastern Australia: Inheritance, fitness and prevalence. Journal of Stored Products Research, 59, 237-244.

Dalal, P. L., Dangi, N. L., Mahla, M. K., Ahir, K. C., Jat, R. (2020). Biology of pulse beetle, *Callosobruchus chinensis* (L.) on stored black gram, Vigna mungo (L.). Journal of Entomology and zoology studies, 8(5): pp. 1798-1800

Damalas, C. A., & Koutroubas, S. D. (2016). Farmers' exposure to pesticides: toxicity types and ways of prevention. *Toxics*, *4*(1), 1.

Danse, L. H. J. C., Van Velsen, F. L., & Van Der Heijden, C. A. (1984). Methylbromide: carcinogenic effects in the rat forestomach. *Toxicology and applied pharmacology*, *72*(2), 262-271.

Dara SK (2017) Insect resistance to biopesticides. UCANR E-Journal of Entomology and Biologicals.

Dara, S. K. (2016). First field study evaluating the impact of the entomopathogenic fungus Beauveriabassiana on strawberry plant growth and yield. *UCANR eJournalStrawberrries and Vegetables*, *7*.

Dara, S. K. (2017). Insect resistance to biopesticides. *UCANR eJournal Strawberrries and Vegetables*.

Dara, S. K., Dara, S. R., &Dara, S. S. (2013). Endophytic colonization and pest management potential of Beauveriabassiana in strawberries. *Journal of Berry Research*, *3*(4), 203-211.

Deeba, F., Sarwar, M., Khuhro, R. D. (2006). Varietal susceptibility of mungbean genotypes to pulse beetle, *Callosobruchus analis* (Fabricius) (Coleoptera: Bruchidae). Pakistan Journal of Zoology, 38(4), 265.

Deng, D., Duan, W., Wang, H., Zhang, K., Guo, J., Yuan, L., ... & Wu, S. (2019). Assessment of the effects of lethal and sublethal exposure to dinotefuran on the wheat aphid Rhopalosiphum padi (Linnaeus). *Ecotoxicology*, *28*, 825-833.

Dent D, Binks RH (2020) *Insect pest management*. Cabi. Wallingford, Oxfordshire, UK. pp353

Devi, M. B., Devi, N. V. (2014). Biology and morphometric measurement of cowpea weevil, *Callosobruchus maculatus* fabr. (Coleoptera: Chrysomelidae) in green gram. Journal of Entomology and Zoology Studies, 2(3), 74-76.

Ding, W., Wang, J. J., Zhao, Z. M., & Tsai, J. H. (2002). Effects of controlled atmosphere and DDVP on population growth and resistance development by the psocid, *Liposcelisbostrychophila*Badonnel (Psocoptera: Liposcelididae). *Journal of Stored Products Research*, *38*(3), 229-237.

Duan, C. X., Li, D. D., Sun, S. L., Wang, X. M., Zhu, Z. D. (2014). Rapid development of microsatellite markers for *Callosobruchus chinensis* using Illumina paired-end sequencing. PLoS One, 9(5), e95458.

Elzun, H. M., Hamady, S. E. E., Gazzy, A. A., & Salem, A. A. (2019). Insecticidal activity of selected chemicals and biopesticides against cowpea and khapra beetles, and their sub-lethal toxicity on the Japanese quail. *Menoufia Journal of Plant Protection*, *4*(5), 189-209.

Emery, R. N., Collins, P. J., &Wallbank, B. E. (2003). Monitoring and managing phosphine resistance in Australia. *Stored Grain in Australia*, 25-27.

Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., & Prasanna, B. M. (2022). Global maize production, consumption and trade: Trends and R&D implications. *Food Security*, *14*(5), 1295-1319.

Fang, Y., Shi, W. Q., Wu, J. T., Li, Y. Y., Xue, J. B., & Zhang, Y. (2019). Resistance to pyrethroid and organophosphate insecticides, and the geographical distribution and polymorphisms of target-site mutations in voltage-gated sodium channel and acetylcholinesterase 1 genes in *Anopheles sinensis* populations in Shanghai, China. *Parasites & vectors*, *12*(1), 396.

FAO. 2019. The State of Food and Agriculture 2019. Moving forward on food loss and waste reduction. Rome. pp182

Fatima, S. M., Usman, A., Sohail, K., Afzaal, M., Shah, B., Adnan, M., & Shah, S. R. A. (2016). Rearing and identification of *Callosobruchus maculatus* (Bruchidae: Coleoptera) in Chickpea. *J. Entomol. Zool. Stud*, *4*, 264-266.

