# INVESTIGATING INSECTICIDE EFFICACY AND DEVELOPMENT OF RESISTANCE IN SPODOPTERA FRUGIPERDA SMITH, 1797(LEPIDOPTERA: NOCTUIDAE)

Synopsis of Ph.D. Thesis

**Ms. Harshita Sharma**
Registration number: **FoS/2161**

DECEMBER 30, 2022

DEPARTMENT OF ZOOLOGY, FACULTY OF SCIENCE

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA 390 002

# INVESTIGATING INSECTICIDE EFFICACY AND DEVELOPMENT OF RESISTANCE IN *SPODOPTERA FRUGIPERDA* SMITH, 1797 (LEPIDOPTERA: NOCTUIDAE)

## INTRODUCTION

*Spodoptera frugiperda* Smith, 1797, is a polyphagous lepidopteron insect and a problematic agricultural pest. *S. frugiperda* has a wide range of host crops, which are of economic concern to us. This includes 353 plant species from 76 families having crops such as corn, tomato, potato, rice, etc. **(Montezano et al., 2018)**.

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is a noctuidae pest, originating from America and eliciting an exorbitant reproductive rate, throughout the year **(Sparks, 1979)**.

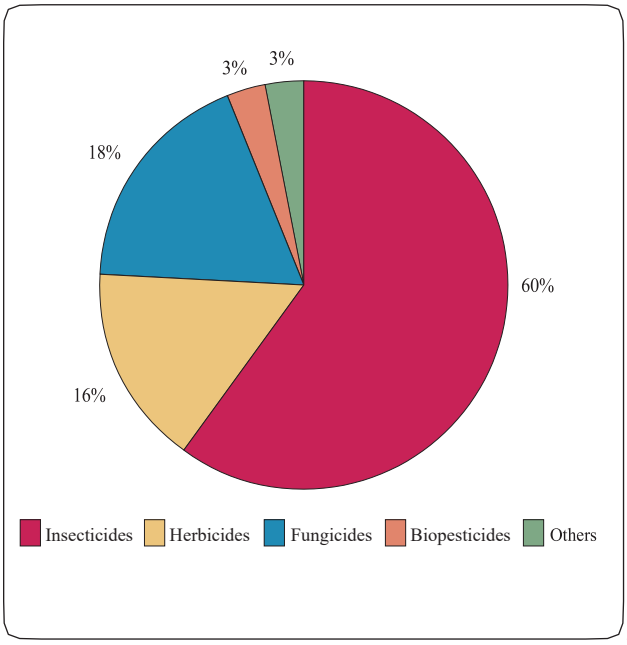
Several West and Central African countries have reported sudden and severe outbreaks of fall armyworm populations, owing to their vast dispersal and strong flying abilities, while this is the first case of invasion outside the American continent **(Goergen et al., 2016)**.

For the first time in 2018, *Spodoptera frugiperda* has shown its occurrence not only in India but also in Asia. In Karnataka, FAW infestation has been reported on maize **(Deshmukh et al., 2018)**. Recently in China, this invasive pest was confirmed to be present, using phylogenetic analysis of biological macromolecules **(Jing et al., 2019)**.

*S. frugiperda* invasion was also first reported in Gujarat on maize in fields in Anand district **(Sisodiya et al., 2018)**. The *Spodoptera frugiperda* infestations on sugarcane and other crops from Maharashtra were confirmed based on the male genital dissection of the insect **(Chormule et al., 2019)**. In Rajasthan, the presence of FAW on maize has been marked **(Babu et al., 2019)**.

Of all the major economically important crops produced in India, maize is the third most important cereal, after wheat and rice. According to a report from Mizoram in May 2019, the fall armyworm has caused a loss of about 200 million INR because it has spread to 122 districts where maize is grown.

Pests are usually controlled by spraying pesticides. There are variations in various pesticides' usage and application. In India, a trend toward maximum usage of insecticide among all pesticides is observed (**Graph 1**).




**Graph 1**: The consumption pattern of pesticides in India **(Agarwal & Garg, 2015)**

Further, *S. frugiperda* is shown to be resistant to commonly sprayed older classes of insecticides, including pyrethroids, organophosphates, and carbamates, which led to the failure of crops in Florida **(Yu, 1991)**. As a result, controlling the pest has become increasingly difficult, as the most commonly used insecticides are ineffective.

Till now, there is no certain solution for the sustainable management of FAW in Africa or Asia **(Padhee & Prasanna, 2019)**. This calls for the need to conduct experiments in the lab, for which mass rearing of the test insect is required. Such rearing requires ideal biotic and abiotic conditions, including temperature, humidity, and diet. Artificial diets are used as a medium for effective insect pest rearing in labs.

Artificial diets have the capacity to fulfil the nutritional requirements of insect pests in the lab. The diet studies also promote knowledge about the biology, behaviour, and nutritional requirements of insects, and such information is fundamental for the development of efficient Integrated Pest Management **(Pinto et al., 2019)**.

Devising a management strategy would involve a clear understanding of physiology, for which rearing them artificially in a lab is prescribed. The insects to be reared in the lab have specific characteristics that can be easily observed in their distinct larval and adult forms, as mentioned by **Sharanabasappa et al., 2018** and shown in **Figure 1**.

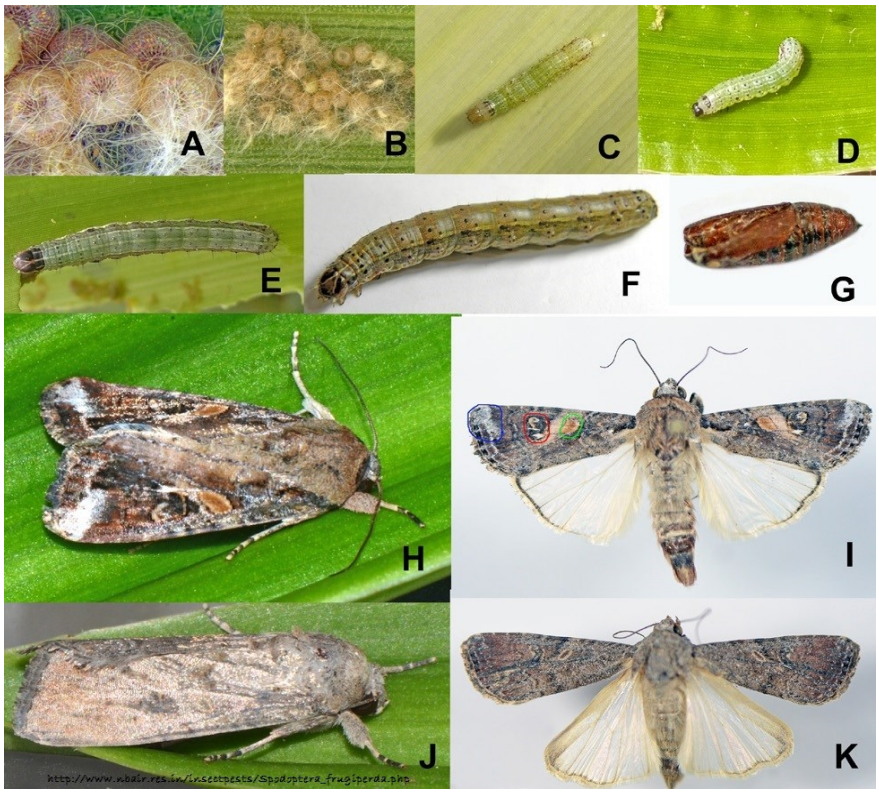



**Figure 1:** *S. frugiperda* lifecycle **A**-B: Eggs, C-F: Larvae, G: Pupa, H-I: Male, J-K: Female

Distinct larval and adult identifying features are present, as shown in **Figure 2.**

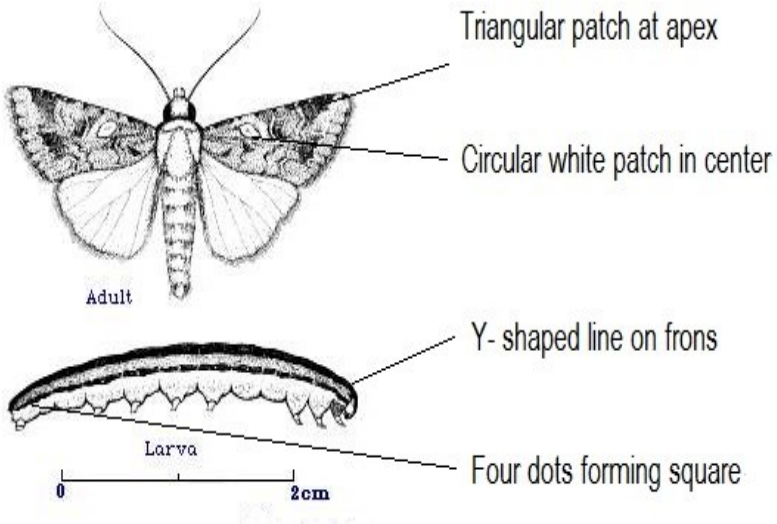



**Figure 2:** Adult (up) & Larval (down) identifying features

**INSECTICIDES**

The new generation of insecticides has the potential to effectively control new invasive insect pests. Two of them are:

1. Chemical formula-C18H14BrCl2N5O2. Chlorantraniliprole is a new reduced-risk insecticide by Dupont. a novel insecticide belonging to the diamide class. used to control lepidopteran pests. function as ryanodine receptor modulators **(Figure 3).**
2. Emamectin benzoate. chemical formula-C56H81NO15. Emamectin benzoate is a novel semi-synthetic insecticide discovered by Syngenta. It is an abamectin (Avermectin Group) derivative that acts as a glutamate-gated chloride channel (GluCl) allosteric modulator against a wide range of lepidopteran pests **(Figure 4)**

To find effectiveness of the control by insecticides in controlling *S. frugiperda*, technical grades of Chlorantraniliprole & Emamectin benzoate were selected as these were:

1. Green Label: very less toxic to non-targets
2. Recommended for controlling lepidopteran pests
3. Only commercial grade has been tested with preliminary data in India

