Investigating insecticide efficacy and development of resistance in *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae)

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Ph.D. Thesis Submitted to
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THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
सत्यं शिवं सुन्दरम्

INVESTIGATING INSECTICIDE EFFICACY AND DEVELOPMENT OF RESISTANCE IN

Spodoptera frugiperda **Smith, 1797**

(LEPIDOPTERA: NOCTUIDAE)

Ms. Harshita Sharma

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Abstract of Ph.D. Thesis in the Field of Zoology

for the Degree of Doctor of Philosophy

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CHAPTER 1

1. INTRODUCTION

1.1. AGRICULTURE FIELDS AND PESTS

"Agriculture was the first occupation of man, and as it embraces the whole earth, it is the foundation of all other industries."

Edward W. Stewart

There are many agricultural fields in and around Vadodara **(Figure 1)**. This includes castor, maize, cotton, cauliflower, and banana. However, farmers cannot make the expected profits due to the invasion and infestation by various pests of the agricultural fields.

Since humans started cultivating wild plants for their own needs, insects, birds, rodents, and other animals, plant pathogens, and weeds have all become competitors for crop yield. The agriculture fields of Vadodara have flourished with several crops of importance. Climate and pest infestation are the biggest challenges, and amongst these, whenever a new challenge comes, like an invasive pest, it adds to the problem.

1.2. VARIOUS INSECT ORDERS CREATE THE PROBLEM

"The first day, one is a guest, the second a burden, and the third a pest."
~ Jean de la Bruyere

The most devastating effects and destruction of already produced crops in agricultural fields with much investment in money and labour are caused by pests in the form of weeds, fungi, nematodes, insects, etc. A constant threat to crops remains due to pests **(Bruce, 2010)**. Amongst all the major pests of the agriculture fields, insects are said to be causing high levels of damage. Global warming will further result in substantial crop losses and food security problems due to low pest control efficacy **(Sharma, 2014)**. The agricultural insect pests mainly belong to Order Orthoptera (e.g. *Gryllotalpa fossor*, *Schistocerca gregaria*), Hemiptera (e.g. *Aphis gossypii*, *Bemisia tabaci*), Coleoptera (e.g. *Holotrichia insularis*, *Oryctes rhinoceros*) and Lepidoptera (e.g. *Spodoptera litura*, *Plutella xylostella*). Coleoptera, followed by Lepidoptera, are the most significant insect order. Therefore, the high diversity of these species is also observed **(Stork, 2018)**.

Castor field

Cotton field in Savli

Cauliflower field

Maize field

Figure 1: Agriculture fields around Vadodara

1.3.MENACE OF INSECTICIDES

"To make agriculture sustainable, the grower has got to be able to make a profit."
Sam Farr

There is a large market for pesticides in the world. These work against a different group of troublemakers. If little is good, more is better. This has been the behaviour of farmers towards pest control by using pesticides. Humans try to control harmful insects with the help of pesticides. This can have some difficulties. Stimulating free radicals, lipid peroxidation, and disturbance of the body's antioxidant capability are mechanisms of toxicity observed in organophosphates, herbicides, and organochlorine pesticides **(Abdollahi et al., 2004)**.

1.4.INSECTICIDE EFFICACY

"The farmer works the soil; the agriculturist works the farmer."
Eugene F. Ware

Insects cause some problems. Control over different pests requires specific treatments. These vary as per the chemical composition as well as the amount required. There can be a pest causing a nuisance in the house of agriculture fields, or they might act as a vector of many diseases. Insects have been majorly contributor to the spread of various severe and fatal diseases. Other than controlling agricultural pests, insecticides are used to control vectors as well. Insecticides, to be effective in the control of insects, must be prepared into a suitable form for a particular application method called – formulation. It involves the addition of various chemical solvents or diluents to improve the effectiveness and properties of the insecticide **(Rathburn, 1985)**.

1.5.RESISTANCE DEVELOPMENT IN INSECTS TO INSECTICIDES

"Natural selection certainly operates. It explains how bacteria will gain antibiotic resistance; it will explain how insects get insecticide resistance, but it does not explain how you get bacteria or insects in the first place."
William A. Dembski

Resistance in insects to insecticides is a phenomenon that occurs when populations of insects become less sensitive to the toxic effects of insecticides over time. This can happen because of several factors, including genetic mutations, adaptation, and changes in behaviour. The presence of pests on different crops throughout the year has widely exposed them to insecticides and resulted in the rapid development of resistance to a range of these insecticides **(Saleem et al., 2008)**.

1.6.MOLECULAR BASIS OF RESISTANCE

"Genes are like the story, and DNA is the language in which the story is written."
Sam Kean

The molecular basis of insect resistance development is primarily due to genetic mutations that affect the target site of the insecticide or the metabolic pathways that detoxify the insecticide. One common mechanism of resistance is the alteration of the target site of the insecticide, detoxification. For example, cytochrome P450-dependent monooxygenases are essential in the catabolism and anabolism of xenobiotics and endogenous compounds. This monooxygenase-mediated metabolism is a common mechanism by which insects become resistant to insecticides **(Scott, 1999)**.

1.7.LEPIDOPTERA

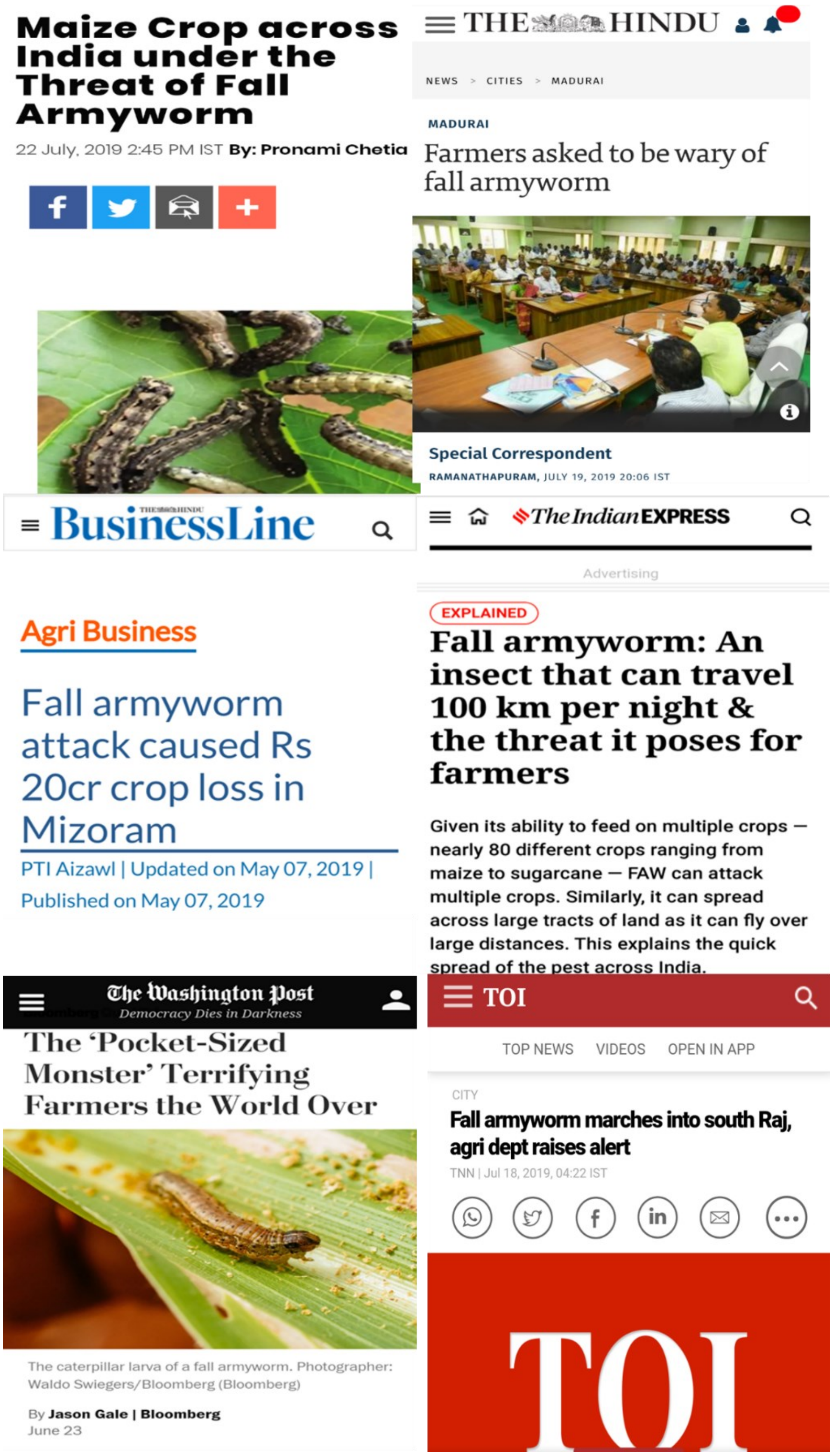
"Just when the caterpillar thought – I am incapable of moving, it became a butterfly."
Annette Thomas

Lepidopterans (butterflies & moths) depend highly on crops for survival. A few widespread are *Spodoptera litura*, *Plutella xylostella*, and *Helicoverpa armigera*. There occurs substantial monetary loss in the control and management of the Lepidopteran pest. One such pest is DBM. Management costs and lost production with diamondback moth are approx US$4 to US$5 billion **(Zalucki et al., 2012)**.