Fragoso, D. B., Guedes, R. N. C., &Peternelli, L. A. (2005). Developmental rates and population growth of insecticide-resistant and susceptible populations of *Sitophiluszeamais*. *Journal of Stored Products Research*, *41*(3), 271-281.

Giga, D. E., Smith, R. H. (1987). Egg production and development of *Callosobruchus rhodesianus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on several commodities at two different temperatures. Journal of Stored Products Research, 23(1), 9-15.

Gilleard, J. S., & Beech, R. N. (2007). Population genetics of anthelmintic resistance in parasitic nematodes. *Parasitology*, *134*(8), 1133.

Golić, M. P., Andrić, G., & Kljajić, P. (2018). Effects of chlorpyrifos-methyl and pirimiphos-methyl applied with 5° C temperature on *Sitophilus oryzae (L.)* (Coleoptera: Curculionidae) in wheat grain. *Julius-Kühn-Archiv*, (463), 878-885.

Gopi, N., Singh, K. I. (2020). Biology and varietal preference of pulse beetle, *Callosobruchus chinensis* L. on stored green gram. Journal of Entomology and zoology studies ,8(5): pp.582-584.

Guedes, N. M. P., Guedes, R. N. C., Ferreira, G. H., & Silva, L. B. (2009). Flight take-off and walking behavior of insecticide-susceptible and-resistant strains of *Sitophiluszeamais* exposed to deltamethrin. *Bulletin of entomological research*, *99*(4), 393.

Guedes, R. C., & Pereira, E. J. G. (2008). Maize weevil x insecticides: pyrethroid resistance, associated fitness costs and mitigation, and management implications. *Crop Protection Research Advances. Nova Science Publishers Inc*, 125-150.

Gujar, G. T., Yadav, T. D. (1978). Feeding of *Callosobruchus maculatus* (Fab.) and *Callosobruchus chinensis* (Linn.) in green gram. Indian Journal of Entomology: pp108-112.

Guo, L., Desneux, N., Sonoda, S., Liang, P., Han, P., & Gao, X. W. (2013). Sublethal and transgenerational effects of chlorantraniliprole on biological traits of the diamondback moth, Plutella xylostella L. *Crop Protection*, *48*, 29-34.

Gupta, S., Apte, S. D. (2016). Relative varietal preference of *Callosobruchus maculatus* (Fab.) in different green gram varieties (free choice test). Annals of Biological Research, 7(8), 1-3.

Haddi, K., Valbon, W. R., Jumbo, L. O. V., de Oliveira, L. O., Guedes, R. N., & Oliveira, E. E. (2018). Diversity and convergence of mechanisms involved in pyrethroid resistance in the stored grain weevils, *Sitophilus* spp. *Scientific reports*, *8*(1), 1-15.

Hagstrum, D. W., & Athanassiou, C. G. (2019). Improving stored product insect pest management: from theory to practice. *Insects*, *10*(10), 332.

Haniotakis, G. E. (2005). Olive pest control: present status and prospects. *IOBC wprs Bulletin*, *28*(9), 1.

Harein, P. K., & Davis, R. (1992). Control of stored-grain insects. *Storage of cereal grains and their products*, *4*, 491-534.

Haritos, V. S., Damcevski, K. A., &Dojchinov, G. (2006). Improved efficacy of ethyl formate against stored grain insects by combination with carbon dioxide in a 'dynamic'application. *Pest Management Science: formerly Pesticide Science*, *62*(4), 325-333.

Hasan, M. M., Aikins, M. J., Schilling, M. W., & Phillips, T. W. (2020). Comparison of methyl bromide and phosphine for fumigation of *Necrobia rufipes* (Coleoptera: Cleridae) and *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae), pests of high-value stored products. *Journal of economic entomology*, *113*(2), 1008-1014.

Hegazy, R. (2013). Post-harvest situation and losses in India. *High Court of Karnataka-Bengaluru Bench: WP*, *24739*, 2012.

Hosamani, G. B., Jagginavar, S. B., Karabhantanal, S. S. (2018). Biology of pulse beetle *Callosobruchus chinensis* on different pulses. Journal of Entomology and Zoology Studies, 6(4), 1898-1900.

Huijben, S., &Paaijmans, K. P. (2018). Putting evolution in elimination: winning our ongoing battle with evolving malaria mosquitoes and parasites. *Evolutionary applications*, *11*(4), 415-430.