4. A detailed study on them including resistance is insufficient

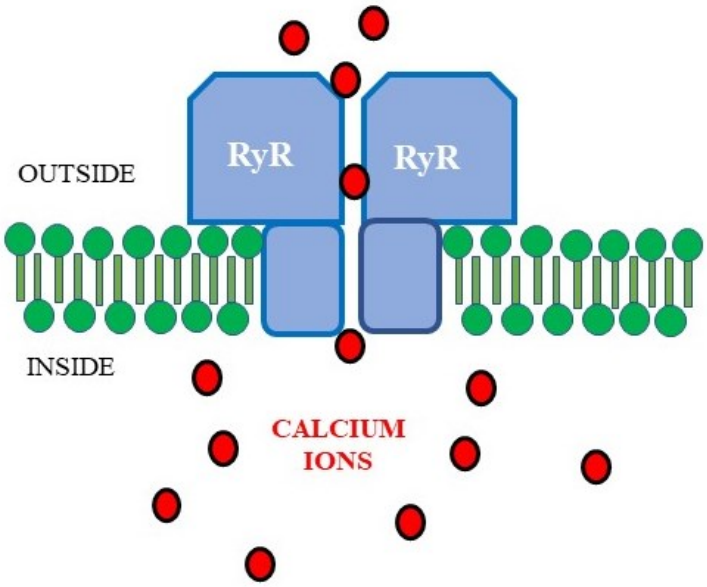



**Figure 3**: Diamide Family (Chlorantraniliprole)

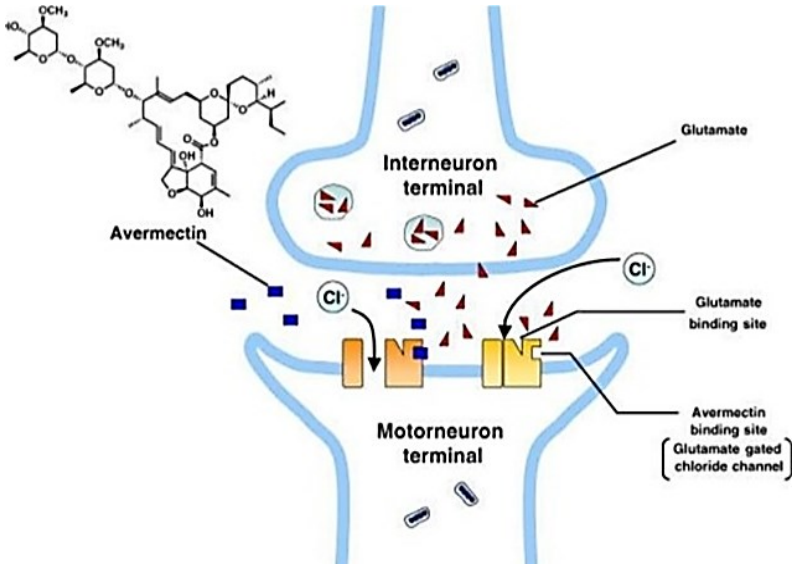



**Figure 4**: Avermectin Family (Emamectin Benzoate)

Specifications of the technical grades of insecticides (Sigma Aldrich) are mentioned in **Table 1**.

| **Specifications** | Chlorantraniliprole, PESTANAL, analytical standard | Emamectin benzoate, PESTANAL, analytical standard |
|---|---|---|
| **Brand** | Sigma-Aldrich | Sigma-Aldrich |
| **Appearance (colour)** | White to off-white | White to off-white |
| **Appearance (form)** | Powder or Crystal | Powder or Crystal |
| **Purity** | ≥95% | ≥85% |
| **Melting point** | 223-228 C | 150-155 C |
| **Water** | ≤1% | ≤3.0% |
| **Purity** | Confirms to structure | Conforms to structure |
| **Formula** | C18H14BrCl2N5O2 | C56H81NO15 |

**Table 1**: Insecticide technical grade properties

## MOLECULAR BASIS OF RESISTANCE

The insecticides, although effective in controlling the pest, have a drawback in the form of resistance developed against them by the pest. The reason for resistance can be known at the gene level. RNA sequence analysis of *Spodoptera litura* midgut and fat body tissues suggested that genes from these sites may play an important role in xenobiotic detoxification **(Li et al., 2019).**

Some molecular factors are behind the resistance mechanism. One such process is the detoxification process. The cytochrome P450-dependent monooxygenases are important 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)**.

There is a certain molecular mechanism underlying the resistance. From a total of 32 D. melanogaster genes and proteins involved in insecticide resistance, 21 genes for resistance have been identified, which include 15 metabolic resistance genes: 11 P450 genes, 2 EST genes, and 2 GST genes, and 6 target site resistance genes: 1 AChE, 1 VGSC, 2 GABA, and 2 JH **(Zhang & Zhang, 2018)**.

Resistance levels in insects should be monitored. Anthranilic diamide insecticides like chlorantraniliprole are widely used against lepidopteran pests. Resistance against chlorantraniliprole in *Chilo suppressalis* was found to have increased as much as above 200 folds in a population. Mechanisms of resistance included detoxification and mutation, which can be identified by molecular studies **(Wei et al., 2019)**.

## *Origin of the problem*

*S. frugiperda* is a massive pest invading a large part of the Indian subcontinent and is observed to be resistant to a wide range of older insecticides, used in other parts of the globe. On the other hand, two drugs, namely chlorantraniliprole and emamectin benzoate, have been invented in recent decades to combat lepidopteran manifestations, but their efficiency to control *S. frugiperda* has not been studied in detail. Further, whether this pest develops resistance against the two drugs is unknown. As a result, this study is an attempt to investigate the combating potential of these drugs against *S. frugiperda*, with an eye toward potential drug resistance that may develop across multiple generations of the insect. The approach here is to decipher the mechanisms through which the resistance might develop to develop a rescue operation beforehand.

**AIMS & OBJECTIVES**

Overall aim: To investigate the insecticide efficacy and resistance for *Spodoptera frugiperda* Smith, 1797 post field survey and standardizing laboratory rearing

To achieve the aim, work has been divided into the following objectives:

Objective 1: A survey of agricultural fields in and around Vadodara to check the presence and infestation of *Spodoptera frugiperda*

Objective 2: 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 insecticide: The molecular basis of resistance

While starting the survey of agriculture fields in and around Vadodara, there was a trend toward using older insecticides against fall armyworms. Because the pest was new and there was no recommended insecticide treatment, farmers mostly sprayed organophosphate, carbamates, and synthetic pyrethroids as single agents or in combination. Farmers were complaining not getting any control using the mentioned insecticides against the fall armyworm. Going through the literature survey, I found that the pest, *Spodoptera frugiperda* has already developed resistance to those insecticides. This led to my choosing and finalising the two new-generation insecticides, which have been proven to be effective against lepidoptera. Experiments began, and in 2020 and 2021, government agencies like CIB also enlisted recommendations for control against fall armyworm, and our insecticides were mentioned in that list. The technical grades of these insecticides have not been thoroughly tested in the Gujarat population or the Indian scenario. Therefore, finding the doses for control of these insecticides was essential. Meanwhile, a field survey was also going on simultaneously. In the years 2021 and 2022, the maximum number of fields in Vadodara were surveyed where maize was grown or *Spodoptera frugiperda* infestations were seen. It was observed that the usage of emamectin benzoate, commercial grade by different names has increased to a drastic level. This calls for the danger of resistance development, which might occur in near future. Such a scenario urges the need for a resistance study wherein the molecular mechanism which would occur in emamectin benzoate-treated *Spodoptera frugiperda* should be known so as to have a rescue beforehand.

## METHODOLOGY

1. **Pest Collection:** *Spodoptera frugiperda* was collected from the agricultural fields of Vadodara. The pest was found in the maize crop. Following a thorough inspection, the pest was collected. (**Figures 5, 6**)

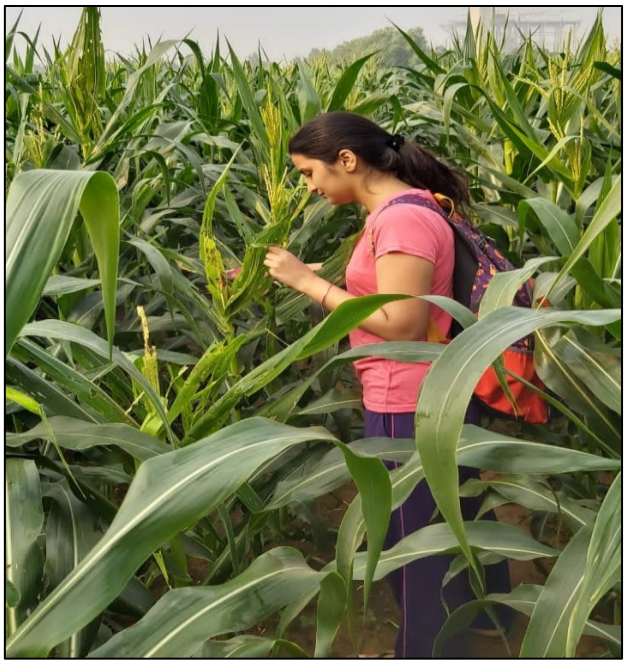


**Figure 5**: Collecting FAW from Maize field in Chhani region

**Figure 6**: Collection of Fall Armyworm

2. **Laboratory set-up:** Different trays, boxes, and cages were made for different stages of the insect pest, i.e., eggs, larvae, pupae, and adults. Appropriate temperature, humidity, and photoperiod were optimised for the artificial rearing and were maintained with the help of the BOD chamber (25±2°C temperature & 70±10% humidity, 12:12 D:L).
3. **Artificial diet**: The larvae were reared on an artificial diet. Before that, natural and artificial diets were compared and different diets were evaluated **(Figures 7, 8)**. After standardization of the most effective diet plan, insects were reared on the diet throughout the experiment **(Tables 2, 4)**. Adults will be given a diet commonly given to adult moths, consisting of honey and sucrose **(Table 3)**.

**Figure 7**: Larvae Reared on Natural Diet

**Figure 8**: Larvae reared on Artificial Diet

**Table 2**: Artificial maize flour-based diet ingredients for larvae

| Sr.No. | Ingredients | Amount |
|---|---|---|
| **1.** | Agar-agar powder | 18g |
| **2.** | Ascorbic acid | 5 g |
| **3.** | Becosule | 6 ml |
| **4.** | Formaldehyde (10%) | 20 ml |
| **5.** | Maize powder | 150 g |
| **6** | Wheat germ | 50 g |
| **7.** | Methyl-paraben | 3 g |
| **8.** | Propionic acid | 2 ml |
| **9.** | Sorbic acid | 2 g |
| **10.** | Yeast powder | 50 g |
| **11.** | Water | 1000ml |

**Table 3**: Artificial diet for adults

| Sr. No. | Ingredients | Amount |
|---|---|---|
| 1. | Honey | 100 g |
| 2. | Sucrose | 100 g |
| 3. | Becosule | 4 g |
| 4. | Methyl paraben | 4 g |
| 5. | Ascorbic acid | 40 g |
| 6. | Water | 1000 ml |

**Table 4**: Different diet's composition in gm or ml for *Spodoptera frugiperda* (1 l diet)

| Sr. No. | Ingredients | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|---|
| **1** | Wheat germ | 60g | 60g | 60g | 60g |
| **2** | Sucrose | 36.36g | 36.36g | 36.36g | 36.36g |
| **3** | Yeast | 53g | 53g | 53g | 53g |
| **4** | Agar | 20g | 20g | 20g | 20g |
| **5** | Sorbic acid | 1.7g | 1.7g | 1.7g | 1.7g |
| **6** | Ascorbic acid | 5.3g | 5.3g | 5.3g | 5.3g |
| **7** | Methyl-p hydroxy-benzoate | 3.3g | 3.3g | 3.3g | 3.3g |

| | | | | | |
|---|---|---|---|---|---|
| **8** | Formaldehyde | 20 ml | 20 ml | 20 ml | 20 ml |
| **9** | Becosule | 12ml | 12ml | 12ml | 12ml |
| **10** | Propionic acid | 2ml | 2ml | 2ml | 2ml |
| **11** | Maize flour | 160g | - | - | - |
| **12** | Soya flour | - | 160g | - | - |
| **13** | Chickpea flour | - | - | 160g | - |
| **14** | Jowar/Sorghum flour | - | - | - | 160g |
| **15** | Water | 1000ml | 1000ml | 1000ml | 1000ml |

4. **Pure culture:** Collected the caterpillar stage of the fall armyworm (3rd to 5th instar larvae) from the fields. Brought the caterpillars into the lab and reared them on a natural diet (fresh maize leaves) till pupal formation. Adults were raised from pupae and fed a honey-based diet. Adults mated, and egg-laying occurred. Young neonates from the eggs were kept on an artificial diet until all the larval stages were complete and adults emerged and laid eggs. Such a process was repeated for three generations, termed F01, F02, and F03. The insects were lab-raised for three generations on an artificial diet free of any insecticides to avoid the effects of insecticide residues from the past. This was termed pure culture and was further used for main experiments**.**

5. **Insecticides & testing:** Technical grade of chlorantraniliprole and emamectin benzoate were bought from sigma aldrich. Insecticides were tested after making stock solutions of the insecticides and preparing serial dilutions for making various ppm of the insecticide solutions. Both insecticides were separately incorporated into the standardized diet as per the guidelines of the Insecticide Resistance Action Committee (IRAC), to devise the LC50 dose for each chemical. The range of concentration was tested for every generation of both insecticides. The generation with the least effect on the population, or the time when the concentration giving LC50 appears to be no longer effective in controlling the population in the required numbers, was considered the onset of resistance. At this generation, the pest was considered to have possibly achieved resistance.