1.8.THE PEST- SPODOPTERA FRUGIPERDA

"I get inspiration from many things around me - nature, hills, people, and even insects."
Ruskin Bond

The insect in the headlines in India and the world in recent years is – The fall armyworm **(Figure 2)**. Its highly destructive nature is a cause of concern for the country. *Spodoptera frugiperda* (J. E. Smith) is a problematic agricultural pest. *S. frugiperda* has a wide range of host crops that are of economic concern to us **(Montezano et al., 2018)**. African countries stated sudden and severe outbreaks of fall armyworm populations due to their vast dispersal and strong flying skills. At the same time, this is the first invasion outside the American continent **(Goergen et al., 2016)**. In India, FAW infestation started in Karnataka in 2018 and was later reported in many states **(Deshmukh et al., 2018)**.



Maize Crop across India under the Threat of Fall Armyworm

22 July, 2019 2:45 PM IST By: Pronami Chetia

THE HINDU

NEWS > CITIES > MADURAI

MADURAI

Farmers asked to be wary of fall armyworm

Special Correspondent

RAMANATHAPURAM, JULY 19, 2019 20:06 IST

BusinessLine

Agri Business

Fall armyworm attack caused Rs 20cr crop loss in Mizoram

PTI Aizawl | Updated on May 07, 2019 | Published on May 07, 2019

The Indian EXPRESS

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EXPLAINED

Fall armyworm: An insect that can travel 100 km per night & the threat it poses for farmers

Given its ability to feed on multiple crops — nearly 80 different crops ranging from maize to sugarcane — FAW can attack multiple crops. Similarly, it can spread across large tracts of land as it can fly over large distances. This explains the quick spread of the pest across India.

The Washington Post

Democracy Dies in Darkness

The 'Pocket-Sized Monster' Terrifying Farmers the World Over

The caterpillar larva of a fall armyworm. Photographer: Waldo Swiegers/Bloomberg (Bloomberg)

By Jason Gale | Bloomberg

June 23

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Fall armyworm marches into south Raj, agri dept raises alert

TNN | Jul 18, 2019, 04:22 IST

TOI

Figure 2: FAW in news

1.9. MAIZE:

"A light wind swept over the corn, and all nature laughed in the sunshine."
Anne Bronte

Maize (*Zea mays* L.) is a cereal food crop of significance to the world with the highest production and productivity. It is an essential crop for food, feed, fodder, and many industrial products. The crop suffers much biotic stress because of its vulnerability to weeds, insect pests, and pathogens. These factors' extent varies tremendously depending upon factors like the cultivar used, the season, and the location. A timely estimation of crop loss can help reduce crop loss.

1.10. CHALLENGES WITH FAW:

"Opportunities to find deeper powers within ourselves come when life seems most challenging."
Joseph Campbell

The fall armyworm (*Spodoptera frugiperda*) is a highly destructive insect pest that feeds on the leaves and stems of crops, causing significant damage to maize, rice, and other crops. Studying insecticide resistance in fall armyworms presents several challenges, including:

- Rapid Evolution of Resistance
- Lack of Standardized Testing Methods
- Difficulty in Identifying the Mechanisms of Resistance
- Limited Information on Population Dynamics
- High Cost of Monitoring and Control

1.11. INSECTICIDES USED IN THE STUDY:

"It is important to concede that modern pesticides have helped to make farming more productive and to increase yield."
Chuck Norris

Using a combination of Chlorantraniliprole and Emamectin benzoate is one approach proposed to combat pesticide resistance in insects. Chlorantraniliprole and Emamectin benzoate are insecticides that target different sites in the insect's nervous system and have different modes of action, making it more difficult for insects to develop resistance to both compounds. Studies have shown that the combination of Chlorantraniliprole and Emamectin benzoate can effectively control a variety of insect pests, including Lepidoptera, Coleoptera, and Hemiptera species. Their mode of action is different from older classes **(Figure 3, 4)**

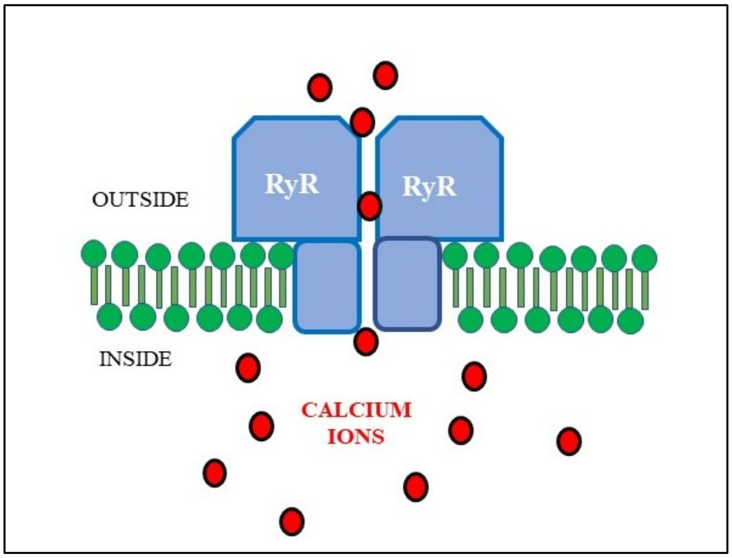


Figure 3: Diamide Family (Chlorantraniliprole)

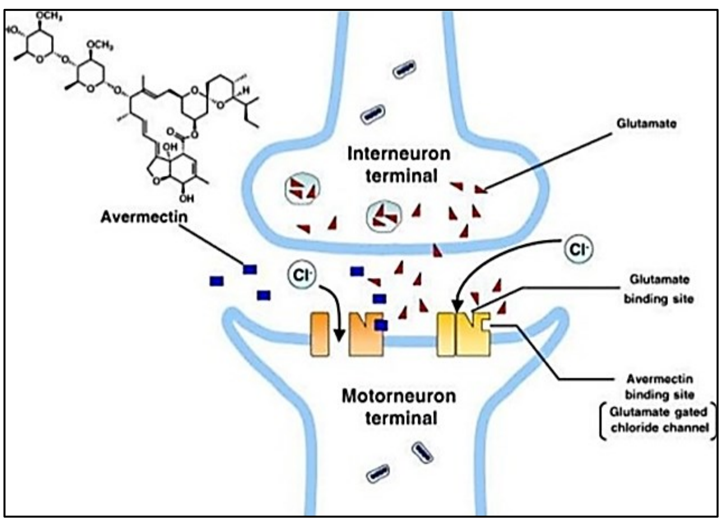


Figure 4: Avermectin Family (Emamectin Benzoate)

Aim of the work:

"A goal without a plan is just a wish."

Antoine de Saint-Exupery

The study aims to investigate the efficacy of two new-generation insecticides and determine if their use against *Spodoptera frugiperda* Smith, 1797 can get controlled or stop responding to these insecticides. To fulfil the aim, the following objectives were undertaken:

Objective 1:

A survey of agricultural fields in and around Vadodara to check the presence and infestation of *Spodoptera frugiperda*

Objective 2:

They were evaluating natural and artificial diets for the biology study of *Spodoptera frugiperda*. Different artificial diet ingredients are compared to find a better lab-reared diet.

Objective 3:

Checking insecticide efficacy (Chlorantraniliprole and Emamectin Benzoate) for the control of *Spodoptera frugiperda*

Objective 4:

Comparing control and insecticide-treated insect midgut tissue by histology.

Objective 5:

Understanding resistance development in *Spodoptera frugiperda* against the insecticides Chlorantraniliprole & Emamectin Benzoate: The molecular basis of resistance

CHAPTER 2

2. REVIEW OF LITERATURE

The *Spodoptera frugiperda* Smith, 1797 (fall armyworm or FAW) is an ecologically important agricultural pest worldwide. It causes damage to several crops of ecological importance. The FAW was limited to the agricultural fields of the American continent before invading Africa in 2016 and Asia in 2018. The first reported case of *Spodoptera frugiperda* was on maize in Karnataka, India, and it was the first report from the continent of Asia.

2.1. THE WORLD

FAW has spread in many countries in Africa and Asia in recent times. The initial detections of the corn strain of *Spodoptera frugiperda* in China were confirmed using the molecular marker method- CO1 and Tpi genes by **Jing and friends (2020)**. Insecticide resistance is making it difficult to control the Lepidoptera pests. **Wei and others (2019)** stated that by studying its resistance mechanisms and invasion pathways, we could better understand the movement of resistance genes and invasion pathways. The midgut plays a role in causing resistance as the detoxification genes play an essential role there. **Sun and team (2019)** found that it happened due to an increase in P450 monooxygenase activity, primarily in the fat body and midgut. **Dumas and friends (2019),** working with the insecticide Chlorantraniliprole on *Leptinotarsa decemlineata*, indicated the presence of a few HSPs (heat shock proteins), whose deletion led to mortality and verified HSP's role in resistance. **Li et al., 2019** discovered the cause for *Spodoptera litua's* resistance to tomatine in tomatoes was discovered through RNA sequence analysis of the animal's fat body and midgut, which implicated DEGs (differentially expressed genes) such as cytochrome P450 **(Figure 5)**. The GSTs or Glutathione transferases are a family of enzymes ubiquitously found in aerobic organisms. **Enayati and team (2005)** stated GST's significant role in detoxifying both endogenous and xenobiotic compounds.

2.2.INDIA

Deshmukh and colleagues (2018) reported *Spodoptera frugiperda* for the first time in India on maize fields and later on other crops in Karnataka state at Agricultural University fields. The confirmation was done through molecular analysis. The first occurrences of the exotic pest fall armyworm in sugarcane were reported by **Srikanth and team (2018**) from the south Indian state of Tamil Nadu. *Spodoptera frugiperda* has been identified in Southern Rajasthan by **Babu and the group (2019)**. It was through morphological and DNA barcoding methods. The percentage damage was between 10 to 40% in different hybrids. The life history of FAW lab conditions was studied at UAHS, Shivamogga, Karnataka, by **Sharanabasappa with the team (2018).** Gravid females were laying eggs with a fecundity of 1064 eggs. Incubation, total larval, and pupal period were observed to be 2-3, 14-19, and 9- 12 days, respectively. The total life cycle of males and females was observed to be 32-43 and 34-46 days, respectively. The larvae fed on hosts like sorghum, cabbage, tomato, groundnut, and sugarcane but not on rice which shows its belonging to the Corn strain.