Ignacimuthu, S., Janarthanan, S., &Balachandran, B. (2000). Chemical basis of resistance in pulses to Callosobruchus maculatus (F.)(Coleoptera: Bruchidae). *Journal of Stored Products Research*, *36*(1), 89-99.

Jacob, A. P., Hadi, M., & Bingham, G. (2014). Deltamethrin contact bioassay and boring/chewing tests with the maize weevil, *Sitophilus zeamais* (Mot). *International Journal of Agricultural Research and Review*, *1*(12), 133-142.

Jagadeesan, R., Collins, P. J., Daglish, G. J., Ebert, P. R., &Schlipalius, D. I. (2012). Phosphine resistance in the rust red flour beetle, *Triboliumcastaneum* (Coleoptera: Tenebrionidae): inheritance, gene interactions and fitness costs. *PLos one*, *7*(2), e31582.

Jaiswal, D. K., Raju, S. V. S., Kumar, D., Vani, V. M. (2018). Studies on biology of pulse beetle, *Callosobruchus chinensis* (L.) on stored chickpea under laboratory conditions. Journal of Pharmacognosy and Phytochemistry, 7(6), 464-467.

Jaiswal, D. K., Raju, S. V. S., Vani, V. M., Sharma, K. R. (2019). Studies on life history and host preference of pulse beetle, *Callosobruchus chinensis* (L.) on different pulses. Journal of Entomological Research, 43(2), 159-164.

Jallow, M. F., Awadh, D. G., Albaho, M. S., Devi, V. Y., & Thomas, B. M. (2017). Pesticide risk behaviors and factors influencing pesticide use among farmers in Kuwait. *Science of the Total Environment*, *574*, 490-498.

Jha, S. N., Vishwakarma, R. K., Ahmad, T., Rai, A., & Dixit, A. K. (2015). Assessment of quantitative harvest and post-harvest losses of major crops and commodities in India. *Ministry of Food Processing Industries (Govt. of India), ICAR-Central Institute of Post-Harvest Engineering and Technology (ICAR-CIPHET), PO: PAU, Ludhiana-141004, Punjab (India)*, 64-67.

Jian, F. (2019). Influences of stored product insect movements on integrated pest management decisions. *Insects*, *10*(4), 100.

Jiang, L., & O'Neill, B. C. (2017). Global urbanization projections for the Shared Socioeconomic Pathways. *Global Environmental Change*, *42*, 193-199.

Kamble, S. M., Bagde, A. S., Patil, R. R. (2016). Growth and development of *C. chinensis* L. on different varieties of chickpea. Advances in Life Sciences, 5(11), 4472-4476.

Kar, A., & Ganguli, J. (2016). Fecundity and preferential oviposition by pulse beetle, *Callosobruchus maculatus* F. on chickpea (*Cicer arietinum* L.) var dollar. Legume Res, 39(2), 310-314.

Karaağaç, S. U. (2012). Insecticide resistance. *Insecticides-advances in integrated pest management*, 469-478.

Kargbo, I. S. (2013). Postharvest Losses And Evaluation Of The Bioefficacy Of *Chromolaena Odorata* and *Jatropha Gossypiifolia* Against *Sitophilus Zeamais* Motsch And *Tribolium Castaneum* Herbst *In The Awutu-Senya District Of The Central Region Of Ghana* (Doctoral dissertation, University of Ghana).

Kaur, R., Schlipalius, D. I., Collins, P. J., Swain, A. J., & Ebert, P. R. (2012). Inheritance and relative dominance expressed as toxicity response and delayed development, of phosphine resistance in immature stages of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Journal of Stored Products Research*, *51*, 74-80.

Kaushik, G., Singhal, P., & Chaturvedi, S. (2018). Food processing for increasing consumption: The case of legumes. In *Food processing for increased quality and consumption* (pp. 1-28). Academic Press.

Kim, B. H., Han, G. D., Kwon, H., Chun, Y. S., Na, J., & Kim, W. (2019). Chlorine dioxide fumigation to control stored product insects in rice stored in a room. *Journal of Stored Products Research*, *84*, 101527.

Kogan, M., & Goeden, R. D. (1970). The host-plant range of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae). Annals of the entomological Society of America, 63(4), 1175-1180.

Kortbeek, R. W., van der Gragt, M., &Bleeker, P. M. (2019). Endogenous plant metabolites against insects. *European Journal of Plant Pathology*, *154*(1), 67-90.

Kumar, D., Kalita, P. (2017). Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods, 6(1), 8.

