CHAPTER 6

6. CONCLUSION

Why study Fall armyworms?

Fall armyworms, *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae), invaded India in 2018, are a major pest of several important crops, including maize, sorghum, cotton, and soybean **(Deshmukh et al., 2018; Balla et al., 2019)**. Also, many agricultural fields 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 major 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.

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 across the globe, the development of resistance to commonly used pesticides have become a major concern. Studying pesticide-resistance development in fall armyworms is important for understanding the mechanisms involved in resistance development, identifying the genes and molecular pathways that are involved in resistance, and developing effective pest management strategies.

Factors influencing pesticide resistance development in fall armyworms

Several factors can influence pesticide resistance development in fall armyworms, including genetic factors, environmental factors, and exposure to pesticides. 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.

MECHANISMS OF PESTICIDE RESISTANCE DEVELOPMENT IN FALL ARMYWORMS

Pesticide resistance in fall armyworms can develop through various mechanisms, including target site mutations, metabolic detoxification, and behavioral resistance. Target site mutations involve changes in the target protein of the pesticide, which can reduce its effectiveness. Metabolic detoxification involves the production of enzymes that can break down or modify the pesticide, making it less toxic to the insect. Behavioral resistance involves changes in the behavior of the insect that can reduce its exposure to the pesticide.

APPROACHES TO STUDYING PESTICIDE RESISTANCE DEVELOPMENT IN FALL ARMYWORMS

There are various approaches to studying pesticide resistance development in fall armyworms, including generation studies in lab, bioassays, gene expression analysis, and genome sequencing. Bioassays involve exposing insects to different concentrations of pesticides; rearing on artificial diet and measuring their survival and/or growth rates. Gene expression analysis can identify genes that are upregulated or downregulated during resistance development, while genome sequencing can identify genetic variations that may be associated with resistance.

IMPLICATIONS FOR PEST MANAGEMENT

Understanding pesticide resistance development in fall armyworms can have important implications for pest management. This knowledge can inform the development of effective pest management strategies that can reduce the impact of this pest on crop yields. This can include the use of alternative control measures that do not rely on pesticides, such as biological control agents, pheromone traps, and cultural control practices. It can also inform the development of pesticide use strategies that minimize the risk of resistance development, such as rotation or combination of pesticides with different modes of action.

In brief, the present work was designed to address the above mentioned issues in an elaborate manner, as 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. It can inform the development of alternative control measures that can reduce reliance on pesticides and minimize the risk of resistance development. This knowledge can also inform the development of pesticide use strategies that are more effective and sustainable in the long term.

LAB REARING OF FAW

As part of IPM, chemical control is one of the components to control any insect pest **(IPM, 2023)**. Therefore, laboratory rearing of *S. frugiperda* was done for multiple generations on an artificial diet. It is a challenge to provide an insecticide-free, natural diet to the insect regularly and many times a day. For that purpose, an artificial diet was standardized for lab rearing **(Table 3.3)**.

A constant temperature of 26 °C, a humidity of 70%, and a photoperiod of 12:12 (day: night) were provided. The fall armyworm has many host crops, such as maize, soya, and jowar (sorghum). Ingredients from the host crop, such as maize flour and soy flour, were used to prepare four diets and test them for rearing.

Both the maize-based artificial diet and the chickpea-based artificial diet gave a high survival percent as well as a high larval growth index, confirming their efficiency as good artificial diets for the laboratory rearing of fall armyworm. There are many different ingredients in artificial diets, as each one of them has a different nutritional value, and overall, they can fulfil various nutritional requirements of the insect. They substitute for various needs: carbohydrates, proteins, lipids, and antimicrobials. A chickpea-based artificial diet was used throughout the lab rearing from then on. For a few months, fall armyworms were reared without any insecticide exposure to create a susceptible population.

PESTICIDE EFFICACY OVER THE GENERATIONS

Generation studies are an important tool for understanding how pesticide resistance develops in insects over time. These studies involve exposing successive generations of insects to sublethal doses of a pesticide 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. There are several types of generation studies that can be used to study pesticide resistance development, including:

Selection studies: In these studies, insects are exposed to gradually increasing doses of a pesticide over several generations. This approach can help identify the genetic mutations and metabolic changes that enable the insects to tolerate higher doses of the pesticide.

Recovery studies: In these studies, insects that have developed resistance to a pesticide are allowed to breed with non-resistant individuals to assess the rate at which resistance is lost. This approach can help identify the fitness costs associated with resistance and the potential for reversion to susceptibility.

Cross-resistance studies: In these studies, insects that have developed resistance to one pesticide are exposed to another pesticide to determine whether resistance to one pesticide confers cross-resistance to other pesticides. This approach can help identify the metabolic pathways and genes involved in resistance development and inform the development of alternative control strategies.

Field studies: In these studies, insects are monitored in their natural habitat to assess changes in their susceptibility to pesticides over time. This approach can help identify the factors that contribute to resistance development in real-world settings and inform the development of integrated pest management strategies.