2.3.GUJARAT

Since its identification, the pest S. frugiperda has been creating a problem in Gujarat. **Sisodiya and colleagues (2018)** reported the first fall armyworm occurrence in Gujarat in the sweet corn fields. Distinct morphological features are the basis of its primary identification. The lepidopteran pests have been tried to control with different pesticides. In the Junagarh area of Gujarat, **Bhut and team (2022)** did field experiments on the efficacy of chemical insecticides against two of the significant castor pests- *Spodoptera litura* and *Achaea janata*. Chlorantraniliprole, Spinosad and Emamectin benzoate provided good control. Combination insecticides often provide effective control against insects. The control by insecticides on other pests was also checked. Insecticide efficacy was checked by **Devashrayee et al. (2022)** in Navsari, Gujarat, against Indian bean pod borers. Of all the insecticides tested, Emamectin benzoate 5SG was the best with maximum pod yield and increase in a pod over control. It was effective against both *Helicoverpa armigera* and *Maruca vitrata.* Also, Indoxacarb 14.5 SC effectively controlled the pests after Emamectin benzoate.

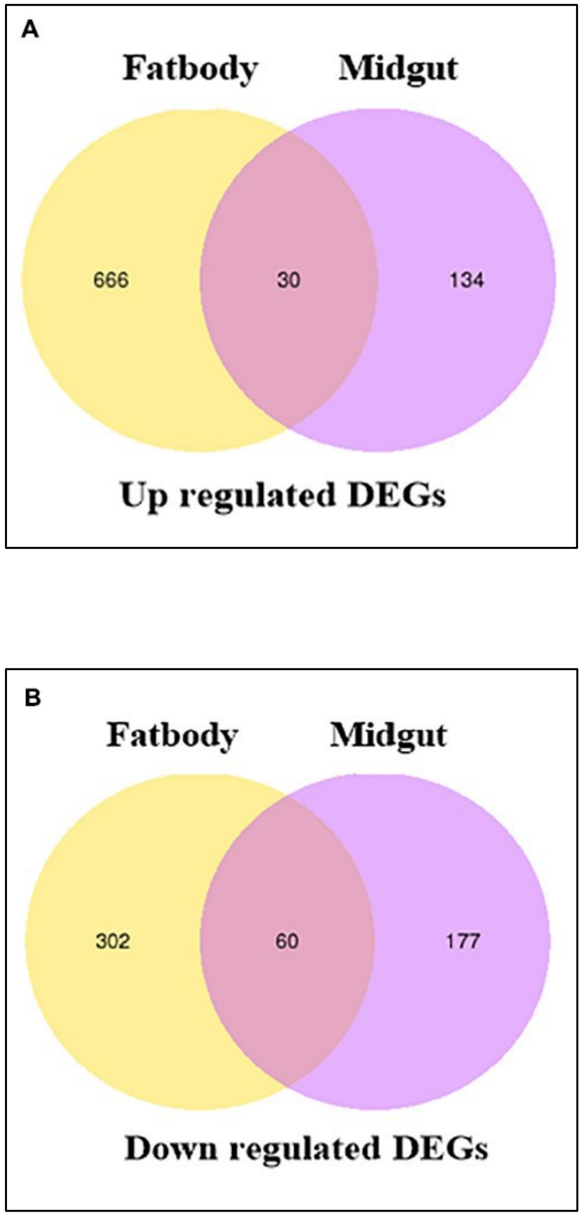


Figure 5: Venn diagram depicting the fat bodies and midguts differentially expressed genes in the of S. litura against treatment with tomatine **(Source: Li et al., 2019)**

CHAPTER 3

3. MATERIALS AND METHODS

3.1.METHODOLOGY FOR OBJECTIVE 1

Survey of agricultural fields: A thorough survey of agriculture fields in the Vadodara district was done, followed by collection **(Figure 6)**. Various fields are in different directions where several crops are grown (Table 3.1). These agricultural fields were surrounded in and around Vadodara. This included various necessary fields where farming is done to a large extent, and vast areas of farms are present. Different types of crops are grown in various fields. In the lab, the assessment and severity of insect pests and the crops they damage in the agricultural fields of Vadodara have been done previously, with details given in the thesis.

3.2 METHODOLOGY FOR OBJECTIVE 2

Rearing in the Lab: The insects were reared on a natural diet for a complete life cycle. The larvae were reared in separate trays, closed with a lid, and maintained at 25±4˚C, 70±10% RH. Each insect was kept in separate cells to avoid cannibalism.

Rearing on different Artificial Diets: A larval diet was prepared with the minimum ingredients possible to make it economically viable. Four diets were made and compared.

3.2METHODOLOGY FOR OBJECTIVE 3

Efficacy studies- The insects used for the study were first collected from the fields and made to acclimatize in the laboratory for a few generations (three generations). Testing started after that.

Insecticides: Technical grades of chlorantraniliprole and emamectin benzoate **(Figure 7)** were made in different ppm and tested. The larvae stage selected for insecticide efficacy experiments was of the 4^{th} instar stage.

Diet incorporation assay: The different concentrations of both pesticides were tested through the "Diet Incorporation Assay" of IRAC. An untreated check was also kept; the diet without insecticides was placed. The setup was checked every day. After 72 hours, observations were taken.

Figure 6: Collection of Fall Armyworm from Agricultural Fields

Technical grade Chlorantraniliprole   Technical grade Emamectin benzoate

Figure 7: Details of Chlorantraniliprole and Emamectin Benzoate: showing the structure and properties

3.3 METHODOLOGY FOR OBJECTIVE 4

Histological studies were conducted for insect control and resistant *Spodoptera frugiperda* Smith. Midguts were taken for analysis. Since the midgut is essential to detoxification, it was selected for histological studies. The control population was kept insecticide-free throughout the study, and the resistant population was seen after exposure to the LD_{50} concentration for four generations.

3.4 METHODOLOGY FOR OBJECTIVE 5

The development of resistance occurs after the target population develops some internal mechanism to fight and inhibit the effect of the insecticides. These internal mechanisms need to be known to understand the cause of resistance. Midgut samples were collected from the control (untreated throughout) and test populations (the ones exposed to Emamectin benzoate treatment for some generations) to identify the genes responsible for resistance development.

DETAILED PROCEDURE FOR MOLECULAR ANALYSIS

Extraction of RNA and quality assessment: Tissues from insect midguts were collected from control and insecticide-treated pests in the TRIzol reagent (Sigma Aldrich, USA) and stored at -80°C until processed. RNA was extracted using the TRIzol method. The concentration of Nanodrop 1000 was measured to check the extraction's purity. Extracted RNA's quality was checked on Qubit 4.0 fluorometer (Thermofisher #Q33238) using an RNAHS assay kit (Thermofisher #Q32851) following the manufacturer's protocol. To measure the purity of the extraction, we also measured the concentration of Nanodrop 1000. In the end, RNA was checked on the TapeStation using HS RNA ScreenTape (Agilent) to obtain RIN values. The library was queried on the Tapestation 4150 (Agilent) using susceptible D1000 screen tapes (Agilent #5067-5582) following the makers' protocol to determine the insert size. All the libraries after passed on were taken for the library sequencing. A flow of work is there **(Figure 8)**.

Overall Statistics: Percent survival on various diets was checked. Larval growth was compared for the diets using the larval growth index. For diets, the descriptive statistic feature of the statistical software Minitab19. Graphical comparison through GraphPad prism 9. Both insecticides' toxicity at different concentrations against Fall armyworm was evaluated. The LD_{50} was checked with the help of Probit analysis of SPSS software. Various plots represented transcriptome analyses. DGE was analyzed by graphical comparison between the control and treated sample. A total of three plots were made, including the MA plot, Volcano plot and Heat map.