Kumari, S., Yadav, S. S., Rolania, K., Dhanda, S. (2020). The biology of pulse beetle, *C. chinensis* on stored mungbean. Journal of Entomology and Zoology Studies, 8(3), 1200-1203.

Lal, M., Ram, B., Tiwari, P. (2017). Botanicals to cope stored grain insect pests: a review. International Journal of Current Microbiology and Applied Sciences, 6(6), 1583-1594.

Lamichhane, J. R., Arseniuk, E., Boonekamp, P., Czembor, J., Decroocq, V., Enjalbert, J., ... & Messéan, A. (2018). Advocating a need for suitable breeding approaches to boost integrated pest management: a European perspective. *Pest management science*, *74*(6), 1219-1227.

Lee, J. S., Kim, H. K., Kyung, Y., Park, G. H., Lee, B. H., Yang, J. O., & Kim, G. H. (2018). Fumigation activity of ethyl formate and phosphine against *Tetranychus urticae* (Acari: Tetranychidae) on imported sweet pumpkin. *Journal of economic entomology*, *111*(4), 1625-1632.

Liu, T. T., Chao, L. K. P., Hong, K. S., Huang, Y. J., Yang, T. S. (2019). Composition and insecticidal activity of essential oil of Bacopa caroliniana and interactive effects of individual compounds on the activity. Insects, 11(1), 23.

Lorini, I., & Galley, D. J. (2001). The cross-resistance spectrum in deltamethrin resistance strains of *Rhyzoperthadominica* (F.)(Coleoptera: Bostrychidae). *Neotropical Entomology*, *30*(2), 321-325.

Manal A, A., Trandil, F. W., Marwa, I. M., & MS, S. (2017). Resistance status and associated resistance mechanisms to certain insecticides in rice weevil *Sitophilusoryzae* (Coleoptera: Curculionidae). *Alexandria Journal of Agricultural Sciences*, *62*(4), 331-340.

Margus, A., Piiroinen, S., Lehmann, P., Tikka, S., Karvanen, J., & Lindström, L. (2019). Sublethal pyrethroid insecticide exposure carries positive fitness effects over generations in a pest insect. *Scientific reports*, *9*(1), 11320.

Martínez, J. L., &Baquero, F. (2002). Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clinical microbiology reviews*, *15*(4), 647-679.

Mason-D'Croz, D., Vervoort, J., Palazzo, A., Islam, S., Lord, S., Helfgott, A., Lipper, L. (2016). Multi-factor, multi-state, multi-model scenarios: exploring food and climate futures for Southeast Asia. Environmental Modelling & Software, 83, 255-270.

Mau, Y. S., Collins, P. J., Daglish, G. J., Nayak, M. K., Pavic, H., & Ebert, P. R. (2012). The rph1 gene is a common contributor to the evolution of phosphine resistance in independent field isolates of Rhyzoperthadominica. *Plos One*, *7*(2), e31541.

Mebarkia, A., Guechi, A., Mekhalif, S., Makhlouf, M. (2009). Biochemical composition effect of some cereal species on the behaviour of *Sitophilus granarius* L. and *Rhyzopertha dominica* F. species in semi-arid zone of Setif, Algeria. Journal of Agronomy, 8(2), 60-66.

Meghwal, H. P., & Singh, V. (2005). A study of the biology of pulse beetle, *Callosobruchus chinensis* (Linn.) on moth bean, *Vigna aconitifolia* (Jacq.) Marechal. Indian Journal of Entomology, 67(4), 334.

Mehta, V., Negi, N. (2020). Biology of pulse beetle *Callosobruchus chinensis* (L.) on three pulses. Indian Journal of Entomology, 82(4), 631-632.

Mesterházy, Á., Oláh, J., & Popp, J. (2020). Losses in the grain supply chain: Causes and solutions. *Sustainability*, *12*(6), 2342.

Mitcham, E., Martin, T., & Zhou, S. (2006). The mode of action of insecticidal controlled atmospheres. *Bulletin of entomological research*, *96*(3), 213.

Muntaha, S. T., Sagheer, M., Hasan, M. U., & Sahi, S. T. (2017). Repellent and growth inhibitory impact of plant extracts and synthetic pyrethroids on three strains of Callosobruchus chinensis L. *Pakistan Journal of Zoology*, *49*(2), 581-581.

Mushobozy, D. M. (2009). Comparative susceptibility of different legume seeds to infestation by cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Plant Protection Science, 45(1), 19.