## HISTOLOGY

The differences between the control, treated, and resistant insects were attempted to be observed through histological analysis. Since the midgut plays an important role in detoxification, it was selected for the histological studies. The control population was kept insecticide-free throughout the study, the treated population was seen after 24 hours of exposure to the LC50 concentration, and the resistant population had undergone insecticide testing for some generations. After proper sectioning, staining with hematoxylin-eosin was done and observed under a bright-field microscope.

## MECHANISMS OF RESISTANCE: TRANSCRIPTOME STUDIES

Midgut samples were collected from both the control (untreated throughout) and test populations (emamectin benzoate treated) in order to identify the genes responsible for resistance development. The reason behind the selection of the emamectin-benzoate treated insects for molecular studies was the extensive increase in use of the insecticides against fall armyworm in Vadodara fields over the last two years. The difference between the control and treatment profiles would reveal the genes responsible for causing resistance. This can be achieved through the transcriptome profiling of the samples.

### Transcriptome analysis of the midgut

RNA isolation: RNA extraction was performed using the Trizol method. Extracted RNA quantity is checked on a 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 measure the concentration in Nanodrop 1000. Finally, to obtain RIN values, RNA was checked on the TapeStation using HS RNA ScreenTape (Agilent). Differential expressions of genes were noted and analysed.

## DATA ANALYSIS

The larval mortalities were recorded after 72 hours. The larvae were considered dead if they were unable to make a coordinated movement when given any stimulus. LC50 was the concentration value where 50% of the population died. LC50 value for Chlorantraniliprole and Emamectin benzoate was checked over generation and calculate with Probit analysis of SPSS software.

# RESULTS

## FIELD SURVEY

A survey of the agriculture fields in Vadodara was done. The fields with crops grown there are mentioned (**Table 5**). Damage was observed in the field **(Figure 9, 10)**.

**Table 5**: Agriculture fields of Vadodara with location and crops grown there

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

**Figure 9:** *S. frugiperda* infestation in maize fields (creating windows)

**Figure 10:** Damage by FAW in Maize (complete holes)

**DIET STUDIES**

Percent Pupation on the two diets is shown in **Table 6**. The larval growth index is shown in **Table 7**. Statistics result in **Table 8** shows descriptive statistics for diets which reveals the average days the insect retains its different life stages (ND=Natural Diet, AD=Artificial Diet, 1 & 2 show the replicates). The maize-based artificial diet & chickpea-based diet were found to be successful and economic for easy laboratory rearing of the pest inside the lab.

The observations for various diets on the survival of *Spodoptera frugiperda* are shown in the table. **(Table 9 & 10)**. The larval growth index was calculated **(Table 11)**. A graphical comparison between survival and completion of the life cycle between natural and artificial diets **(Graph 2)**, and various artificial diets have been done **(Graph 3).**

**Table 6**: Percent pupation in two different diets

| Diet | Larvae/ tray | No. of trays | Total Larvae released | No. of Pupa formed | % Pupation |
|---|---|---|---|---|---|
| **1 (Natural)** | 10 | 2 | 20 | 17 | 85% |
| **2 (Artificial)** | 10 | 2 | 20 | 19 | 95% |

**Table 7**: Larval Growth Index of two different diets

| Diet | Percent Pupation | Larval period | Larval Growth Index |
|---|---|---|---|
| **1 (Natural)** | 85% | 19.6 | 4.34 |
| **2 (Artificial)** | 95% | 17.85 | 5.32 |

Larval Growth Index (LGI) = Percent pupation/ Larval period (days)

**Table 8**: Descriptive statistics for diets

| Variable | Stage | N | N* | Mean | StDev |
|---|---|---|---|---|---|
| **ND(A)Days** | Adult | 9 | 1 | 5.667 | 0.500 |
| | Egg | 10 | 0 | 3.800 | 0.632 |
| | Larva | 10 | 0 | 19.200 | 0.789 |
| | Pupa | 10 | 0 | 8.400 | 2.989 |
| | | | | | |
| **ND(B)Days Days** | Adult | 8 | 2 | 5.875 | 0.354 |
| | Egg | 10 | 0 | 3.900 | 0.568 |
| | Larva | 10 | 0 | 19.300 | 0.675 |
| | Pupa | 10 | 0 | 7.60 | 4.06 |
| | | | | | |
| **AD(A)Days Days** | Adult | 10 | 0 | 6.600 | 0.516 |
| | Egg | 10 | 0 | 2.900 | 0.568 |
| | Larva | 10 | 0 | 17.900 | 0.568 |
| | Pupa | 10 | 0 | 7.800 | 0.422 |
| | | | | | |
| **AD(B)Days** | Adult | 9 | 1 | 6.667 | 0.866 |
| | Egg | 10 | 0 | 3.700 | 0.483 |
| | Larva | 10 | 0 | 17.800 | 0.632 |
| | Pupa | 10 | 0 | 6.800 | 2.486 |

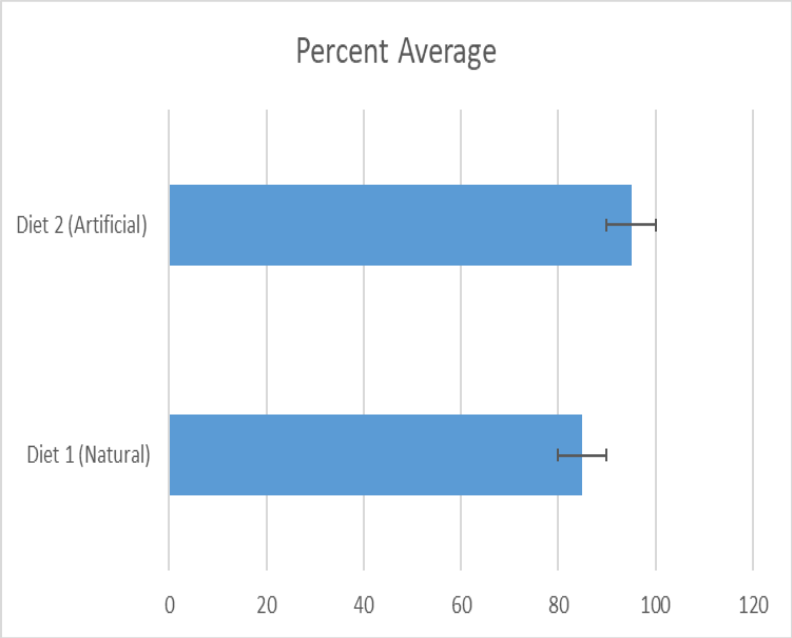
**Table 9**: Percent survival of *Spodoptera frugiperda* larva on diets

| Diets | 2nd instar larvae/ cell | Total larvae released | Total pupa formed | Percent survival |
|---|---|---|---|---|
| **1** | 1 | 30 | 29 | 96.66% |
| **2** | 1 | 30 | 26 | 86.66% |
| **3** | 1 | 30 | 29 | 96.66% |
| **4** | 1 | 30 | 24 | 80.00% |

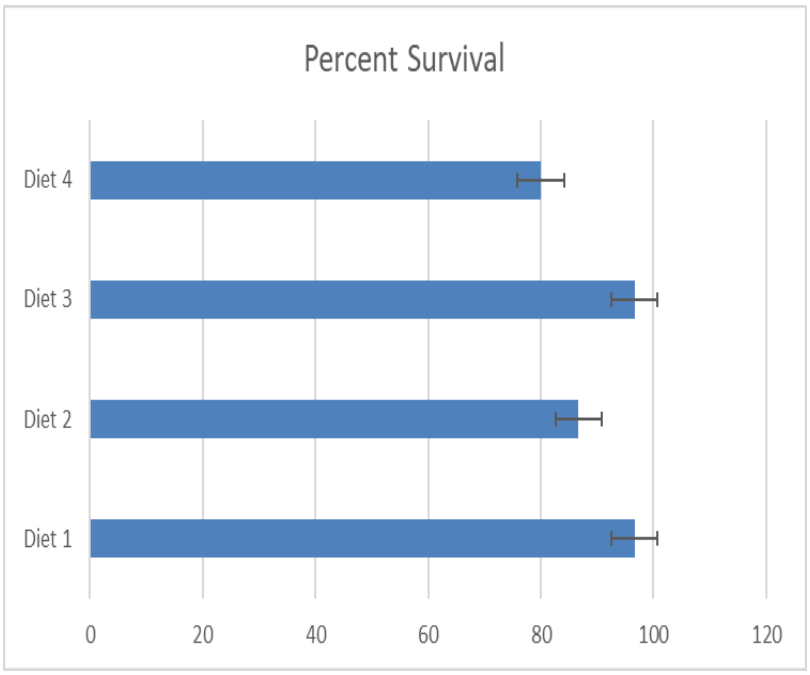
**Table 10**: Larval growth index for *Spodoptera frugiperda*

| Diet | Percent survival | Larval period | Larval Growth Index |
|---|---|---|---|
| **1** | 96.66% | 14.61 | 6.61 |
| **2** | 86.66% | 15.67 | 5.53 |
| **3** | 96.66% | 14.52 | 6.65 |
| **4** | 80.00% | 16.15 | 4.95 |

Larval Growth Index (LGI) = Percent pupation/ Larval periods (days)




**Graph 2:** Survival on natural & artificial diet




**Graph 3:** Survival on different artificial diets

## INSECTICIDE EFFICACY

The bioassays performed on the pest *Spodoptera frugiperda* indicated efficacy present in both the insecticides to control fall armyworm. The insecticides selected were chlorantraniliprole and emamectin benzoate. Both insecticides were individually checked for assessing mortality values for this pest. The mother culture was simultaneously maintained to have a susceptible population. After 72 hours of insects being fed artificial diets containing different ppm of the two insecticides, the test trays were checked. At 72 hours, the observation was taken using forceps that had been pre-sterilized. The observations of mortality from the insecticides are mentioned. Separate tools were used for untreated and treated insects to avoid contamination. They were checked for any kind of bacterial or fungal infection. A larva was considered dead if there was no movement after contact with a brush. A larva was considered to be moribund if it showed less and uncoordinated movement as compared to an untreated larva. A larva was considered alive if it showed normal movement when compared to an untreated larva after getting a stimulus with a brush (or any physical stimulus). The ideal conditions of temperature and humidity were maintained to obtain the actual results without interference from external factors.