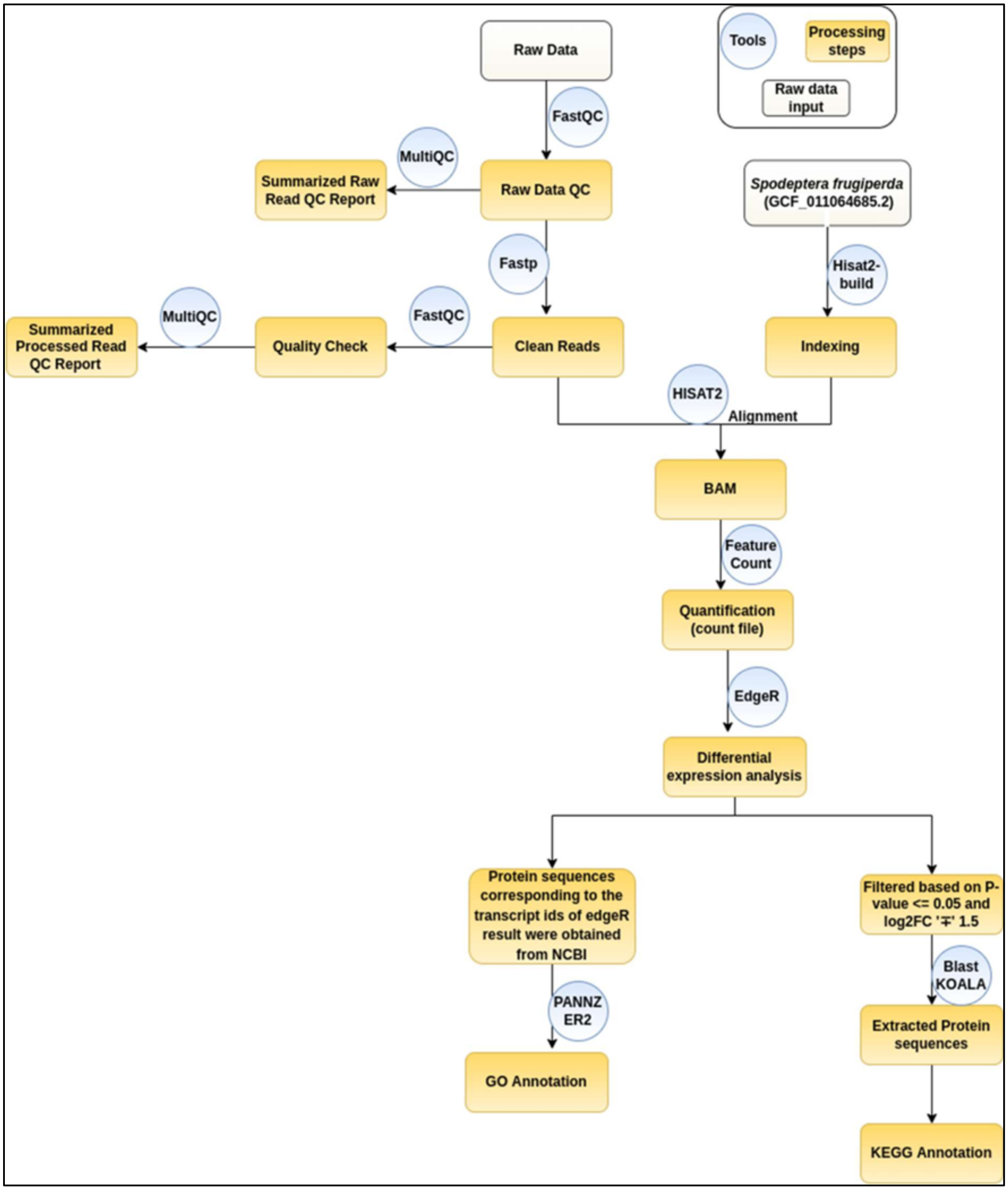


Figure 8: Flow of transcriptome analysis

CHAPTER 4

4. RESULTS

4.1 RESULTS FROM THE FIELD SURVEY

A survey of the agriculture fields in Vadodara was done. Many crops of importance are grown in the agricultural fields in and around Vadodara. The fields with crops grown there are mentioned **(Table 1)**. In each maize field, fall armyworm was found to be present and was causing severe destruction to the crop **(Figure 9)**. Rearing was done in the lab for all life stages **(Figure 10)**.

Table 1: Agriculture fields of Vadodara with location and crops grown there

Study sites	Location	Type of crops
Chhani	11 km North of Vadodara	Maize, Cotton, Castor, Brinjal, Pigeon pea, Sorghum, Ladyfinger, Potato, Brinjal, Radish & Cauliflower
Sherkhi	13 km in the North West of Vadodara	Maize, Cotton, Castor, Pigeon pea, Sugarcane, Cauliflower
Waghodia	10 km East of Vadodara	Maize, Cotton, Castor, Sugarcane & Brinjal
Padra	17 km. South West of Vadodara	Maize, Cotton, Castor, Pigeon pea, Cabbage, Paddy
Savli	30 km North of Vadodara	Maize, Cotton, Castor, Rice, Banana, Cauliflower
Chapad	11 km South of Vadodara	Maize, Cotton, Castor
Dandiapura	80 km East of Vadodara	Maize, Chickpea, Cotton, Castor

Figure 9: Damage by FAW in agricultural fields

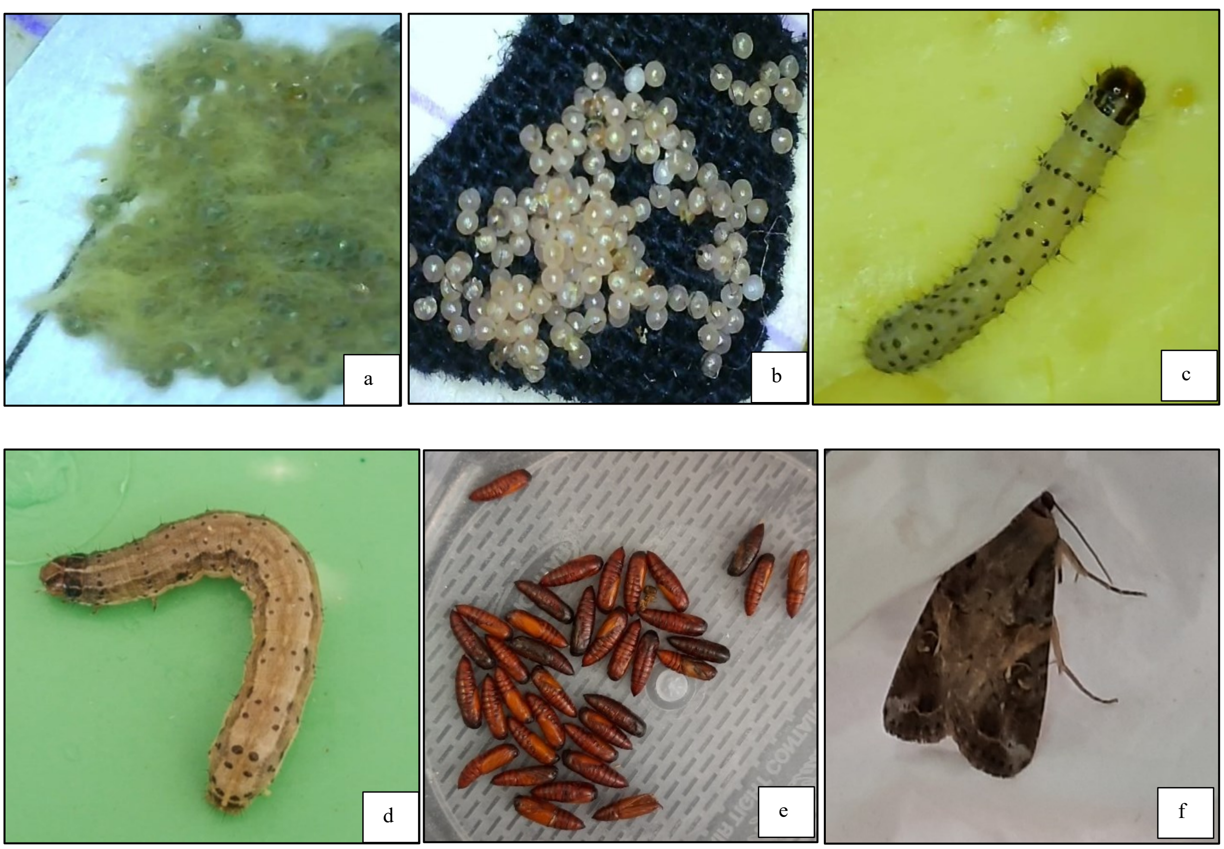
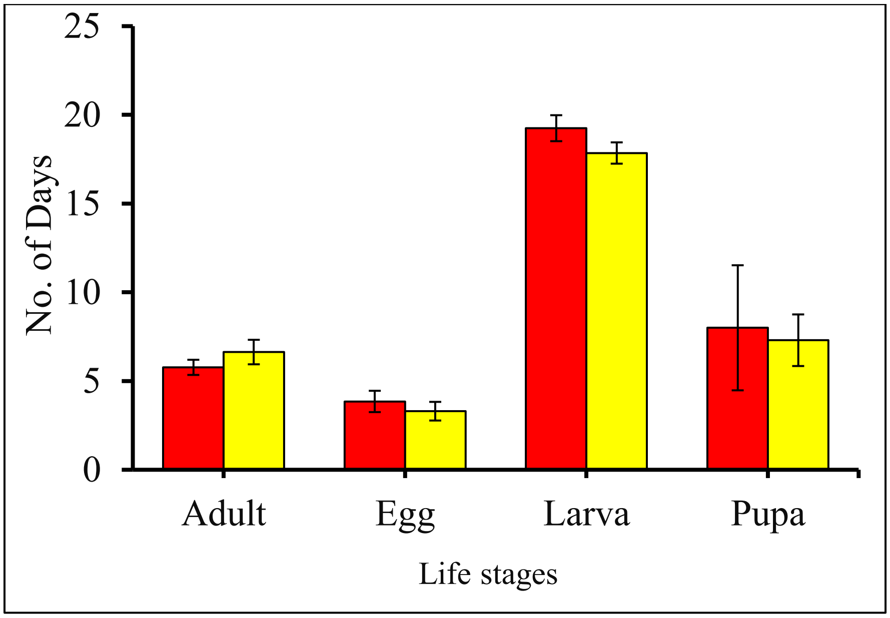


Figure 10: Various stages of FAW reared in the lab

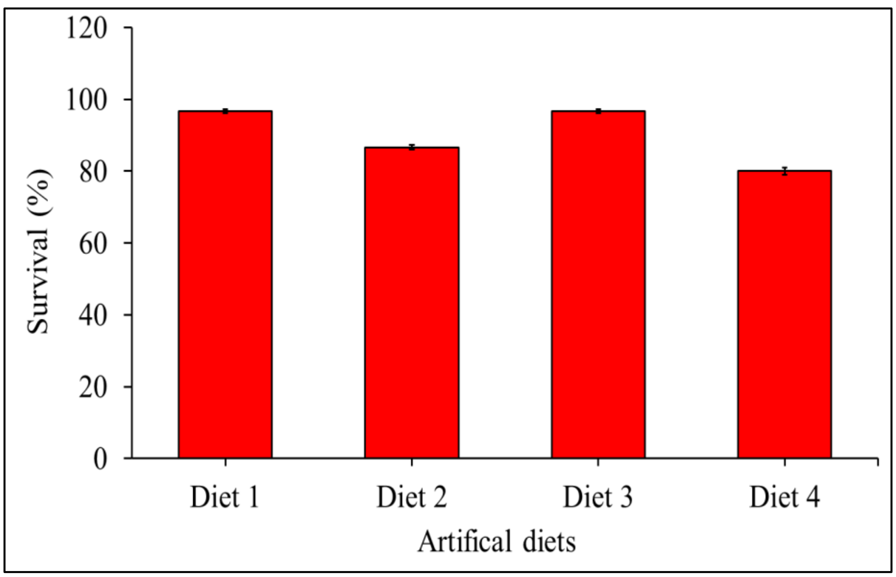
(a; early egg mass, b: late egg mass, c: early instar, d: late instar, e: pupa, f: adult)