Muthusamy, R., Suganya, R., Gowri, M., &Shivakumar, M. S. (2013). Biochemical mechanisms of organophosphate and pyrethroid resistance in red hairy caterpillar *Amsactaalbistriga* (Lepidoptera: Arctiidae). *Journal of the Saudi Society of Agricultural Sciences*, *12*(1), 47-52

Nagpal, M., & Kumar, A. (2012). Grain losses in India and government policies. *Quality Assurance and Safety of Crops & Foods*, *4*(3), 143-143.

Nansen, C., Baissac, O., Nansen, M., Powis, K., & Baker, G. (2016). Behavioral avoidance-will physiological insecticide resistance level of insect strains affect their oviposition and movement responses?. *PloS one*, *11*(3), e0149994.

Navarro, S. (2012). The use of modified and controlled atmospheres for the disinfestation of stored products. *Journal of Pest Science*, *85*(3), 301-322.

Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R. (2020). Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. *Annual Review of Entomology*, *65*, 333-350.

Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. E. (2009). Repellent activity of essential oils from seven aromatic plants grown in Colombia against Sitophilus zeamais Motschulsky (Coleoptera). Journal of Stored Products Research, 45(3), 212-214.

Ngom, D., Fauconnier, M. L., Malumba, P., Thiaw, C., Brévault, T., & Sembène, M. (2021). Morphophysical and biochemical traits involved in maize grain varietal susceptibility to the maize weevil, Sitophilus zeamais (Coleoptera, Curculionidae).

Nguyen, T. T., Collins, P. J., & Ebert, P. R. (2015). Inheritance and characterization of strong resistance to phosphine in *Sitophilusoryzae* (L.). *PloS one*, *10*(4), e0124335.

Ningombam, A., Ahluwalia, V., Srivastava, C., &Walia, S. (2017). Antifeedant activity and phytochemical investigation of Millettiapachycarpa extracts against tobacco leaf eating caterpillar, *Spodopteralitura* (Fabricius)(Lepidoptera: Noctuidae). *Journal of Asia-Pacific Entomology*, *20*(2), 381-385.

Nisar, M. S., Haq, I. U., Ramzan, H., Aljedani, D. M., Qasim, M., Islam, W., Khan, K. A. (2021). Screening of different legumes for the developmental preference of *Callosobruchus maculatus* (Bruchidae: Coleoptera). International Journal of Tropical Insect Science, 41(4), 3129-3136.

Odeyemi, O. O., Ashamo, M. O., Akinkurolere, R. O., &Olatunji, A. A. (2010). Resistance of strains of rice weevil, *Sitophilusoryzae* (Coleoptera: Curculionidae) to pirimiphos methyl. *Julius-Kühn-Archiv*, (425), 167.

Padmasri, A., Srinivas, C., Lakshmi, K. V., Pradeep, T., Rameash, K., Anuradha, C., Anil, B. (2017). Management of rice weevil (*Sitophilus oryzae* L.) in maize by botanical seed treatments. International Journal of Current Microbiology and Applied Sciences, 6(12), 3543-3555.

Pandya, N., Thakkar, B., Pandya, P., & Parikh, P. (2021). Evaluation of insecticidal potential of organochemicals on SF9 cell line. *The Journal of Basic and Applied Zoology*, *82*(1), 1-10.

Patel, V. K., Chaudhuri, N., Senapati, S. K. (2005). Biology of pulse beetle (*Callosobruchus chinensis* Linn.) as influenced by feeding of different grain pulses. Agricultural Science Digest, 25(4), 254-256.

Paudyal, S., Opit, G. P., Arthur, F. H., Bingham, G. V., & Gautam, S. G. (2016). Contact toxicity of deltamethrin against tribolium castaneum (coleoptera: Tenebrionidae), sitophilus oryzae (coleoptera: Curculionidae), and rhyzopertha dominica (coleoptera: Bostrichidae) adults. *Journal of Economic Entomology*, *109*(4), 1936-1942.

Paudyal, S., Opit, G. P., Osekre, E. A., Arthur, F. H., Bingham, G. V., Payton, M. E., ... & Nsiah, E. P. (2017). Field evaluation of the long-lasting treated storage bag, deltamethrin incorporated, (ZeroFly® Storage Bag) as a barrier to insect pest infestation. *Journal of Stored Products Research*, *70*, 44-52.

Perez-Mendoza, J. (1999). Survey of insecticide resistance in Mexican populations of maize weevil, *SitophiluszeamaisMotschulsky* (Coleoptera: Curculionidae). *Journal of Stored Products Research*, *35*(1), 107-115.