**TESTING OBSERVATIONS**

Insecticide testing was done by selecting a range of concentrations for both insecticides and the same range of dose was repeated for four generations. Mortalities over the generations for the insecticides were noted down and analysed.

In the case of chlorantraniliprole-treated insects, around 50% of the test insects were found to be dead (LC50) at 0.05 ppm in the first-generation testing. There was no mortality observed in the control population **(Table 11).** In the case of emamectin benzoate-treated insects, around 50% of the test insects were found to be dead at 0.1 ppm in the first-generation testing. There was no mortality observed in the control population **(Table 12)**.

Over the generations, chlorantraniliprole-treated insect mortality was seen to be constant or near the same in the second generation and third generations with 46.66% mortality at 0.05 ppm **(Table 13 and Table 15)**. There was observed a decrease in mortality in the fourth generation of insecticide testing wherein only 20% mortality was achieved with 0.05 ppm **(Table 17)**.

Over the generations, emamectin benzoate-treated insect mortality was seen to be constant or near the same in the second generation and third generation with 50% mortality at 0.1 ppm and 43.33% mortality at 0.1 ppm, respectively **(Table 14 and Table 16)**. There was observed a decrease in mortality in the fourth generation of insecticide testing wherein only 30% mortality was achieved with 0.05 ppm **(Table 18)**.

**Table 11**: Mortality (%) obtained in *S. frugiperda* against Chlorantraniliprole (1st Generation)

| Sr. no | Concentration (ppm) | Total Larvae | Dead | Moribund | Total (Dead +Moribund) | % Mortality |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 30 | 0 | 30 | 100.00 |
| **3** | 1 | 30 | 30 | 0 | 30 | 100.00 |
| **4** | 0.5 | 30 | 26 | 2 | 28 | 93.33 |
| **5** | 0.1 | 30 | 15 | 3 | 18 | 60.00 |
| **6** | 0.05 | 30 | 14 | 2 | 16 | 53.33 |
| **7** | 0.02 | 30 | 13 | 1 | 14 | 46.66 |
| **8** | 0.01 | 30 | 4 | 2 | 6 | 20.00 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 12**: Mortality (%) obtained in *S. frugiperda* against Emamectin benzoate (1st Generation)

| Sr.no | Concentration (ppm) | Total Larvae | Dead | Moribund | Total (Dead +Moribund) | % Mortality |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 30 | 0 | 30 | 100.00 |
| **3** | 1 | 30 | 26 | 2 | 28 | 93.33 |
| **4** | 0.5 | 30 | 16 | 2 | 18 | 60.00 |
| **5** | 0.1 | 30 | 11 | 3 | 14 | 46.66 |
| **6** | 0.05 | 30 | 11 | 1 | 12 | 40.00 |
| **7** | 0.02 | 30 | 2 | 1 | 3 | 10.00 |
| **8** | 0.01 | 30 | 0 | 0 | 0 | 0.00 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

The surviving insects from the exposure to insecticide 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. **(Table 13-18)**. Probit analysis using SPSS was done with mortality values over the generations were found out **(Table 19-20)**.

**Table 13**: Mortality (%) obtained in *S. frugiperda* against Chlorantraniliprole (2nd Generation)

| **Sr.no** | **Concentration (ppm)** | **Total Larvae** | **Dead** | **Moribund** | **Total (Dead +Moribund)** | **% Mortality** |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100 |
| **2** | 5 | 30 | 30 | 0 | 30 | 100 |
| **3** | 1 | 30 | 28 | 2 | 30 | 100 |
| **4** | 0.5 | 30 | 24 | 4 | 28 | 93.33 |
| **5** | 0.1 | 30 | 16 | 2 | 18 | 60.00 |
| **6** | 0.02 | 30 | 14 | 1 | 15 | 50.00 |
| **7** | 0.05 | 30 | 13 | 1 | 14 | 46.66 |
| **8** | 0.01 | 30 | 3 | 2 | 5 | 16.67 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 14**: Mortality (%) obtained in *S. frugiperda* against Emamectin benzoate (2nd Generation)

| **Sr.no** | **Concentration (ppm)** | **Total Larvae** | **Dead** | **Moribund** | **Total (Dead +Moribund)** | **% Mortality** |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 28 | 2 | 30 | 100.00 |
| **3** | 1 | 30 | 23 | 4 | 27 | 90.00 |
| **4** | 0.5 | 30 | 14 | 3 | 17 | 56.66 |
| **5** | 0.1 | 30 | 14 | 1 | 15 | 50.00 |
| **6** | 0.05 | 30 | 9 | 1 | 10 | 46.66 |
| **7** | 0.02 | 30 | 4 | 2 | 6 | 33.33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **8** | 0.01 | 30 | 0 | 0 | 0 | 0.00 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 15**: Mortality (%) obtained in *S. frugiperda* against Chlorantraniliprole (3rd Generation)

| **Sr.no** | **Concentration (ppm)** | **Total Larvae** | **Dead** | **Moribund** | **Total (Dead +Moribund)** | **% Mortality** |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 27 | 1 | 28 | 93.33 |
| **3** | 1 | 30 | 23 | 2 | 25 | 86.66 |
| **4** | 0.5 | 30 | 14 | 4 | 18 | 60.00 |
| **5** | 0.1 | 30 | 14 | 1 | 15 | 50.00 |
| **6** | 0.05 | 30 | 13 | 1 | 14 | 46.66 |
| **7** | 0.02 | 30 | 5 | 1 | 6 | 20.00 |
| **8** | 0.01 | 30 | 2 | 1 | 3 | 10.00 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 16**: Mortality (%) obtained in *S. frugiperda* against Emamectin benzoate (3rd Generation)

| **Sr.no** | **Concentration (ppm)** | **Total Larvae** | **Dead** | **Moribund** | **Total (Dead +Moribund)** | **% Mortality** |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 28 | 2 | 30 | 100.00 |
| **3** | 1 | 30 | 22 | 1 | 23 | 86.00 |
| **4** | 0.5 | 30 | 14 | 2 | 16 | 53.33 |
| **5** | 0.1 | 30 | 11 | 2 | 13 | 43.33 |
| **6** | 0.05 | 30 | 10 | 0 | 10 | 33.33 |
| **7** | 0.02 | 30 | 4 | 2 | 6 | 20.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **8** | 0.01 | 30 | 0 | 0 | 0 | 0.00 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 17**: Mortality (%) obtained in *S. frugiperda* against Chlorantraniliprole (4th Generation)

| **Sr.no** | **Concentration (ppm)** | **Total Larvae** | **Dead** | **Moribund** | **Total (Dead +Moribund)** | **% Mortality** |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 23 | 4 | 27 | 90.00 |
| **3** | 1 | 30 | 15 | 3 | 18 | 60.00 |
| **4** | 0.5 | 30 | 13 | 2 | 15 | 50.00 |
| **5** | 0.1 | 30 | 13 | 1 | 14 | 46.66 |
| **6** | 0.05 | 30 | 4 | 2 | 6 | 20.00 |
| **7** | 0.02 | 30 | 0 | 0 | 0 | 0.00 |
| **8** | 0.01 | 30 | 0 | 0 | 0 | 0.00 |
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 18**: Mortality (%) obtained in *S. frugiperda* against Emamectin benzoate (4th Generation)

| **Sr.no** | **Concentration (ppm)** | **Total Larvae** | **Dead** | **Moribund** | **Total (Dead +Moribund)** | **% Mortality** |
|---|---|---|---|---|---|---|
| **1** | 10 | 30 | 30 | 0 | 30 | 100.00 |
| **2** | 5 | 30 | 22 | 4 | 26 | 86.00 |
| **3** | 1 | 30 | 15 | 2 | 17 | 56.66 |
| **4** | 0.5 | 30 | 13 | 1 | 14 | 46.66 |
| **5** | 0.1 | 30 | 7 | 2 | 9 | 30.00 |
| **6** | 0.05 | 30 | 4 | 1 | 5 | 16.66 |
| **7** | 0.02 | 30 | 0 | 0 | 0 | 0.00 |
| **8** | 0.01 | 30 | 0 | 0 | 0 | 0.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **9** | Control (or Untreated) | 30 | 0 | 0 | 0 | 0.00 |

**Table 19**: Mortality values of *S. frugiperda* against Chlorantraniliprole over generations (G 1-4)

| **Sr. No.** | **Concentration (ppm)** | **G-1** | **G-2** | **G-3** | **G-4** |
|---|---|---|---|---|---|
| **1** | 10 | 100.00 | 100 | 100.00 | 100.00 |
| **2** | 5 | 100.00 | 100 | 93.33 | 90.00 |
| **3** | 1 | 100.00 | 100 | 86.66 | 60.00 |
| **4** | 0.5 | 93.33 | 93.33 | 60.00 | 50.00 |
| **5** | 0.1 | 60.00 | 60.00 | 50.00 | 46.66 |
| **6** | 0.05 | 53.33 | 50.00 | 46.66 | 20.00 |
| **7** | 0.02 | 46.66 | 46.66 | 20.00 | 0.00 |
| **8** | 0.01 | 20.00 | 16.67 | 10.00 | 0.00 |
| **9** | Control (or Untreated) | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 20**: Mortality values of *S. frugiperda* against Emamectin benzoate over generations (G 1-4)

| **Sr. No.** | **Concentration (ppm)** | **G-1** | **G-2** | **G-3** | **G-4** |
|---|---|---|---|---|---|
| **1** | 10 | 100.00 | 100.00 | 100.00 | 100.00 |
| **2** | 5 | 100.00 | 100.00 | 100.00 | 86.00 |
| **3** | 1 | 93.33 | 90.00 | 86.00 | 56.66 |
| **4** | 0.5 | 60.00 | 56.66 | 53.33 | 46.66 |
| **5** | 0.1 | 46.66 | 50.00 | 43.33 | 30.00 |
| **6** | 0.05 | 40.00 | 46.66 | 33.33 | 16.66 |
| **7** | 0.02 | 10.00 | 33.33 | 20.00 | 0.00 |
| **8** | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | |
|---|---|---|---|---|---|
| **9** | Control (or Untreated) | 0.00 | 0.00 | 0.00 | 0.00 |

In case of both chlorantraniliprole and emamectin benzoate, it was observed that high mortality was observed in the first generation. Mortality for 0.05 ppm dose of chlorantraniliprole was 53.33% for the 1st generation, 50.00% for 2nd generation, 46.66% for 3rd generation and 20.00% for 4th generation. Mortality for 0.1 ppm of emamectin benzoate was 46.66% in the 1st generation, 50.00% for 2nd generation, 43.33% for 3rd generation and 30.00% for 4th generation. Over the course of four generations, the mortality rate started declining. This could be due to the initiation of resistance development in the insect against the insecticides. In the fourth generation, mortality as low as 20% for chlorantraniliprole and 30% for emamectin benzoate was achieved. Increased use of emamectin benzoate in fields against fall armyworm is a matter of concern as this might create high level of resistance in the future. To understand resistance mechanism in fall armyworm, we took the fourth generation emamectin treated insect (4th instar) and control (4th instar). Dissection was done and midgut extracted and RNA isolated which was checked for transcriptome analysis.