4.2 RESULTS FROM THE DIET STUDIES

The observations for various diets on the survival of *Spodoptera frugiperda* are shown in a graphical comparison between survival and completion of the life cycle between natural and artificial diets **(Graph 1)**, and various artificial diets have been done **(Graph 2).**



Graph 1: Survival on natural & artificial diet



Graph 2: Survival on different artificial diets

4.3 RESULTS FROM THE INSECTICIDE EFFICACY

The pest *Spodoptera frugiperda* was treated with chlorantraniliprole and emamectin benzoate at different concentrations. The concentrations, i.e., 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm, were used. No mortality in control was observed. The surviving insects from the exposure were cultured from further generations with testing and exposure to various doses on the 3rd or 4th instar in every generation, similar to the first generation. Overall results for both are shown **(Table 2, 3)**.

Table 2: Mortality values of *S. frugiperda* against Chlorantraniliprole over generations (G 1-4)

Concentration (ppm)	G-1	G-2	G-3	G-4
10	100.00	100	100.00	100.00
5	100.00	100	93.33	90.00
1	100.00	100	86.66	60.00
0.5	93.33	93.33	60.00	50.00
0.1	60.00	60.00	50.00	46.66
0.05	53.33	50.00	46.66	20.00
0.02	46.66	46.66	20.00	0.00
0.01	20.00	16.67	10.00	0.00
Control (or Untreated)	0.00	0.00	0.00	0.00

Table 3: Mortality values of *S. frugiperda* against Emamectin benzoate over generations (G1-4)

Concentration (ppm)	G-1	G-2	G-3	G-4
10	100.00	100.00	100.00	100.00
5	100.00	100.00	100.00	86.00
1	93.33	90.00	86.00	56.66
0.5	60.00	56.66	53.33	46.66
0.1	46.66	50.00	43.33	30.00
0.05	40.00	46.66	33.33	16.66
0.02	10.00	33.33	20.00	0.00
0.01	0.00	0.00	0.00	0.00
Control (or Untreated)	0.00	0.00	0.00	0.00

4.4 RESULTS FROM THE HISTOLOGY

The histology of *Spodoptera frugiperda* midgut was observed in a brightfield microscope- DM 750, Leica. Photography was done for all three types of midguts- control (susceptible) and resistance (Emamectin-resistant insect). Observations were taken at three magnifications, namely, 10X, 20X and 100X.

Control: *S. frugiperda*'s midgut had an epithelial layer, and the digestive cells' cytoplasm had a uniform, well-developed nuclei. These cell surfaces were well-striated, and the peritrophic matrix in the midgut lumen was well-developed. Muscular layers lined its basal surface **(Figure 11- A, B, C)**.

Resistant: The midgut region had fewer deformities observed than the treated ones. The structure seemed largely intact. The longer and more regular exposure to the insecticide might have kept the structure more stable than the initial exposure. However, little vacuolization was observed with a slight deformation in shape **(Figure 11- D, E, F)**.

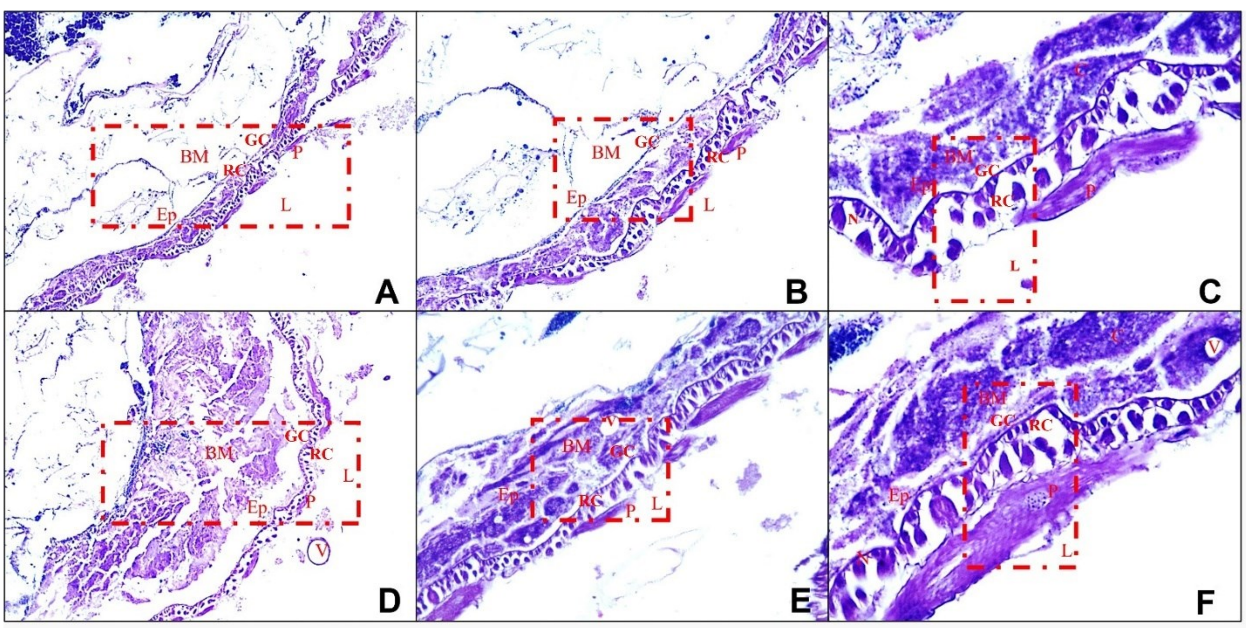


Figure 11: Histology of control and resistant midgut of *Spodoptera frugiperda*

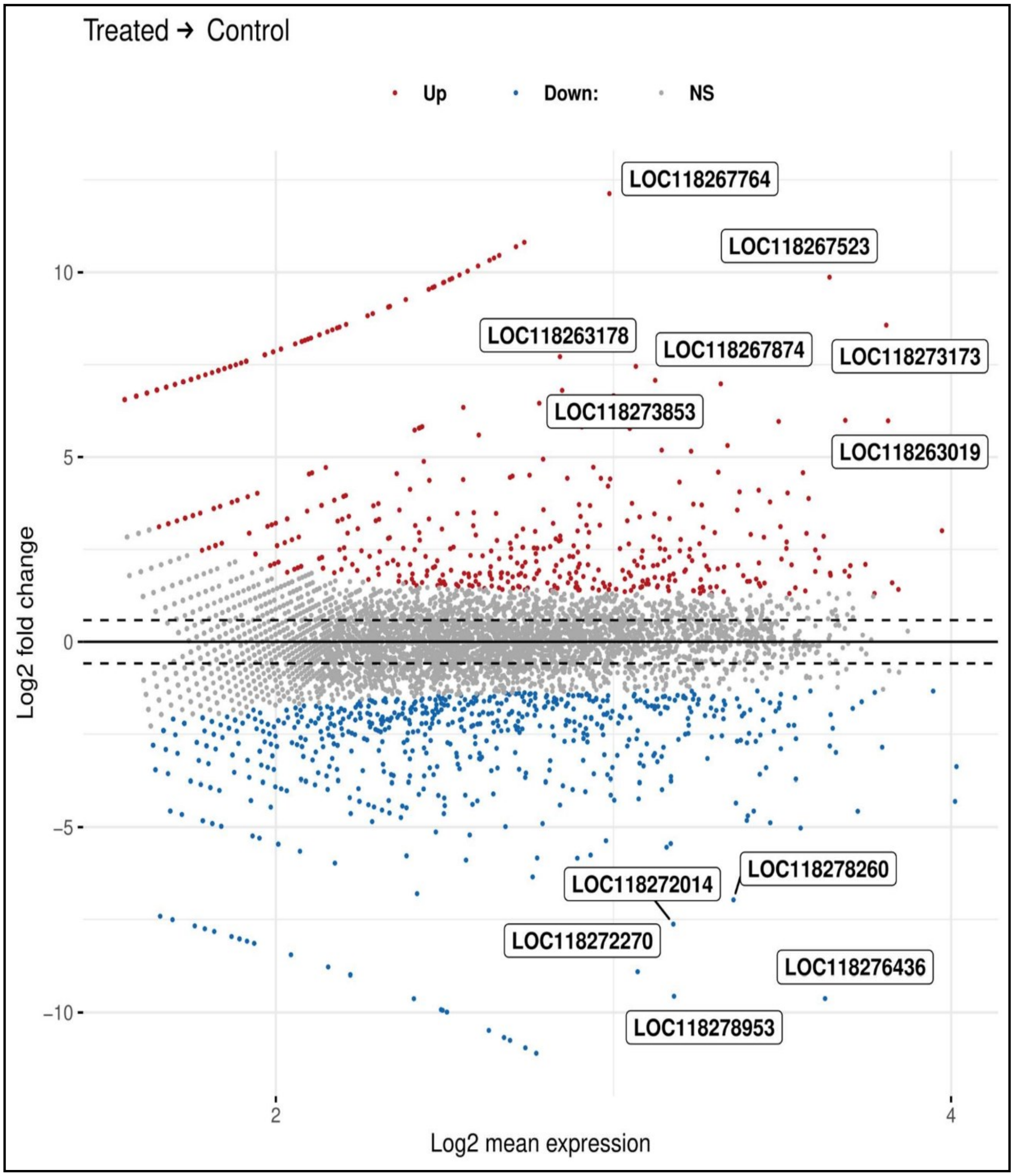
4.5RESULTS FROM THE TRANSCRIPTOME STUDIES

A total of 464 genes were up-regulated, and 607 genes were downregulated in treated (emamectin) compared to control (susceptible) insects. It includes various categories of genes performing various vital functions inside the insect's body. This difference in the profile between the control and treated sample serves as a basis for the comparison of the two. It helps to find out the reasons helping the insect to increase its tolerance to the lethal chemical. Further, these up-regulated and down-regulated genes are analyzed and discussed. Some plots were made to analyze the differential gene expression.