Phillips, T. W., & Throne, J. E. (2010). Biorational approaches to managing stored-product insects. *Annual review of entomology*, *55*.

Pimentel, M. A. G., Faroni, L. R. D. A., Batista, M. D., & Silva, F. H. D. (2008). Resistance of stored-product insects to phosphine. *PesquisaAgropecuáriaBrasileira*, *43*(12), 1671-1676.

Pipariya, G., Sharma, P., Pandya, N., Parikh, P. (2022). Insecticidal Activity of Essential Oils from Mint and Ajwain against Pulse Beetle *Callosobruchus chinensis* (L). Indian Journal of Entomology, 1-5.

Popp, J., Pető, K., & Nagy, J. (2013). Pesticide productivity and food security. A review. *Agronomy for sustainable development*, *33*(1), 243-255.

Qu, Y., Xiao, D., Liu, J., Chen, Z., Song, L., Desneux, N., ... & Song, D. (2017). Sublethal and hormesis effects of beta-cypermethrin on the biology, life table parameters and reproductive potential of soybean aphid Aphis glycines. *Ecotoxicology*, *26*, 1002-1009.

Radha, R., Susheela, P. (2014). Studies on the life history and ovipositional preference of *Callosobruchus maculatus* reared on different pulses. Research Journal of Animal, Veterinary and Fishery Sciences, 2(6), 1-5.

Ramesh Babu, S., DV, S. R. K., &Madhumathi, T. (2018). Toxicity of newer insecticide molecules against lesser grain borer, *Rhyzoperthadominica* (Fabricius)(Bostrichidae: Coleoptera).

Rana, D. K., Mishra, S. P., Vishal, A., Katlam, B. P., Jaba, J., Sathish, K. (2020). Biology and Morphometrics of Pulse Beetle, *Callosobruchus chinensis* (L.) on Chickpea. International Research Journal of Pure and Applied Chemistry, 21(23), 161-165.

Ren, Y., Lee, B., &Padovan, B. (2011). Penetration of methyl bromide, sulfuryl fluoride, ethanedinitrile and phosphine into timber blocks and the sorption rate of the fumigants. *Journal of Stored Products Research*, *47*(2), 63-68.

Ribeiro, B. M., Guedes, R. N. C., Oliveira, E. E., & Santos, J. P. (2003). Insecticide resistance and synergism in Brazilian populations of *Sitophiluszeamais* (Coleoptera: Curculionidae). *Journal of Stored Products Research*, *39*(1), 21-31.

Rumbos, C. I., Dutton, A. C., & Athanassiou, C. G. (2018). Insecticidal effect of spinetoram and thiamethoxam applied alone or in combination for the control of major stored-product beetle species. *Journal of Stored Products Research*, *75*, 56-63.

Sadozai, A., Naeem, M., Shah, M., Ali, A. (2003). Host preference of pulse beetle *Callosobruchus maculatus* in different legumes. Sarhad Journal of Agriculture (Pakistan).19:557–561

Sarfati, M. S., Kelly, D., &Brockerhoff, E. G. (2010). The parasitoids of seed predators attacking snow tussocks, *Chionochloa spp*.(Poaceae). *New Zealand Journal of Zoology*, *37*(1), 19-33.

Sathish, K., Jaba, J., Katlam, B. P., Mishra, S. P., Rana, D. K. (2020). Evaluation of chickpea, Cicer arietinum, genotypes for resistance to the pulse beetle, *Callosobruchus chinensis* (L.). Journal of Entomology and Zoology Studies, 8(3), 1002-1006.

Sayadi, A., Immonen, E., Bayram, H., & Arnqvist, G. (2016). The de novo transcriptome and its functional annotation in the seed beetle Callosobruchus maculatus. *PLoS One*, *11*(7), e0158565.

Schlipalius, D. I., Cheng, Q., Reilly, P. E., Collins, P. J., & Ebert, P. R. (2002). Genetic linkage analysis of the lesser grain borer *Rhyzoperthadominica* identifies two

Schöller, M., Prozell, S., Suma, P., & Russo, A. (2018). Biological control of stored-product insects. Recent advances in stored product protection, 183-209.

Sekender, S., Sultana, S., Akter, T., & Begum, S. (2020). Susceptibility of different stored pulses infested by pulse beetle, *Callosobruchus chinensis* (Lin.). Dhaka University Journal of Biological Sciences, 29(1), 19-25.

Sewsaran, R., Khan, A., Stone, R., & John, K. (2019). Resistance screening of 14 *Cajanus cajan* (L.) Millsp. cultivars to *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research, 82, 67-72.