While starting the survey of agriculture fields in and around Vadodara, there was a trend toward using older insecticides against fall armyworms. Because the pest was new and there was no recommended insecticide treatment, farmers mostly sprayed organophosphate, carbamates, and synthetic pyrethroids as single agents or in combination. Farmers were complaining not getting any control using the mentioned insecticides against the fall armyworm. Going through the literature survey, I found that the pest, *Spodoptera frugiperda* has already developed resistance to those insecticides. This led to my choosing and finalising the two new-generation insecticides, which have been proven to be effective against lepidoptera. Experiments began, and in 2020 and 2021, government agencies like CIB also enlisted recommendations for control against fall armyworm, and our insecticides were mentioned in that list. The technical grades of these insecticides have not been thoroughly tested in the Gujarat population or the Indian scenario. Therefore, finding the doses for control of these insecticides was essential. Meanwhile, a field survey was also going on simultaneously. In the years 2021 and 2022, the maximum number of fields in Vadodara were surveyed where maize was grown or *Spodoptera frugiperda* infestations were seen. It was observed that the usage of emamectin benzoate, commercial grade by different names has increased to a drastic level. This calls for the danger of resistance development, which might occur in near future.

Such a scenario urges the need for a resistance study wherein the molecular mechanism which would occur in emamectin benzoate-treated *Spodoptera frugiperda* should be known so as to have a rescue beforehand.

**BEHAVIOURAL CHANGES**

The following change was observed in case of a treated insect as compared to the control:

- There was observed repellence of the insect from the food/diet containing insecticides
- Feeding decreased or even stopped in case of diet constituting higher concentrations of insecticides
- Shrinking of the body was observed, size decreased
- Insects exposed to insecticides also turned blackish
- On providing any stimulus, there was lethargy and no movement as compared to control where a sharp response was seen on providing stimulus

**HISTOLOGY**

**Control:** *S. frugiperda*'s midgut had an epithelial layer, and the cytoplasm of digestive cells has uniform, well-developed nuclei. These cell surfaces were well striated, and the peritrophic matrix in the midgut lumen was well-developed. It had muscular layers lining their basal surface **(Figure 11a)**.

**Treated:** The midgut epithelium of the insect was uneven, and the cytoplasm was severely vacuolized, loss of lining, straited border disorganization, cell disintegration **(Figure 11b)**.

Resistant: The midgut region here had fewer deformities observed as compared to the treated ones. The structure seemed largely intact. The longer and regular exposure to the insecticide might have played a role in keeping more stable as compared to initial exposure. However, little vacuolization was observed with a slight deformation in shape **(Figure 11c)**.

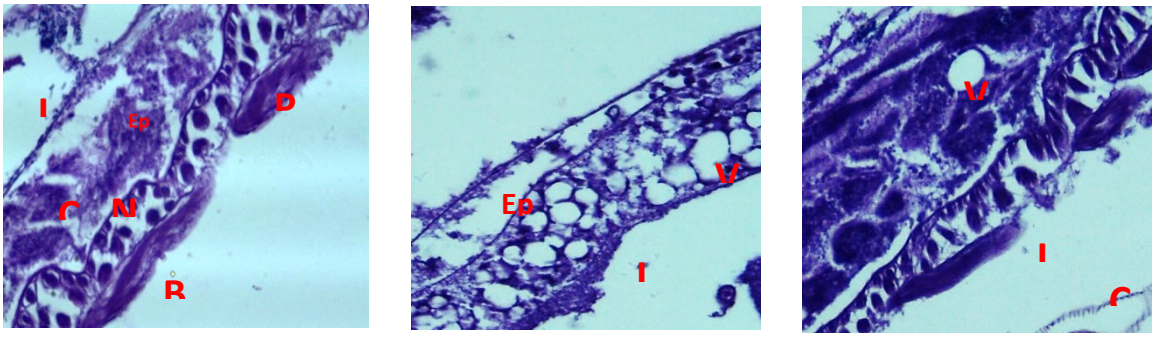



**Figure 11:** Midgut section; a. Control, b. Treated, c. Resistant (10X)

[L=lumen, Ep=epithelium, P=peritrophic matrix, B=basal membrane, C=cytoplasm, N=nucleus, V=vacuole, L=loss of lining]

# TRANSCRIPTOME ANALYSIS

## SAMPLE TAKEN FROM MIDGUT REGION

The total set of transcripts present in a cell for a particular developmental stage or physiological condition is known as the transcriptome. To interpret the functional components of the genome, expose the molecular components of cells and tissues, and to comprehend development and illness, one must have a thorough comprehension of the transcriptome. (Wang et al., 2010)

Sequencing of RNA transcripts is a useful method for RNA profiling because-

- Differential gene expression (DGE) analysis – sensitive quantification of gene expression levels and transcriptional activity and comparison between control and treatments. Analysis across a wide dynamic range – detection of more differentially expressed genes with higher fold change
- Some uncharacterized, unidentified genes might also show up from it. Identification of both known and novel transcripts
- Profile the RNA transcripts of nearly any organism. A complete view of the entire transcriptome i.e., every gene
- A complete set of genes working inside can be known at once
- There have been many studies from the past wherein midgut has been extracted from the caterpillars for conducting studies related to histology and transcriptomics previously.
- A study done on another noctuidae pest *Spodoptera litura* used midgut and fat bodies for RNA sequencing wherein they found that genes from these sites play a role in providing resistance against tomatine (Li et al., 2019). Midgut from *Spodoptera litura* has also been checked for detoxification genes against xenobiotic compounds and bacteria (Huang et al., 2011). The midgut

site is thus known to be important for providing resistance as containing supporting genes for providing resistance.

To begin with, RNA was extracted and its quality checked (**Table 21**). The difference between the gene expression patterns of the two samples was noted. One sample was from the susceptible population, which was made through several generations of rearing inside laboratories without any exposure to insecticides. Another sample was the fourth generation of emamectin benzoate, which was surviving even after insecticide exposure. The upregulated and downregulated genes in the resistant population as compared to the susceptible population were observed (**Table 23**).

**Table 21:** Sample QC

| Sr. No. | Sample Name | Nanodrop (ng/ul) | A260/280 | A260/230 | Qubit (ng/ul) | RIN Value | QC Remarks |
|---|---|---|---|---|---|---|---|
| **1** | MG C 1 | 530.6 | 1.97 | 2.03 | 752 | 9.4 | Pass |
| **2** | MG C 2 | 365.5 | 2.04 | 2 | 524 | 9.3 | Pass |
| **3** | MG T 1 | 811.6 | 2.06 | 2.44 | 918 | 9.3 | Pass |
| **4** | MG T 2 | 709.5 | 2.03 | 2.44 | 1026 | 9.2 | Pass |

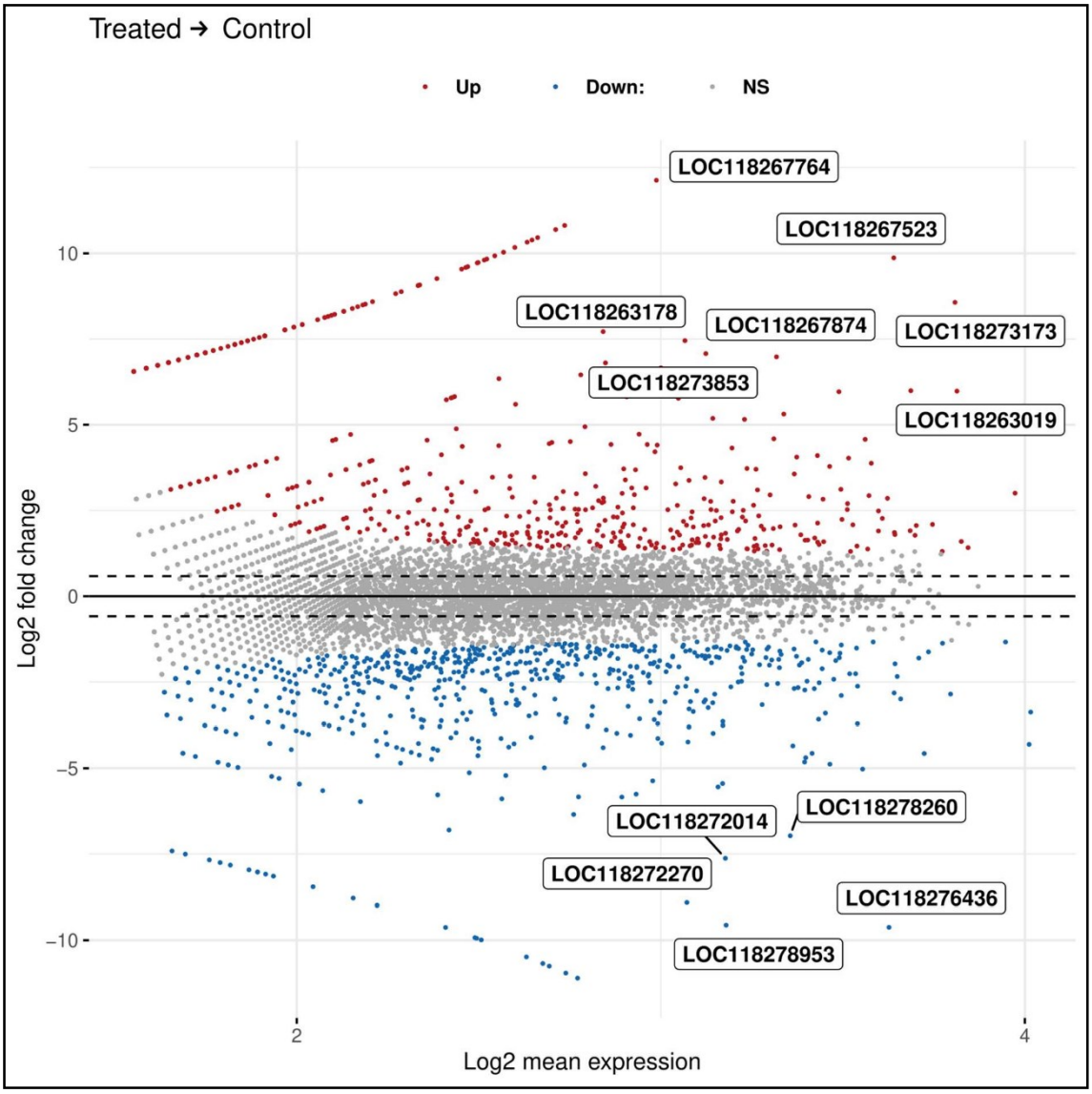
**Table 22**: Up regulated and down regulated count based on p-value (unadjusted value), FDR (adjusted p-value) and Log2FoldChange

| Filter Parameters | Treated vs Control | |
|---|---|---|
| | Up regulated genes | Down regulated genes |
| P Value <= 0.05 & log2FC ∓ 1.5 | 464 | 607 |

A total of 464 genes were found upregulated and 607 genes were found downregulated in treated (emamectin) as compared to control (susceptible) insect. Details of few of the up and downregulated genes have been shown in the MA plot **(Plot 1)**.

**CYTOCHROME P450: ONE OF THE MAJOR SET OF GENES FOR DEVELOPING RESISTANCE**

An incredibly significant metabolic system involved in the catabolism and anabolism of xenobiotics and endogenous substances is the cytochrome P450-dependent monooxygenases (monooxygenases). The large number of insect species and insecticides affected demonstrate that monooxygenase-mediated metabolism is a typical mechanism through which insects develop resistance to insecticides (Scott, 1999). In our study, around 78 cytochromes have been upregulated and 66 cytochromes are downregulated. Some representative genes showing most variation in the fold change with their names as found up or down regulated are displayed in the table (**Table 23)**.