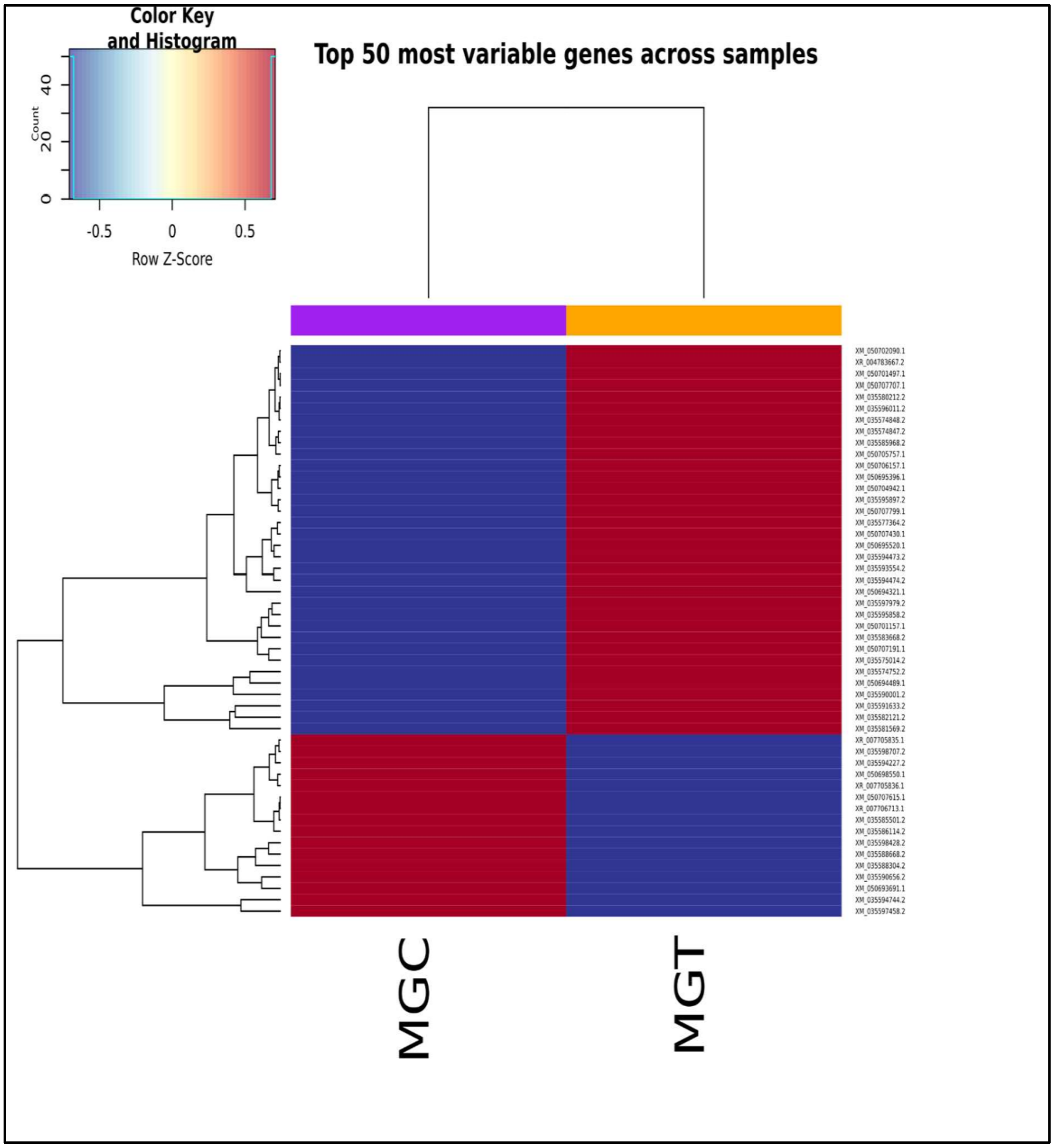
One such is the MA plot. MA-plot shows the distribution of the gene expression between the groups' MGT (treated) and MGC (control). The Y-axis shows the Log2fold change (M), and the X-axis represents the log of the mean of normalized expression counts (A) of the samples. Red dots correspond to genes up-regulated (>+1.5), and blue dots correspond to the down-regulated genes (<- 1.5) based on the p-value `0.05`. The grey dot corresponds to the non-significant genes where the p-value > 0.05 **(Graph 3)**.

A heat map was also made. The top 50 most variable genes are shown in the heatmap across samples. Heatmap shows the top 50 genes with the highest variance across samples between the group of the two samples **(Graph 4)**.

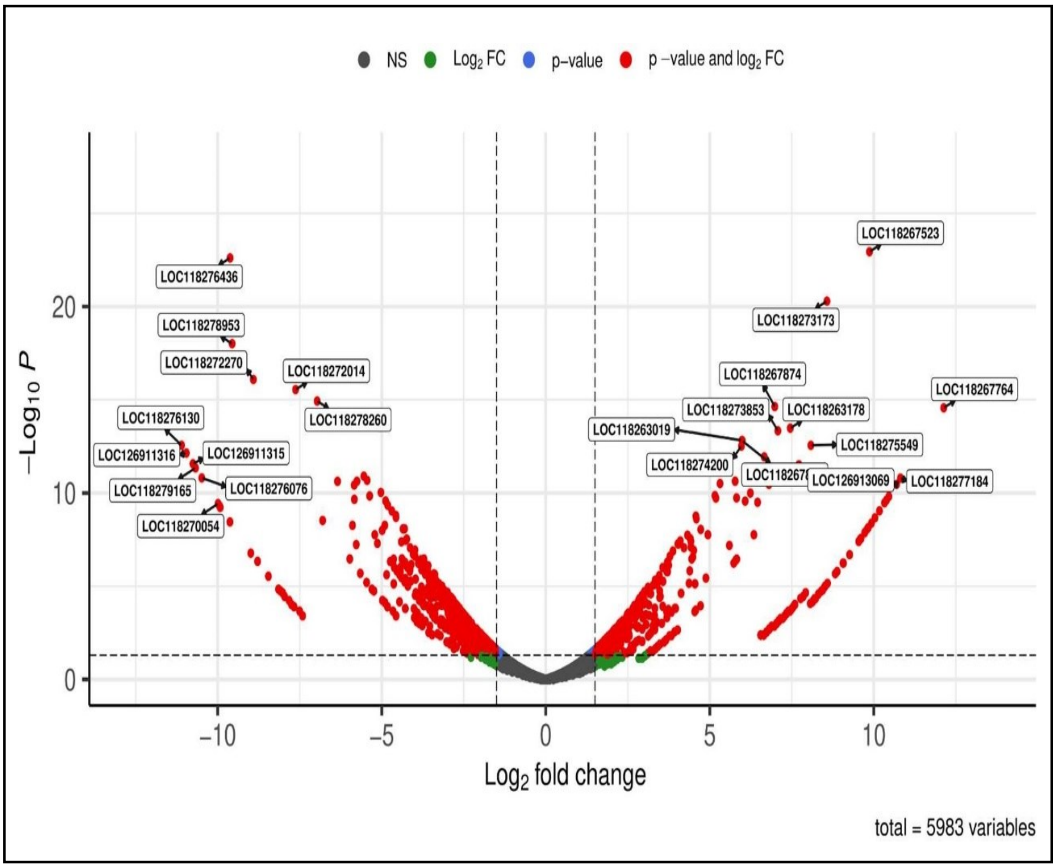
Another plot is the volcano plot. Volcano plot of MGT (treatment) and MC expressed genes (control). The x-axis represents a log 2-fold change in gene expression between the treated and control groups, and the y-axis displays log 10 p values. Significant genes are represented by the red points (p-value 0.05) **(Graph 5)**.



Graph 3: MA Plot of Treated vs Control



Graph 4: Top 50 most variable genes heatmap across samples heatmap showing the top 50 genes with the highest variance across samples between the group of the two samples.



Graph 5: Volcano Plot of Treated vs Control

All the genes in our study which got differentially expressed, including 607 downregulated and 464 up-regulated ones. Each of them plays a different role in the physiology of the insect. Some of the various up-regulated genes, such as collagenase, cholinesterase 1-like, and brachyurin-like have proteolysis and hydrolyze activity. Some of the various downregulated genes, such as essential juvenile hormone-suppressible proteins 1 and 2, have nutrient reservoir activity. A number of differentially expressed genes are well known for their role in metabolism and metabolic detoxification. Up-regulated genes included glutathione S-transferase 1, acetylcholinesterase, cytochromes, esterases, and transporters. This suggests their role in providing resistance against emamectin benzoate treatment as well.