Sharma, R., Devi, R., Soni, A., Sharma, U., Yadav, S., Sharma, R., Kumar, A. (2016). Growth and developmental responses of *Callosobruchus maculatus* (F.) on various pulses. Legume Research, 39(5), 840-843.

Shende, K. S., & Lifeter, Y. B. (2017). Post-harvest challenges of food crops in Jakairi subdivision, Cameroon. A threat to food security. *International Journal of Agriculture and Environmental Research*, *7*(10), 2455-6939.

Shrivastava, B., Shrivastava, A., Kumar, A., Bhatt, J. L., Bajpai, S. P., Parihar, S. S., & Bhatnagar, V. (2011). Impact of Deltamethrin on Environment, use as an Insecticide and its Bacterial degradation-A preliminary study. *International Journal of Environmental Sciences*, *1*(5), 977-985.

Shukla, U. N., Mishra, M. L. (2020). Present scenario, bottlenecks and expansion of pulse production in India: A review. Legume Research: An International Journal, 43(4).

Siddiqui, J. A., Fan, R., Naz, H., Bamisile, B. S., Hafeez, M., Ghani, M. I., & Chen, X. (2023). Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. Frontiers in Physiology, 13, 1112278.

Singh, A. K., Singh, S. S., Prakash, V. E. D., Kumar, S., Dwivedi, S. K. (2015). Pulses production in India: Present status, sent status, bottleneck and way forward. Journal of AgriSearch, 2(2), 75-83.

Singh, R. P., Prasad, P. V., Reddy, K. R. (2013). Impacts of changing climate and climate variability on seed production and seed industry. Advances in Agronomy, 118, 49-110.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), 389-394.

Sivakumar, C., Chandrasekaran, S., Vijayaraghavan, C., &Selvaraj, S. (2010). Fumigant toxicity of essential oils against pulse beetle, *Callosobrucrhusmaculatus* (F.)(Coleoptera: Bruchidae). *Journal of Biopesticides*, *3*(1), 317.

Stathers, T. E., Arnold, S. E., Rumney, C. J., Hopson, C. (2020). Measuring the nutritional cost of insect infestation of stored maize and cowpea. Food Security, 12(2), 285-308.

Su, C., & Xia, X. (2020). Sublethal effects of methylthio-diafenthiuron on the life table parameters and enzymatic properties of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Pesticide biochemistry and physiology*, *162*, 43-51.

Takla, S. S., El-Dars, F. M., Amien, A. S., Rizk, M. A. (2021). Prospects of Neem Essential Oil as Bio-Pesticide and Determination of Its Residues in Eggplant Plants During Crop Production Cycle. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 13(2), 1-15.

Talukder, F. (2009). Pesticide resistance in stored-product insects and alternative biorational management: a brief review. *Journal of Agricultural and Marine Sciences [JAMS]*, *14*, 9-15.

Tamilselvan, R., Kennedy, J. S., & Suganthi, A. (2021). Sublethal and transgenerational effects of spinetoram on the biological traits of Plutella xylostella (L.)(Lepidoptera: Plutellidae). *Ecotoxicology*, *30*, 667-677.

Tapondjou, L. A., Adler, C. L. A. C., Bouda, H., &Fontem, D. A. (2002). Efficacy of powder and essential oil from *Chenopodiumambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *Journal of stored products research*, *38*(4), 395-402.

Tefera, T., Mugo, S., Beyene, Y. (2016). Developing and deploying insect resistant maize varieties to reduce pre-and post-harvest food losses in Africa. Food Security, 8(1), 211-220.

Thakkar, B., & Parikh, P. (2018). Effects of host grains on the rice weevil *Sitophilus oryzae* (L.). Indian Journal of Entomology, 80(4), 1427-1430.

Thakkar, B., Pandya, P. & Parikh, P. (2020). Isolation, establishment and characterization of new insect cell line derived from midgut of rice weevil, *Sitophilus oryzae*. *In Vitro Cell.Dev.Biol.-Animal*.

Tripathi, A. K. (2018). Pests of stored grains. *Pests and their Management*, 311-359.

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., & Kumar, S. (2001). Toxicity, feeding deterrence, and effect of activity of 1, 8-cineole from *Artemisia annua*on progeny production of *Triboliumcastanaeum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology*, *94*(4), 979-983.