**Plot 1**: MA Plot of Treated vs Control

(The 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 normalised expression counts (A) of the samples. Red dots correspond to genes up-regulated (>+1.5) and blue dots correspond to genes which are down-regulated (<- 1.5) based on the unadj p-value `0.05`. Grey dot corresponds to the non-significant genes where unadj p-value > 0.05.)

**Table 23:** Genes showing most variation in fold change

| Sr. No. | UPREGULATED | DOWNREGULATED |
|---|---|---|
| **1** | cytochrome c oxidase subunit 3-like | cytochrome P450 4C1 |
| **2** | cytochrome P450 4C1-like | cytochrome P450 4c3-like |
| **3** | cytochrome P450 6B2 | cytochrome P450 4d2-like |
| **4** | cytochrome P450 6B6-like | cytochrome P450 4g15 |
| **5** | cytochrome P450 6B7, transcript variant X11 | cytochrome P450 6B2, transcript variant X2 |
| **6** | cytochrome P450 6B7, transcript variant X2 | cytochrome P450 6B4 |
| **7** | cytochrome P450 9e2-like | cytochrome P450 6k1 |

Apart from the genes mentioned above, there have some with a wide differential expression. Amongst them, top 25 upregulated genes and top 25 downregulated genes have been identified and mentioned in **Table 24**

**Table 24:** Differentially expressed genes from the transcriptome analysis

| Sr.No. | Transcript_id | Start | End | Strand | Gene | Product | GeneID | protein_id | PValue |
|---|---|---|---|---|---|---|---|---|---|
| **UPREGULATED GENES** | | | | | | | | | |
| **1** | XM_050706229.1 | 3852125 | 3865697 | + | LOC118268459 | synaptic vesicle glycoprotein 2B | 118268459 | XP_050562186.1 | 0.049763277 |
| **2** | XM_035593481.2 | 7327178 | 7336155 | + | LOC118275495 | uncharacterized LOC118275495 | 118275495 | XP_035449374.2 | 0.049618178 |
| **3** | XM_035593518.2 | 6238039 | 6269380 | - | LOC118275525 | zinc transporter ZIP1 | 118275525 | XP_035449411.1 | 0.049539603 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **4** | XM_035589421.2 | 4616950 | 4623748 | + | LOC11827275 8 | pyrimidodiazepine synthase | 118272758 | XP_035445314.2 | 0.049454694 |
| **5** | XM_035583751.2 | 8991096 | 8993996 | - | LOC11826890 8 | keratin, type I cytoskeletal 10 | 118268908 | XP_035439644.1 | 0.049439689 |
| **6** | XM_035597498.2 | 6235517 | 6236370 | + | LOC11827827 8 | coiled-coil domain-containing protein 115 | 118278278 | XP_035453391.1 | 0.049271322 |
| **7** | XM_035593058.2 | 8845256 | 8850469 | + | LOC11827518 8 | fatty acid-binding protein 1-like | 118275188 | XP_035448951.1 | 0.049115466 |
| **8** | XM_035585375.2 | 12219871 | 12235415 | - | LOC11826997 1 | putative carbonic anhydrase 3 | 118269971 | XP_035441268.1 | 0.048036813 |
| **9** | XM_050694368.1 | 3430357 | 3433237 | + | LOC12691076 6 | uncharacterized LOC12691076 6, transcript variant X3 | 126910766 | XP_050550325.1 | 0.047581525 |
| **10** | XM_035575232.2 | 7764744 | 7771243 | + | LOC11826331 7 | ethanolamine kinase | 118263317 | XP_035431125.1 | 0.047387679 |
| **11** | XM_050696551.1 | 8266742 | 8283690 | - | LOC11827731 6 | ethanolaminephosphotransferase 1 | 118277316 | XP_050552508.1 | 0.046704903 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **12** | XM_050698012.1 | 5515023 | 5688133 | + | LOC118270339 | 5-hydroxytryptamine receptor 1 | 118270339 | XP_050553969.1 | 0.046704285 |
| **13** | XM_035575639.2 | 4646071 | 4652077 | - | LOC118263567 | ubiquitin-conjugating enzyme E2 G2 | 118263567 | XP_035431532.1 | 0.046430242 |
| **14** | XM_035576490.2 | 3615967 | 3617153 | - | LOC118264101 | prostaglandin reductase 1-like | 118264101 | XP_035432383.2 | 0.046415465 |
| **15** | XM_050707788.1 | 4459840 | 4480249 | + | LOC118274408 | mucin-2-like | 118274408 | XP_050563745.1 | 0.045749055 |
| **16** | XM_035595491.2 | 10722601 | 10728588 | - | LOC118276885 | UPF0489 protein C5orf22 | 118276885 | XP_035451384.2 | 0.044841285 |
| **17** | XR_007706897.1 | 1737264 | 1875288 | + | LOC126912277 | uncharacterized LOC126912277 | 126912277 | | 0.0447917 |
| **18** | XM_035602697.2 | 6123378 | 6124516 | - | LOC118281904 | mitochondrial import inner membrane translocase subunit Tim9 | 118281904 | XP_035458590.1 | 0.044178093 |
| **19** | XM_035573163.2 | 6364368 | 6365184 | - | LOC118262058 | 39S ribosomal protein L20, | 118262058 | XP_035429056.1 | 0.043613955 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | mitochondrial | | | |
| **20** | XM_035589784.2 | 370418 | 379004 | - | LOC118273036 | CKLF-like MARVEL transmembrane domain-containing protein 7 | 118273036 | XP_035445677.1 | 0.043613955 |
| **21** | XM_035580523.2 | 8640739 | 8642732 | - | LOC118266901 | 39S ribosomal protein L16, mitochondrial | 118266901 | XP_035436416.1 | 0.043367409 |
| **22** | XM_035587689.2 | 10893159 | 10894295 | + | LOC118271599 | fumarylacetoacetate hydrolase domain-containing protein 2-like | 118271599 | XP_035443582.2 | 0.042727823 |
| **23** | XM_050702463.1 | 3887662 | 3893065 | + | LOC118279544 | uncharacterized LOC118279544 | 118279544 | XP_050558420.1 | 0.042699475 |
| **24** | XR_007706807.1 | 12655258 | 12657023 | + | LOC118266924 | MICOS complex subunit Mic10, transcript variant X2 | 118266924 | | 0.042250704 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **25** | XM_05070661.1 | 11821569 | 11833859 | + | LOC11827391 5 | cytochrome P450 6B7, transcript variant X15 | 118273915 | XP_050562568.1 | 0.041837243 |
| **DOWNREGULATED GENES** | | | | | | | | | |
| **26** | XM_05069847 6.1 | 6673669 | 6716728 | - | LOC11827925 5 | anoctamin-8-like | 118279255 | XP_050554433.1 | 0.04970526 |
| **27** | XM_05069538 3.1 | 4588693 | 4685711 | + | LOC11827557 6 | hemicentin-1-like | 118275576 | XP_050551340.1 | 0.049631622 |
| **28** | XR_00770583 8.1 | 7724788 | 7726803 | - | LOC12691132 1 | uncharacterized LOC12691132 1 | 126911321 | | 0.049612969 |
| **29** | XM_03559588 4.2 | 4971093 | 4974773 | - | LOC11827717 0 | uncharacterized LOC11827717 0 | 118277170 | XP_035451777.2 | 0.04910684 |
| **30** | XM_03558699 9.2 | 5048448 | 5052815 | + | LOC11827110 2 | piggyBac transposable element-derived protein 4-like | 118271102 | XP_035442892.2 | 0.048770873 |
| **31** | XM_03559105 6.2 | 671221 | 673654 | + | LOC11827387 9 | dnaJ protein homolog 1 | 118273879 | XP_035446949.1 | 0.048172076 |
| **32** | XM_03557815 7.2 | 4713848 | 4719545 | + | LOC11826533 3 | plasminogen activator inhibitor 1 | 118265333 | XP_035434050.2 | 0.046559831 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **33** | XM_035583503.2 | 3220598 | 3228530 | + | LOC11826882 1 | protein henna | 118268821 | XP_035439396.1 | 0.046313622 |
| **34** | XM_035576392.2 | 3884901 | 3895012 | - | LOC11826405 2 | mitochondrial glycine transporter, transcript variant X3 | 118264052 | XP_035432285.1 | 0.046289229 |
| **35** | XM_050703618.1 | 5176204 | 5188665 | - | LOC11827884 9 | UDP-glucosyltransferase 2-like | 118278849 | XP_050559575.1 | 0.04578509 |
| **36** | XM_050706927.1 | 12525277 | 12582543 | - | LOC11826996 0 | cadherin-87A | 118269960 | XP_050562884.1 | 0.04578509 |
| **37** | XM_035580179.2 | 7406095 | 7408813 | - | LOC11826669 3 | uncharacterized LOC11826669 3 | 118266693 | XP_035436072.2 | 0.045651874 |
| **38** | XM_035581819.2 | 12615701 | 12621598 | + | LOC11826769 3 | uncharacterized LOC11826769 3 | 118267693 | XP_035437712.1 | 0.045651874 |
| **39** | XM_035596082.2 | 3853545 | 3881117 | - | LOC11827733 3 | transmembrane protein 68, transcript variant X1 | 118277333 | XP_035451975.2 | 0.045651874 |
| **40** | XM_050698379.1 | 8300406 | 8326649 | + | LOC11827906 8 | ribosomal protein S6 kinase delta-1, transcript | 118279068 | XP_050554336.1 | 0.045651874 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | variant X1 | | | |
| **41** | XM_035597708.2 | 9115082 | 9115898 | - | LOC118278492 | nuclear protein 1 | 118278492 | XP_035453601.1 | 0.045523716 |
| **42** | XM_035585904.2 | 8970723 | 9008122 | + | LOC118270343 | homeotic protein empty spiracles | 118270343 | XP_035441797.1 | 0.045254176 |
| **43** | XM_035586554.2 | 10326912 | 10338632 | - | LOC118270780 | protein Skeletor, isoforms D/E | 118270780 | XP_035442447.2 | 0.045254176 |
| **44** | XM_050697007.1 | 2748697 | 2767742 | + | LOC118265122 | fatty acid synthase-like | 118265122 | XP_050552964.1 | 0.045137961 |
| **45** | XM_035583108.2 | 1432471 | 1434637 | - | LOC118268571 | carcinine transporter-like | 118268571 | XP_035439001.2 | 0.045127361 |
| **46** | XM_050695657.1 | 5871796 | 5875680 | - | LOC126910976 | uncharacterized LOC126910976 | 126910976 | XP_050551614.1 | 0.04488643 |
| **47** | XM_035580164.2 | 7798124 | 7801117 | + | LOC118266683 | argininosuccinate lyase | 118266683 | XP_035436057.2 | 0.044497416 |
| **48** | XM_035593168.2 | 9206589 | 9225703 | - | LOC118275268 | uncharacterized LOC118275268 | 118275268 | XP_035449061.2 | 0.044472514 |
| **49** | XM_035574957.2 | 3607782 | 3628031 | + | LOC118263144 | phytanoyl-CoA dioxygenase domain-containing | 118263144 | XP_035430850.1 | 0.044176692 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | protein 1 homolog | | | |
| **50** | XM_035591965.2 | 4293304 | 4302521 | + | LOC11827447 6 | peptidoglycan-recognition protein LB | 118274476 | XP_035447858.2 | 0.044167711 |

## 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 as a result of investing in seeds, growing, fertilizers, and pesticides but not receiving the expected yield. Such a lack of supply would in turn increase the price and cause problems for consumers. Pests thus have an impact not only on ecology but also on the economy. A number of insect pests have already been causing crop damage in the agricultural fields of Vadodara.