CHAPTER 5

5. DISCUSSION

Agricultural insect pests like *S. frugiperda* cause high crop losses that result in a lack of food, animals for fodder, and economic loss to farmers and consumers. Human survival necessitates a steady supply of food, fodder, and other agricultural supplies. With pests like the fall armyworm invading fields and destroying crops, there is the possibility of a resource shortage. Farmers suffer losses from investing in seeds, growing, fertilizers, and pesticides but not receiving the expected yield. Such a lack of supply would increase the price and cause problems for consumers. Pests thus have an impact not only on ecology but also on the economy. Several insect pests have already been causing crop damage in the agricultural fields of Vadodara.

5.1. GLUTATHIONE S-TRANSFERASE:

In insects, the overexpression of the GST gene has been shown to confer resistance to various classes of pesticides, including organophosphates, pyrethroids, and carbamates. The overexpression of the GST gene in insects can be induced by exposure to sublethal concentrations of pesticides. Activating the Nrf2 pathway can also induce the overexpression of the GST gene in insects. The overexpression of the GST gene in insects can result in the metabolism and detoxification of various classes of pesticides, including avermectins **(Li et al., 2022)**.

5.2.ZINC TRANSPORTER ZIP1:

Zinc transporter ZIP1 is a membrane protein that facilitates zinc transport into cells. Although its primary function is related to zinc homeostasis, recent studies have suggested that ZIP1 may play a role in the detoxification of certain pesticides **(Bafaro et al., 2017)**.

5.3.CYTOCHROME P450 ENZYMES:

Cytochrome P450 enzymes are a superfamily of heme-containing proteins involved in metabolizing a wide range of endogenous and exogenous compounds. These enzymes are primarily located in the endoplasmic reticulum of the liver and intestinal cells, but they are also found in other tissues, such as the lung, kidney, and brain **(Elfaki et al., 2018)**.

5.4.ACETYLCHOLINESTERASE:

Overexpression of the AChE gene Mi-ace-1 was associated with resistance to abamectin. The overexpression of this gene was found to increase the expression and activity of AChE in the nematode, leading to an increased ability to detoxify abamectin and a decreased sensitivity to this compound **(Huang et al., 2016)**.

5.5.GLYOXYLATE/HYDROXYPYRUVATE REDUCTASE:

GHPR is an NADPH-dependent enzyme that catalyzes the reduction of glyoxylate and hydroxypyruvate to glycolate and glycerate, respectively. This enzyme is widely distributed in various organisms, including bacteria, plants, and animals. It is essential in various metabolic processes and detoxification, such as the glyoxylate cycle, serine metabolism, and photorespiration **(Givan & Kleczkowski, 1992)**.

5.6.JUVENILE HORMONE ESTERASE:

JHE catalyzes the hydrolysis of J.H. into its inactive form, which is essential for the termination of J.H. signalling and the initiation of metamorphosis. However, recent studies have also suggested that JHE may play a role in the detoxification of xenobiotics, including avermectins **(Kamita & Hammock, 2010)**.

5.7.APYRASE:

One approach to enhance the effectiveness of avermectins is to identify enzymes that can detoxify or modify them, thereby reducing their toxicity or enhancing their pharmacokinetic properties. One such enzyme is apyrase, a ubiquitous enzyme that catalyzes the hydrolysis of ATP and other nucleoside triphosphates to their corresponding diphosphates and inorganic phosphate **(Komoszyński, 1996)**.

5.8.ESTERASE:

To mitigate the potential harm of Avermectin to non-target organisms, various detoxification mechanisms have evolved in these organisms. One of these mechanisms involves the action of VCEs, which catalyze the hydrolysis of Avermectin into less toxic metabolites. VCEs are found in many organisms, including mammals, birds, insects, and plants, and are highly conserved in their structure and function **(Salman et al., 2022)**.

5.9. SYNAPTIC VESICLE GLYCOPROTEIN:

SVG has been known to play a role in avermectin detoxification in various species, including mites, nematodes, and aphids. Molecular mechanisms of avermectin resistance in the mite *Tetranychus*, a significant pest of crops worldwide. They identified a mutation in the gene encoding SV2, which they named Tu-SV2, associated with resistance to avermectins **(Xu et al., 2017)**.

5.10. UBIQUITIN-CONJUGATING ENZYME:

Ubiquitin-conjugating enzymes (UBCs) are a group of enzymes that play a crucial role in regulating protein degradation through the ubiquitin-proteasome pathway. There expression of a UBC gene was up-regulated in the liver of rats exposed to ivermectin, a commonly used avermectin drug. The study also showed that the administration of a UBC inhibitor increased the toxicity of ivermectin in rats, suggesting that UBCs play a protective role in avermectin detoxification **(Liu et al., 2020).**

As little research has been conducted on the pest, a comprehensive study spanning everything from infestation through reproduction, pesticide management, and the evolution of resistance was required for a better understanding.

CHAPTER 6

6. CONCLUSION

6.1. WHY STUDY FALL ARMYWORMS?

Fall armyworms, *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae), invaded India in 2018 and are a significant pest of several important crops, including maize, sorghum, cotton, and soybean. Many agricultural fields are in different parts of Vadodara, mainly Chhani, Waghodia, Padra, Savli, and various districts throughout Gujarat. In recent years, the rapid spread of pesticide resistance in fall armyworm populations has become a significant concern for farmers and agricultural researchers. Studying pesticide-resistance development in fall armyworms is essential for developing effective pest management strategies that can mitigate the impact of this pest on crop yields.

6.2. IMPORTANCE OF STUDYING PESTICIDE-RESISTANCE DEVELOPMENT IN FALL ARMYWORMS

Pesticide resistance in fall armyworms is a significant challenge for farmers and agricultural researchers. As fall armyworms continue to spread globally, the development of resistance to commonly used pesticides has become a significant concern. Studying pesticide-resistance development in fall armyworms is essential for understanding the mechanisms involved in resistance development, identifying the genes and molecular pathways involved in resistance, and developing effective pest management strategies.

6.3. FACTORS INFLUENCING PESTICIDE RESISTANCE DEVELOPMENT IN FALL ARMYWORMS

Several factors can influence pesticide resistance development in fall armyworms, including genetic factors, environmental factors, and pesticide exposure. Genetic factors such as gene flow and selection can affect the frequency of resistance alleles in populations. Environmental factors such as temperature, humidity, and photoperiod can influence the expression of resistance genes. Exposure to pesticides can select for resistant individuals and increase the frequency of resistance alleles in populations.

6.4. PESTICIDE EFFICACY OVER THE GENERATIONS

Generation studies are an essential tool for understanding how pesticide resistance develops in insects over time. These studies involve exposing successive generations of insects to sublethal pesticide doses and monitoring changes in their susceptibility to the pesticide. By doing so, researchers can gain insights into the mechanisms and dynamics of resistance development, which can inform the development of effective strategies to combat pesticide resistance. We tested the two insecticides (chlorantraniliprole and emamectin benzoate) on fall armyworms, and both were efficient in controlling the pest.

6.5.HISTOLOGICAL STUDIES

Followed by this, tissue architecture studies were carried out on control and resistance-developed test organisms. The area of focus was the midgut region which suggests that the midgut of insects plays a crucial role in the absorption of nutrients and elimination of waste products. It is also the primary site of contact with ingested pesticides. The development of pesticide resistance in insects is often associated with morphological changes in the midgut. These changes affect the ability of pesticides to penetrate the midgut epithelium, be absorbed into the hemolymph, and reach their target site.

6.6.CHANGES IN THE ACTIVITY OF DETOXIFICATION ENZYMES

Detoxification enzymes, such as cytochrome P450s, esterases, and glutathione S-transferases, play a crucial role in the metabolism and elimination of insect pesticides. Studies have shown that the activity of these enzymes can change during the development of pesticide resistance. In resistant insects, the activity of detoxification enzymes can increase, enhancing the metabolism and elimination of pesticides and reducing their toxic effects.

Our results suggest the development of pesticide resistance in insects which is associated with morphological changes in the midgut. These changes might have affected the ability of pesticides to penetrate the midgut epithelium, be absorbed into the hemolymph, and reach their target site.

Compared to the control group insects, there was a decrease in mortality rate and changes in the midgut region in the test group. These alterations direct us to the changes that might be brought up by the deviations occurring at the genetic level. To understand this, we performed transcriptome studies on both groups. These differentially expressed genes (DEGs) can provide valuable insights into the mechanisms underlying pesticide resistance and help to develop new strategies for controlling insect pests. The importance of transcriptome data is given below.

6.7.IDENTIFICATION OF TARGETS FOR NEW PESTICIDES

Studying differentially expressed genes can also help to identify potential targets for new pesticides. Understanding the molecular mechanisms of pesticide resistance can provide insight into the vulnerabilities of insects that new chemicals can exploit. For example, identifying genes involved in the detoxification of pesticides can lead to the development of chemicals that are not metabolized by these pathways and are more effective at killing resistant insects.

6.8.DEVELOPMENT OF NEW PEST MANAGEMENT STRATEGIES

Studying differentially expressed genes can also help to develop new pest management strategies. Understanding the molecular mechanisms of pesticide resistance can provide insights into alternative approaches that can be used to control insect pests. For example, using natural enemies of insect pests, such as parasitoids or predators, can be an effective strategy for controlling resistant pests. Studying the genes involved in the interactions between insects and their natural enemies can provide insights into the mechanisms underlying these interactions and help to develop more effective biological control strategies.