Trostanetsky, A., Quinn, E., Rapaport, A., Harush, A., & Gottlieb, D. (2023). Efficacy of deltamethrin emulsifiable concentrate against stored-product insects. *Journal of Stored Products Research*, *101*, 102072.

Tubiello, F. N., Soussana, J. F., & Howden, S. M. (2007). Crop and pasture response to climate change. *Proceedings of the National Academy of Sciences*, *104*(50), 19686-19690.

Upadhyay, N., Dwivedy, A. K., Kumar, M., Prakash, B., & Dubey, N. K. (2018). Essential oils as eco-friendly alternatives to synthetic pesticides for the control of *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae). *Journal of Essential Oil Bearing Plants*, *21*(2), 282-297.

Vassilakos, T. N., Athanassiou, C. G., & Tsiropoulos, N. G. (2015). Influence of grain type on the efficacy of spinetoram for the control of *Rhyzopertha dominica*, *Sitophilus granarius* and *Sitophilus oryzae*. *Journal of Stored Products Research*, *64*, 1-7.

Vassilakos, T. N., Athanassiou, C. G., Saglam, O., Chloridis, A. S., & Dripps, J. E. (2012). Insecticidal effect of spinetoram against six major stored grain insect species. *Journal of Stored Products Research*, *51*, 69-73.

Vayias, B. J., Kavallieratos, N. G., Athanassiou, C. G., & Tatsi, G. (2010, June). Insecticidal action of the combined use of spinosad and deltamethrin against three stored product pests in two stored hear-wheat varieties. In *Proceedings of the 10th international working conference on stored product protection* (Vol. 27, pp. 921-924).

Velki, M., Plavšin, I., Dragojević, J., & Hackenberger, B. K. (2014). Toxicity and repellency of dimethoate, pirimiphos-methyl and deltamethrin against *Tribolium castaneum* (Herbst) using different exposure methods. *Journal of Stored Products Research*, *59*, 36-41.

Wanjala, C. L., Mbugi, J.P., Ototo, E., Gesuge, M., Afrane, Y. A., Atieli, H. E., ... & Yan, G. (2015). Pyrethroid and DDT resistance and organophosphate susceptibility among *Anopheles* spp. mosquitoes, western Kenya. Emerging infectious diseases, 21 (12), 2178.

World Bank Report, (1999) Post-harvest Management -Fights hunger with FAO, India Grains, March 2002, 4(3): 20-22.

World Health Organization. (2019). *The state of food security and nutrition in the world 2019: safeguarding against economic slowdowns and downturns* (Vol. 2019). Food & Agriculture Organisation.

Xu, C., Zhang, Z., Cui, K., Zhao, Y., Han, J., Liu, F., & Mu, W. (2016). Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). *PLoS One*, *11*(6), e0156555.

Xu, P., Shu, R., Gong, P., Li, W., Wan, H., & Li, J. (2019). Sublethal and transgenerational effects of triflumezopyrim on the biological traits of the brown planthopper, Nilaparvata lugens (Stål)(Hemiptera: Delphacidae). *Crop Protection*, *117*, 63-68.

Yaseen, M., Kausar, T., Praween, B., Shah, S. J., Jan, Y., Shekhawat, S. S., Azaz Ahmad Azad, Z. R. (2019). Insect pest infestation during storage of cereal grains, pulses and oilseeds. In Health and Safety Aspects of Food Processing Technologies (pp. 209-234). Springer, Cham.

Yin, X. H., Wu, Q. J., Li, X. F., Zhang, Y. J., & Xu, B. Y. (2008). Sublethal effects of spinosad on *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Crop Protection*, *27*(10), 1385-1391.

Zhang, R. M., Dong, J. F., Chen, J. H., Ji, Q. E., & Cui, J. J. (2013). The sublethal effects of chlorantraniliprole on *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Integrative Agriculture*, *12*(3), 457-466.

Zhu, F., Gujar, H., Gordon, J. R., Haynes, K. F.·, Potter, M. F., & Palli, S. R. (2013). Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Scientific Reports, 3, 1456.

Zhu, F., Lavine, L., O'Neal, S., Lavine, M., Foss, C., & Walsh, D. (2016). Insecticide resistance and management strategies in urban ecosystems. Insects, 7(1), 2.

Zhu, Q., He, Y., Yao, J., Liu, Y., Tao, L., & Huang, Q. (2012). Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology of the cutworm, *Spodoptera litura*. *Journal of Insect Science*, *12*(1), 27.

Sharma Pankaj M.	Dr. Parth Pandya	Prof. Pragna Parikh
Ph.D. Candidate	Co-guide	Guide