The current ways to control new pests effectively require good chemical control. We selected two insecticides recommended for use against lepidopteran pests. These two should be tested in detail for their efficacy and control against the new invasive pest, *S. frigiperda.* In addition, we need to know how long and to what extent the efficacy will last. As it has been shown in many studies, pesticides fail over a long period of time as pests stop showing much response in the long run. A study like this would help to assess the potential of popular insecticides on the market for prescribing the correct dose. A comparative resistance study will reveal which pesticides developed resistance first, which can also be used to compare the efficacy of the two drugs. The required dose providing half mortality, also called LC50, helps to know the concentration at which efficient control can be achieved. A "generation study," i.e., rearing insects for many generations inside a lab and testing insecticides on them, can aid in knowing how much the efficacy changes over generations. Molecular study of resistance will help to find the gene causing resistance. This can be further used in future studies to design chemicals or insecticides capable of counteracting the resistance *Spodoptera frugiperda*, also known as the fall armyworm, is a globally significant pest.

In a study conducted in Brazil, **Montezano et al., 2018** compiled a list of up to 353 plant hosts. During our field assessment, we discovered only maize fields to be significantly affected. Before conquering Africa and Asia in 2016 and 2018, the FAW was restricted to the American continent alone. **Deshmukh et al., 2018** reported *Spodoptera frugiperda* for the first time in India on maize in the state of Karnataka. This was the first record of a fall armyworm on the Asian continent. Subsequently, several reports of *S. frugiperda* emanated from different regions of India. Since its discovery in India in 2018, the insect has wreaked havoc in Gujarat. **Naganna et al., 2020** conducted research on the prevalence of FAW in

Junagadh. **Damasia et al., 2021**, detected fall armyworm in finger millet crops in the Dangs area of Gujarat. **Srikanth et al., 2018** reported the first incidence of the exotic pest fall armyworm on sugarcane outside of Gujarat in the southern Indian state of Tamil Nadu. **Babu et al., 2019** have discovered *Spodoptera frugiperda* in southwestern Rajasthan. In the Sangli District, **Chormule et al., 2019** detected FAW grazing on a two-month-old sugarcane crop (Co 86032) variety. Such incidents continued to occur, particularly in the country's maize-growing areas. **Padhee & Prasanna, 2019** analysed the instance of fall armyworm infestation in India, highlighting the countrywide spread. **Kumar et al., 2022**, described the present methods of regulating FAW in India. FAW's huge capacity is supported by various characteristics, including a strong dispersion capacity and a broad host range. Having previously worked in labs with various lepidopteran pests, we could also see the hyperactive behaviour of *S. frugiperda* in contrast to *S. litura*. **Haenniger et al., 2020**, reported that there was little variation in sexual communication between the maize and rice strains. Before beginning practical control, the status of any pest must be determined. Similar reviews of the state of pests have been done in the past, such as **Rao, 2020**, which investigated the situation of the pink bollworm on Bt in India. High FAW losses need the use of control mechanisms. According to **Harrison et al., 2019**, an FAW estimate of over US$13 billion in Africa drives farmers to use more and more pesticides as a preventative measure. A similar estimation was done previously by **Zalucki et al., 2012**, where an estimate of US$4 billion to US$5 billion is associated with the total costs of managing diamondback moths. The damage caused by insects must be quantified. **Groote et al., 2020**, projected agricultural losses in the Kenya area of Africa. This requires efficient management. We must be certain that such management may produce positive outcomes. The connection between yield and management should be examined. **Tambo et al., 2020** determined that FAW may be effectively managed, resulting in a substantial increase in crop productivity. Before performing research, the life cycle of any organism must be understood. Due to the duration of a pest's life cycle, it is crucial to have thorough knowledge about the pest. The FAW life cycle lasts around a month. It is essential to understand the whole life cycle of a pest in order to determine the different phases of management. **Sharanabasappa et al., 2018** examined the life cycle of FAW lab conditions at UAHS in Shivamogga, Karnataka. In our research, we also examined the length of the life cycle. The recent invasion and extensive damage to the India's agricultural fields by the FAW has demanded a thorough investigation of all feasible control measures. Numerous studies have been conducted on formerly existing agricultural pests in Gujarat, such as lepidopteran pests such as *Spodoptera litura* and *Plutella xylostella*. In 2018, the preliminary research of FAW began in Gujarat. In regions like Anand, Vadodara, Navsari, and Junagadh, work on the new

invasive species has subsequently commenced. Both biotic and abiotic variables influence the growth, development, and reproduction of insects. **Patel et al., 2020**, investigated the relationship between mango thrips and abiotic parameters in the Kesar mango plantation. In this research, we considered the most important biotic and abiotic parameters for FAWS rearing: temperature, humidity, and nutrition. There are several methods of pest management, including chemical control and biocontrol, among others. **Thumar et al., 2020** have also conducted field research on the chemical control of FAW using widely available pesticides. They conducted field tests using the insecticides chlorantraniliprole, emamectin benzoate, spinetoram, and thiodicard during Kharif. Various insecticides have also been used to combat other lepidopteran pests. **Bhut et al., 2022**, evaluated the effectiveness of chemical pesticides against two of the most significant castor pests, *Spodoptera litura* and *Achaea janata*. Combination insecticides may be used for control purposes. **Kamaraju et al., 2021**, used a mixture of neonicotinoids and pyrethroids against the rural malaria vector, *Anopheles culcifacies*. The use of pesticides against various pests was also evaluated. **Devashrayee et al., 2022**, investigated the effectiveness of many pesticides, including emamectin benzoate. There are two species of bean pod borer in India: *Helicoverpa armigera* and *Maruca vitrea*. New pesticides are beneficial, but their hazards must be assessed. **Paramasivam et al., 2022** Tamil Nadu, India, evaluated the risk assessment of chlorantraniliprole in chilli crops. Emamectin is efficient in eliminating other lepidopteran pests. **Singh et al., 2022**, examined the effectiveness of spinosad and emamectin benzoate against *Helicoverpa armigera* on tomato in Varanasi, U.P. Even though a significant amount of research has been conducted with chemical pesticides, a study using the technical grades of insecticides chlorantraniliprole and emamectin benzoate on the Gujarat FAW population is new; so, we conducted this investigation. Here, we also grew insects in the laboratory and tested them across many generations.

In addition to spraying pesticides on plants, other additional approaches for pest control have been investigated. Initial control may be accomplished by spraying pesticides on seeds. **Dobariya & Sisodiya, 2022** evaluated the efficacy of a pesticide as a seed treatment against fall armyworm. By enticing pests with baits containing toxic compounds, instantaneous extinction may be achieved. **Lunagariya et al., 2020**, undertook field trials for poison bait assessment against *Spodoptera frugiperda*. Other than chemical pesticides, even biopesticides may provide effective control. **Dhobi et al., 2020** put 2020-Bio pesticides to the test against autumn armyworm. No matter how effective insecticides are, they will ultimately fail. This is related to the problem of resistance formation. Resistance varies across crop genotypes, as shown

by **Subbireddy et al., 2018**'s evaluation of the resistance potential of numerous okra cultivars and genotypes.

For instance, plant growth regulators have the capacity to suppress pests. **Nagaratna et al., 2022,** conducted tests to ascertain the impact of PGRs and Si on FAW. FAW is also responsible for the devastation of sorghum crops. **Lad & Pawar, 2022**, assessed the effectiveness of pesticides against FAW in sorghum fields. Biocontrol agents are organisms that are capable of eliminating pests. **Aarthi et al., 2022**, examined the bioefficacy of biocontrol agents against several stages of the fall armyworm in the laboratory. In addition to chemical approaches, management includes monitoring, scouting, and mechanical control. **Verma et al., 2016** in Bihar and Uttar Pradesh proposed an environmentally friendly method for controlling the fall armyworm. By examining the resistant population, the molecular mechanism may be determined. There are two known strains of FAW, and their behaviour must be understood. Several substances are evaluated to generate a control. **Fernandes et al., 2018** evaluated the effectiveness of chemical insecticides for both standalone and combination chemicals. We evaluated the efficacy of chlorantraniliprole and emamectin benzoate against fall armyworm after many generations of laboratory breeding. There is observed development of resistance in insect pests in the past. One such pest is the diamondback moth, *Plutella xylostella* which has been known for the initial insect that developed resistance to *Bt.* **Liu & Tabashnik, 1997** saw DBM's increased resistance in Arizona to the Bacillus thuringiensis toxin Cry1C. **Boaventura et al., 2019** sought to determine the molecular mechanism using two resistant populations. Once the responsible gene has been identified, resistance may be overcome. Using CRISPR/Cas9 editing, **Kaduskar et al., 2022** produced knockdown resistance mutations in isogenic laboratory Drosophila strains. Resistance varies in various places. **Wang et al., 2022**, monitored the resilience of sixteen geographical populations in China in Beijing. The function of detox enzymes is well understood. **Li et al., 2022**, attempted to determine the purpose of GSTs in Henan, China. The resistant mutation does not impact the complete gene family. According to **Nauen & Denholm, 2005**, only a subset of P450 genes are associated with pesticide resistance. In our research, we have identified the numerous genes that confer pesticide resistance to the fall armyworm. 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 is required for a better understanding. In addition, the common new-generation pesticides and their long-term impacts must be thoroughly assessed. It was vital to analyse in depth the different elements of the fall armyworm and associated insects, such as their

existence, related pesticides, and resistance, which have been extensively examined. In order to test for any pesticide efficiency or in order to conduct any experimental work on the insect pests, we require huge numbers of them in laboratory. The various components of diet have different nutritional values. Some of these such as chickpea, wheat germ serves as the main carbohydrate provider in diet while formaldehyde, methyl-p-hydroxy benzoate, sorbic acid serve as antimicrobials and the yeast, becosule provide vitamins in the diet. Such a study would help to get good and effective culture of the two insect pests in lab for conducting experiments. Both the insecticides can provide control against fall armyworm. In our study, transcriptome profile revealed that as much as 464 genes were found to be upregulated and 607 genes to be downregulated in the resistant insect as compared to the control population. Some of the genes which showed differential expression included those like cytochrome P450s which are known to have causing detoxification and ultimately resistance. Genes like collagenase and cholinesterase 1-like were found to upregulated while genes like hemolin and basic juvenile hormone-suppressible protein 2 were found to be downregulated. The observations from various studies can be utilized for an effective pest management.

## CONCLUSION & SIGNIFICANT FINDINGS

1. *Spodoptera frugiperda* is a recent pest in India, first case was observed in 2018 in Karnataka. Now the pest, due to its high migration power, has captured most of the Indian states, including Gujarat, with the first case seen in Anand district in 2018.
2. There is a heavy infestation of fall armyworm in the agricultural fields of Vadodara. Mainly, FAW are infesting maize fields. We observed FAW in 2019 in and around agricultural fields in Vadodara.
3. Famers are facing problem in controlling the pest as the already available insecticides are ineffective in controlling it.
4. To find alternative solutions for the control of FAW, they need to be reared in huge numbers in laboratories so that many experiments can be conducted on them.
5. A comparative diet test was performed to determine which diet was superior for lab rearing. Although a natural diet is generally preferred for rearing, there are some challenges. It is difficult to maintain or obtain pesticide-free maize leaves (natural food) regularly. So an artificial diet is better, which can be prepared inside a lab with organic ingredients and the required nutrition.
6. When reared in lab conditions, both artificial and natural diets provided similar survival rates.