Our transcriptome analysis showed the following:

- In DEGs, 464 genes were found to be up-regulated and 607 genes to be downregulated when compared to the control population. These results were plotted in the form of the MA plot, the volcano plot, and the heat map.
- The genes already known to play a significant role in detoxification were also found to be up-regulated, such as GSTs, cytochromes, esterases, and transferases. Other than those, genes playing a role in the structural formation, such as cuticle-forming proteins, were also expressed differentially. Some up-regulated genes include cytochrome P450 6B7, transcript variant X15, synaptic vesicle glycoprotein 2B, zinc transporter ZIP1, and ubiquitin-conjugating enzyme E2 G2. Some downregulated genes include anoctamin-8-like plasminogen activator inhibitor 1, cadherin-87A, and argininosuccinate lyase.
- Several cytochromes were found to be differentially expressed in the results, confirming their role in providing resistance. Some genes already known to provide resistance in various insects to insecticides were also found to have been up-regulated in our results, including glutathione S-transferase 1, acetylcholinesterase, glyoxylate/hydroxypyruvate reductase, and juvenile hormone esterase.

The various genes getting up- and down-regulated are responsible for creating resistance, and they play roles in various physiological functions of the insects.

Chapter 7

7. SIGNIFICANT FINDINGS AND RECOMMENDATIONS

7.1. Significant findings

- The presence of *Spodoptera frugiperda* Smith, the fall armyworm, in many agricultural fields in Vadodara has been noted.
- The fall armyworm was observed to be causing heavy damage to the maize field in Vadodara. The larva (caterpillar) of fall armyworm has been damaging the leaves, cobs, and tassels through their feeding.
- The differential gene expression showed 464 up-regulated genes and 607 downregulated genes.
- Among other groups, genes involved in the structural formation and detoxification were found to be up-regulated.
- Various GSTs, cytochromes, esterases, and transferases play roles in detoxification. Cuticle and chitin proteins performing structural formation were also found to be differentially expressed.
- The importance of cytochromes, glutathione S-transferase 1, acetylcholinesterase, glyoxylate/hydroxypyruvate reductase, juvenile hormone esterase, and many others was confirmed to provide resistance in the insect fall armyworm against Emamectin Benzoate.

7.2.Recommendations

Pesticide resistance in insects is a complex phenomenon that involves a range of metabolic processes that enable the insects to detoxify or eliminate the pesticide. Various genes and enzymes regulate these metabolic processes, and their activity can be affected by genetic mutations, changes in gene expression, and other environmental factors.

METABOLIC PROCESSES INVOLVED IN PESTICIDE RESISTANCE DEVELOPMENT:

Detoxification enzymes: One of the primary mechanisms by which insects develop resistance to pesticides is through the increased production or activity of detoxification enzymes, which enable them to break down and eliminate the pesticide from their system. The three major classes of detoxification enzymes are esterases, glutathione S-transferases, and cytochrome P450s.

Target-site mutations: Another mechanism of pesticide resistance is through mutations in the target site of the pesticide. For example, resistance to pyrethroids, a commonly used class of insecticides, can be conferred by mutations in the sodium channel gene, which reduces the binding affinity of the pesticide to the target site.

Reduced penetration: Insects can also develop resistance to pesticides by reducing the ability of the pesticide to penetrate their cuticle or cell membranes. This can be achieved through structural changes in the cuticle or by up-regulating efflux pumps that pump the pesticide out of the cell.

Reduced metabolic rate: Some insects can develop resistance to pesticides by reducing their metabolic rate, which can slow down the detoxification of the pesticide. This can be achieved through the downregulation of genes involved in energy metabolism or by activating hibernation-like states.

Increased excretion: Insects can also develop resistance to pesticides by increasing their ability to excrete the pesticide. This can be achieved through the upregulation of transporters involved in eliminating the pesticide from the body.

Overall, pesticide resistance development is a multifaceted process involving various metabolic processes. Understanding these metabolic processes is critical for developing

based on resistance knowledge. Various tools for control other than insecticides (chemical control) can also be designed. The Lepidoptera pest management methods with an integrated approach comprising biological control can be tested and implemented in the case of Spodoptera litura (Divya, 2016). Further work on related species from the Noctuidae family, such as other caterpillars (*S. litura*, *S. exigua*, etc.), which are pests on various crops of importance, can be carried out. Our essential findings from the field survey, lab rearing, testing, and gene expression can aid in such research works.

USE OF CRISPR-CAS9 TOOL

CRISPR-Cas9 is a powerful gene-editing tool that has revolutionized various science fields, including agriculture. Recently, researchers have been exploring the potential use of CRISPR-Cas9 to combat pesticide resistance in insect populations.

The CRISPR-Cas9 technique involves using a guide RNA molecule to target a specific gene of interest and a Cas9 enzyme to cut and modify the gene sequence. By doing so, researchers can selectively edit an organism's genome, including insects, and induce changes that can combat pesticide resistance.

One potential use of CRISPR-Cas9 in pest control is to target the genes responsible for developing pesticide resistance in insects. Researchers have identified several genes that play a role in insecticide resistance, including detoxification enzymes, target-site mutations, and transporters. By using CRISPR-Cas9 to modify these genes, researchers could create insects that are less likely to develop resistance to certain insecticides.

Another potential application of CRISPR-Cas9 in pest control is to create genetically modified insects that can control pest populations. For example, researchers have proposed using CRISPR-Cas9 to create sterile male insects or only produce male offspring. By releasing these insects into a pest population, researchers could reduce the population's reproductive capacity and ultimately control their numbers.

CRISPR-Cas9 can also be used to create genetically modified crops that are more resistant to pests, reducing the need for pesticides. For example, researchers could use CRISPR-Cas9 to introduce genes from pest-resistant plants into crop plants or modify existing genes to enhance resistance.

Despite the potential benefits of CRISPR-Cas9 in pest control, there are also potential risks and ethical considerations. The technology could be misused for unintended purposes, such as creating genetically modified organisms with unintended ecological consequences. There is also the risk of disrupting natural ecosystems and biodiversity if genetically modified organisms are released into the environment.

In conclusion, CRISPR-Cas9 holds great promise to combat pesticide resistance in insects and control pest populations. However, further research is needed to fully understand the potential risks and benefits of using this technology in the agricultural sector. Regulatory bodies and policymakers will need to carefully evaluate the ethical and ecological implications of using CRISPR-Cas9 in pest control before it is widely implemented **(Kaduskar et al., 2022)**

USE OF COMBINATION INSECTICIDES

When insect populations are repeatedly exposed to a single insecticide, they can develop genetic changes that make them resistant to the pesticide. This resistance can then be passed on to future generations of insects, making control of pest populations difficult. To address this issue, researchers and farmers have been exploring the use of combination insecticides as a strategy to combat resistance.

Combination insecticides involve the use of two or more different insecticides with distinct modes of action, applied simultaneously or sequentially, to control pests. By using multiple insecticides with different modes of action, the selection pressure on the pest population is reduced, making it less likely that they will develop resistance to either of the insecticides.

There are several benefits to using combination insecticides to combat pesticide resistance development. Firstly, they offer a more effective means of pest control. By combining different insecticides, the likelihood of pests developing resistance to any of the insecticides is reduced, which helps prevent infestations and preserve crop yields.

Secondly, combination insecticides can help to extend the lifespan of existing insecticides. By reducing the rate at which insects develop resistance, combination insecticides can help to prolong the usefulness of currently available insecticides, which is critical as new insecticides take time to develop and bring to market.

Thirdly, combination insecticides can help reduce the overall use of insecticides, which can benefit the environment. By using multiple insecticides with different modes of action, the amount of each insecticide needed can be reduced, which can help to mitigate the risk of environmental contamination and reduce the exposure of non-target organisms to insecticides.

Finally, combination insecticides can be an economically viable option for farmers. Although the cost of using two or more insecticides may be higher than using a single insecticide, the benefits of increased pest control and reduced resistance development can outweigh the costs in the long run, leading to increased crop yields and profits.

Conclusion

In conclusion, combination insecticides offer an effective means of controlling pest populations and combating pesticide resistance development. By reducing the selection pressure on pest populations, combination insecticides can help to extend the lifespan of existing insecticides, reduce overall insecticide use, and be a viable option for farmers economically. As such, combination insecticides should be considered an important tool in the fight against pesticide resistance.

OTHER RELATED PESTS

Lastly, on the basis of resistance knowledge, various tools for control other than insecticides (chemical control) can also be designed. The lepidoptera pest management methods with an integrated approach comprising biological control can be tested as well as implemented in case of *Spodoptera litura* **(Divya, 2016)**. Further work on related species from the noctuidae family, such as other caterpillars (*S. litura*, *S. exigua*, etc.), which are pests on various crops of importance, can be carried out. Our important findings from the field survey, lab rearing, testing, gene expression can aid in such research wo

CHAPTER 8

8. REFERENCES

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CONFERENCE PRESENTATION FROM THESIS

1. National Symposium on multilateral initiatives against arboviral diseases (NSAD-2020), 4 January to 6 January 2020, presented at Mohanlal Sukhadia University, Udaipur, Rajasthan **(Oral presentation-2nd position)**

2. Present status and future trends in Entomological and Wildlife studies: An emerging platform for Zoologists (PENWI-21), 13-14 July, 2021, DEPARTMENT OF ZOOLOGY

Mohanlal Sukhadia University, Udaipur, Raj. **(Oral presentation-3rd position)**

PUBLICATION FROM THESIS

1 Sharma, H., & Kumar, D. (2020). Comparative Analysis of the Life Cycle of Fall Armyworm *Spodoptera frugiperda* Smith, 1797 (Lepidoptera, Noctuidae) on Natural and Artificial Maize Diet under Laboratory Conditions. *Research Journal of Agricultural Sciences*, *11*(6): 1426–1429.

2 Sharma, H., & Kumar, D. (2022). Standardizing the artificial diet and diet preference for lab rearing of *Spodoptera frugiperda* Smith, 1797 (Lepidoptera, Noctuidae). *International Journal of Entomology Research*, *7*(7): 1–5.

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