Overall, generation studies are a valuable tool for understanding how pesticide resistance develops in insects and identifying the mechanisms and factors that contribute to resistance. By gaining a deeper understanding of resistance development, researchers can develop more effective and sustainable strategies for pest control that reduce reliance on pesticides and minimize the risk of resistance development. Therefore, to test the insecticides for their efficacy, a diet incorporation assay of IRAC was used **(IRAC, 2011)**. The diet was already standardized, and insecticide solutions were made and added to the diet in measured amounts.

WHY ARE DOSE RANGE STUDIES REQUIRED?

Pesticide resistance is a significant concern for the agricultural industry, as it can lead to decreased effectiveness of pesticide applications and ultimately crop loss. Pesticide resistance occurs when a pest population evolves genetic changes that enable them to survive exposure to a pesticide that would typically be lethal. The development of resistance is influenced by various factors, including the dose of the pesticide used.

Dose range studies are essential for understanding the impact of pesticide exposure on pest populations and the development of resistance. These studies involve testing the response of pests to different doses of a pesticide, ranging from low to high concentrations. By doing so, researchers can determine the minimum effective dose required to control the pest population and the maximum dose that may cause resistance to develop.

One of the main benefits of conducting dose range studies is the identification of the threshold level of exposure required to induce resistance. This information can help farmers and pesticide applicators to make more informed decisions about pesticide application rates and frequency. It can also aid in the development of resistance management strategies, which aim to minimize the development of resistance by rotating different pesticide classes or using alternative control methods.

Furthermore, dose range studies can help to identify potential mechanisms of resistance development. By exposing pests to increasing doses of a pesticide, researchers can observe changes in the physiological and genetic responses of the pest population. This can provide insight into the genetic and biochemical pathways involved in resistance development and guide the development of new pesticides that target different mechanisms of action.

Dose range studies can also aid in the development of pesticide resistance monitoring programs. These programs involve regularly monitoring pest populations for signs of resistance development and adjusting pesticide application rates accordingly. By conducting dose range studies, researchers can determine the appropriate doses to use in resistance monitoring programs and establish a baseline for assessing changes in resistance over time.

In the current study, a stock solution was prepared for the insecticides Chlorantraniliprole and Emamectin Benzoate. A concentration range was selected for both insecticides. Various concentrations between 0.01 and 10 ppm (10 ppm, 5 ppm, 1 ppm, 0.5 ppm, 0.1 ppm, 0.02 ppm, 0.05 ppm, and 0.01 ppm) were made and tested.

PESTICIDE RESISTANCE DURING THIRD & FOURTH INSTAR STAGES

The transition from the third to fourth instar stage is a critical period in the development of many insect species, and it is during this period that many insects develop resistance to pesticides. Studying pesticide-resistance development between the third and fourth instar stages is important for understanding the mechanisms involved in resistance development and for developing effective pest management strategies.

IMPORTANCE OF STUDYING PESTICIDE-RESISTANCE DEVELOPMENT BETWEEN THE THIRD AND FOURTH INSTAR STAGES

The third to fourth instar stage transition is a critical period in the development of many insects, and it is during this period that many insects develop resistance to pesticides. Studying pesticide-resistance development between the third and fourth instar stages is important for understanding the mechanisms involved in resistance development, identifying the genes involved in resistance, and developing effective pest management strategies that can target this critical period in the life cycle of pests.

FACTORS INFLUENCING PESTICIDE RESISTANCE DEVELOPMENT BETWEEN THE THIRD AND FOURTH INSTAR STAGES

Several factors can influence pesticide resistance development between the third and fourth instar stages, including environmental factors, genetic factors, and exposure to pesticides. Environmental factors such as temperature, humidity, and photoperiod can influence the expression of resistance genes, while genetic factors such as gene flow and selection can affect the frequency of resistance alleles in populations. Exposure to pesticides during this critical period can also contribute to resistance development.

MECHANISMS OF PESTICIDE RESISTANCE DEVELOPMENT BETWEEN THE THIRD AND FOURTH INSTAR STAGES

Pesticide resistance can develop through various mechanisms, including target site mutations, metabolic detoxification, and behavioral resistance. Target site mutations involve changes in the target protein of the pesticide, which can reduce its effectiveness. Metabolic detoxification involves the production of enzymes that can break down or modify the pesticide, making it less toxic to the insect. Behavioral resistance involves changes in the behavior of the insect that can reduce its exposure to the pesticide.

APPROACHES TO STUDYING PESTICIDE RESISTANCE DEVELOPMENT BETWEEN THE THIRD AND FOURTH INSTAR STAGES

There are various approaches to studying pesticide resistance development between the third and fourth instar stages, including bioassays, gene expression analysis, and genome sequencing. Bioassays involve exposing insects to different concentrations of pesticides and measuring their survival and/or growth rates. Gene expression analysis can identify genes that are upregulated or downregulated during resistance development, while genome sequencing can identify genetic variations that may be associated with resistance.

The present study states that, when the first generation of fourth instar larvae when subjected to Chlorantraniliprole testing, had a LC_{50} close to 0.05 ppm of the insecticide concentration, whereas the first generation of fourth instar of larvae when subjected to the first generation of Emamectin Benzoate testing, had a LC_{50} close to 0.1 ppm of the insecticide concentration.

The insects that survived the testing were made to grow and reproduce. Subsequent offspring from them were further subjected to insecticide testing.

Both insecticides were tested on fourth-instar larvae of each generation regularly. There was a drop in larval mortality in both cases. In later generations, a shift in LC_{50} was observed in the case of Chlorantraniliprole as well as Emamectin Benzoate.

In fourth-generation testing of Chlorantraniliprole, only 20% mortality was observed with the initial LC_{50} dose, which was 0.05 ppm. The LC_{50} concentration at this generation was around 0.5 ppm. In fourth-generation testing of Emamectin Benzoate, only 30% mortality was observed with the initial LC_{50} dose, which was 0.1 ppm. The LC_{50} concentration at this generation was around 1 ppm. Such a decrease in the expected mortality of the pest insect raises the possibility of resistance development being initiated in the pest against the insecticides.

A field survey was going on throughout the study, and recent trends show an increase in the use of Emamectin Benzoate in the agricultural fields of Vadodara. Farmers were interviewed, and they revealed that commercial insecticides containing Emamectin Benzoate were the only ones that provided effective control of fall armyworms **(NIH, 2023)**. If Emamectin Benzoate is continuously sprayed for an extended period, resistance will develop. As a result, we must delve into the specifics of the fall armyworm's resistance mechanism to Emamectin Benzoate.

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.

THICKENING OF THE PERITROPHIC MEMBRANE

The peritrophic membrane is a chitinous layer that lines the midgut and provides a physical barrier between ingested food particles and the midgut epithelium. Studies have shown that the peritrophic membrane of resistant insects is often thicker than that of susceptible insects. This thickening can reduce the penetration of pesticides into the midgut epithelium and limit their toxic effects.

Changes in the morphology of microvilli

The microvilli are small finger-like projections that line the midgut epithelium and increase its surface area for nutrient absorption. Studies have shown that the morphology of microvilli can change during the development of pesticide resistance. In resistant insects, the microvilli can become shorter and thicker, which can reduce the absorption of pesticides into the midgut epithelium.

Alterations in the structure of the midgut epithelium

The midgut epithelium is composed of different cell types, including columnar cells, goblet cells, and regenerative cells. Studies have shown that the structure of the midgut epithelium can change during the development of pesticide resistance. In resistant insects, the number of columnar cells can increase, while the number of goblet cells can decrease. This can affect the absorption and metabolism of pesticides in the midgut.

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 pesticides in insects. 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, which can enhance the metabolism and elimination of pesticides and reduce their toxic effects.

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

When comparing to the control group insects, there was a decrease in mortality rate and changes in the midgut region in test group. These alterations direct us to the changes that might be a result brought up by the deviations occurring at genetic level. To understand this, we performed the transcriptome studies on both the 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.

UNDERSTANDING THE MOLECULAR MECHANISMS OF PESTICIDE RESISTANCE

Pesticide resistance is the ability of insects to survive exposure to a pesticide that would normally kill them. This resistance can arise through a variety of mechanisms, including changes in the target site of the pesticide, enhanced metabolism of the pesticide, and changes in behavior that reduce exposure to the pesticide. Studying differentially expressed genes can provide insight into the molecular mechanisms underlying pesticide resistance. For example, changes in the expression of genes involved in detoxification pathways can lead to increased metabolism of pesticides and reduced susceptibility to their toxic effects.

IDENTIFICATION OF BIOMARKERS FOR PESTICIDE RESISTANCE

Studying differentially expressed genes can also help to identify biomarkers that can be used to detect and monitor pesticide resistance. Biomarkers are specific molecules or characteristics that can be used to indicate the presence or severity of a condition. Identifying biomarkers for pesticide resistance can help to detect the development of resistance early and improve the effectiveness of pest management strategies. For example, the identification of specific genes involved in detoxification pathways that are upregulated in response to pesticide exposure can be used as biomarkers for pesticide resistance.

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 can be exploited by new chemicals. 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.

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, the use of 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.

The transcriptome analysis showed the following:

In DEGs, 464 genes were found to be upregulated 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 that are already known to play a major role in detoxification were also found to be upregulated, such as GSTs, cytochromes, esterases, and transferases. Other than those, genes playing a role in structural formation, such as cuticle-forming proteins, were also expressed differentially. Some upregulated 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.

A number of cytochromes were found to be differentially expressed in the results, confirming their role in providing resistance. Some genes that are already known to provide resistance in various insects to insecticides were also found to have been upregulated 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.