7. Among artificial diets, maize- and chickpea-based diets were more preferred by insects. However, we maintained and used a chickpea-based artificial diet as it was also showing good survival and there was easier availability of chickpea flour throughout the year.
8. When the efficacy of the two insecticides against the pest fall armyworm was tested, it was discovered that both were capable of controlling the pest at the optimum concentration.
9. One big problem with insecticide implementation in IPM programmes is that they develop quick resistance to insecticides.
10. Three cultures were maintained: a control (susceptible), chlorantraniliprole, and emamectin benzoate. Emamectin-treated insects of the fourth generation (4th instar),
11. The midguts of control and emamectin benzoate-treated larvae were extracted, and histology and differential gene expression were examined.
12. Control and resistant insects had similar structures, while freshly treated insects had more disruptive structures, according to histology analysis.
13. In DEGs, 464 genes were found to be upregulated and 607 genes to be downregulated when compared to the control population.

## Publication from the thesis- RJAS (UGC CARE Group 1, Web of Science)

*Res. Jr. of Agril. Sci.* **11**(6): 1426-1429, Nov-Dec 2020

ISSN: 0976-1675

www.rjas.org

**Research Journal of Agricultural Sciences**

© Centre for Advanced Research in Agricultural Sciences

**Short Communication**

DI: 6277-2707-283

# Comparative Analysis of the Life Cycle of Fall Armyworm *Spodoptera frugiperda* Smith, 1797 (Lepidoptera, Noctuidae) on Natural and Artificial Maize Diet under Laboratory Conditions

**Harshita Sharma and Dolly Kumar***

Division of Entomology, Department of Zoology,

Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara - 390 002, Gujarat, India

Received: 27 July 2020; Revised accepted: 08 September 2020

**Key words:** *Spodoptera frugiperda*, Control, Fall armyworm, Larvae, Maize diet, Pest

*Spodoptera frugiperda* is a serious pest of American origin present throughout the world. It is a polyphagous pest preferring maize, causes white elongated patches and windows on leaves and fecal pellets deposited in the whorls. The adult male has brown forewing with a white spot and triangular white patch while the female has plain greyish wings (Sharanabasappa *et al*. 2018). In India, the first report of the FAW came from Karnataka (Deshmukh *et al*. 2019). Other Indian states reported its presence like that of Maharashtra, Gujarat, Rajasthan, Tamil Nadu (Deshmukh *et al*. 2019) (Sisodiya *et al*. 2018, Babu *et al*. 2019, Srikanth *et al*. 2018). A genetic study from Africa and India revealed the possibility of a common source of invasion between the countries (Nagoshi *et al*. 2019). Regions where the worm is most damaging, yields increase by an insecticide application (Andrews 2014). There are two main types of FAW strain designated as corn and rice strain which are morphologically identical, so molecular techniques are used for identity (Nagoshi and Meagher 2004). It is important to know the biology of FAW to identify the life stages to control (Sharanabasappa *et al*. 2018). In Lepidopteran pest, *Plutella xylostella* a threat to crucifers, knowledge of the biology influences the host plant quality and helps in its management (Gowri and Manimegalai 2016). In Florida, field FAW strain was resistant to classes of insecticides including synthetic pyrethroids, organophosphate, and carbamates (Yu 1991). Alternative methods in the form of chemical insecticides, biologicals, botanicals for the control of FAW is a must. Such a study requires knowledge of its stages and its rearing to conduct experiments. The present study was undertaken to study the biology of *Spodoptera frugiperda* on two diets.

***Corresponding author:** Prof. Dolly Kumar, Head, Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara - 390 002, Gujarat

**e-mail:** dollymsu@gmail.com

*Collection*: A detailed survey of various agricultural fields in and around Vadodara was done. It was amongst the initial observation for the occurrence of fall armyworm from few agricultural fields of Vadodara in June 2019. The insects were collected from the agricultural fields where the attack of FAW was observed. Survey and collection revealed high infestation of FAW in maize fields of Vadodara (Fig 1-2). The stage selected for the collection was the larval stage (in caterpillar form). The identifying feature of the FAW caterpillar being the Y shaped white line on the front and the four dots which form a square at the posterior end. The colour is shades of brown with black lines on its body. The caterpillars were allowed to feed on the natural leaves after the collection was done.

Fig 1 Maize field in Channi region of Vadodara

Fig 2 Collection of fall Armyworm

*Rearing*: The insects were reared on a natural diet for a complete life cycle. The larvae were reared in insect culture trays containing the maize-based artificial diet and natural diet in separate trays closed with a lid and maintained at

## Publication from thesis IJER- (Web of Science, Clarivate analytics)

International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 29-05-2022, Accepted: 15-06-2022, Published: 01-07-2022
Volume 7, Issue 7, 2022, Page No. 1-5

# Standardizing the artificial diet and diet preference for lab rearing of *Spodoptera frugiperda* Smith, 1797 (Lepidoptera, Noctuidae)

**Harshita Sharma*, Dolly Kumar**
Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

**Abstract**
*Spodoptera frugiperda* Smith, 1797 (Lepidoptera, Noctuidae) was announced as an invasive species in India as recently as 2018. Commonly it is known by the name fall armyworm. It is causing nuisance because of its highly polyphagous nature. *S. frugiperda* is a pest of American origin migrating to other parts of the world including Africa and Asia. In recent years, this insect has invaded many states of India including Gujarat. Mostly chemical pesticides and a few biopesticides have been the most reliable means to control the pest. This has led to resistance development in this pest. The resistance related studies against fall armyworm has been studied in countries like the USA and Brazil. Before *Spodoptera frugiperda* becomes havoc in the agricultural fields and turns resistant to all available insecticides; evaluating the alternative methods, their efficiency to control the pest, resistance development against newer insecticides and its mechanism needs to be studied. This would require mass rearing of pests in the laboratory. Laboratory mass rearing requires ideal biotic and abiotic factors. The diet is the chief component for the rearing of pests. In this study, four artificial diets are analyzed and compared for efficient rearing of pests in the laboratory.

**Keywords:** diet, Gujarat, invasive, polyphagous, rearing

**Introduction**
*Spodoptera frugiperda* is a polyphagous insect and a problematic agricultural pest. *S. frugiperda* has a wide range of host crops which are of economic concern to us. This includes 353 plant species from 76 plant families having crops such as corn, tomato, millets, and potato. (Montezano *et al*, 2018) [8]. The fall armyworm, *Spodoptera frugiperda* is a lepidopteran insect, originating from America eliciting an exorbitant reproductive rate, throughout the year (Sparks, 1979) [13]. The incidences of sudden and severe outbreaks of fall armyworm populations from several West and Central African countries have been observed. This is due to the virtue of their vast dispersal and strong flying skills, while this is the first case of invasion outside the American continent (Goergen *et al*, 2016) [4]. For the first time in 2018, *Spodoptera frugiperda* has shown its occurrence in India in the state of Karnataka (Deshmukh *et al*, 2018) [3]. In China, this invasive pest was recently confirmed to be present, by using phylogenetic analysis of biological macromolecules (Jing *et al*., 2019) [6]. FAW invasion was also first reported from Gujarat maize fields in the Anand district (Sisodiya *et al*., 2018) [12]. In Karnataka, FAW infestation has already been reported on maize and paddy. Its biology has been studied there as well (Sharanabasappa *et al*, 2018) *Spodoptera frugiperda* invasion of sugarcane and other crops from Maharashtra was confirmed based on the male genital dissection of the insect (Chormule *et al*., 2019) [2]. In Rajasthan, the presence of FAW on maize has been marked (Babu *et al*., 2019) [1]. Amongst the major economically important crops produced in India, after wheat and rice, maize is the third most important cereal. An estimated loss of 200 million INR has been incurred, as reported from Mizoram in May 2019 after 122 districts with maize cultivation were infested by the fall armyworm. Further, *S. frugiperda* is shown to be resistant to commonly sprayed older classes of insecticides including carbamates, organophosphates and pyrethroids which lead to the failure of crops in Florida (Yu, 1991). The pest management due to these have become difficult since the most commonly used insecticides are unable in controlling it. Midgut and fat body tissues RNA Sequence analysis of another *Spodoptera* species i.e. *Spodoptera litura* suggested that the genes from these sites may play a substantial role in xenobiotic detoxification in these caterpillars (Li *et al*., 2019) [10].
Till now there is no certain solution for sustainable management of FAW in Africa or Asia (Padhee & Prasanna, 2019) [9]. Such a situation calls for experiments and research for alternate control methods. This will require the availability of a numerous insects which can be achieved by mass rearing. Artificial diets for insects are used as a medium for the effective rearing of pests in the lab. Studies related to diet also provide knowledge about the **insect's biology, behavior, and nutritional require**ments and such information are fundamental for the development of efficient Integrated Pest Management (Pinto *et al*., 2019) [10]. Devising a management strategy would involve a clear understanding of physiology, for which rearing them artificially in the lab is prescribed.

**Conference presentation from the thesis (Oral- 2nd position)**



# CERTIFICATE

4-6 January, 2020

National Symposium on
MULTILATERAL INITIATIVES AGAINST
ARBOVIRAL DISEASES

NSAD-2020

This Certificate is awarded to ..... HARSHITA SHARMA .....

of DIVISION OF ENTOMOLOGY, DEPT. OF ZOOLOGY, THE MAHARAJA SAYAJIRAO, VADODARA

in appreciation of his/her participation in National Symposium on MULTILATERAL INITIATIVES AGAINST ARBOVIRAL DISEASES (NSAD-2020).

~~He~~/She ~~Chaired~~ Session/~~Delivered~~ ~~Invited Talk~~/Presented Paper (Oral/~~Poster~~)/Attended the Conference.

II nd Position

Prof. Arti Prasad
Organizing Secretary

R S Yadav
Dr. Rajpal Yadav
WHO, Geneva

Organized by
Laboratory of Public Health Entomology
Department of Zoology, University College of Science
Mohanlal Sukhadia University, Udaipur (Raj.) 313001

**Conference- MLSU (Oral- 3rd position)**



DEPARTMENT OF ZOOLOGY

Mohanlal Sukhadia University, Udaipur (Raj.)

Two days International Virtual Conference

on

Present status and future trends in Entomological and Wildlife studies: An emerging platform for Zoologists (PENWI-21)

13-14 July, 2021

This certificate is awarded to Harshita Sharma, Ph.D. Scholar, Department of Zoology, The Maharaja Sayajirao University of Baroda in appreciation of his/her participation in two days International Virtual Conference on "Present status and future trends in Entomological and Wildlife studies: An emerging platform for Zoologists" organized by Department of Zoology, Mohanlal Sukhadia University, Udaipur (Raj.) from July 13 - 14, 2021. He/She attended the conference.

He/She has presented a research paper titled Investigating Insecticide Efficacy for the Control of Spodoptera frugiperda Smith, 1797 (Lepidoptera: Noctuidae) in the oral presentation. He/She is awarded Third position in oral presentation.

Dr. R. S. Sharma
Additional Director, National Disease Vector Control Program, New Delhi

Dr. Vijay Kumar Koli
Organizing Secretary

Prof. Arti Prasad
Convener

**Conference- BHU**

International Virtual Conference

on

Recent Trends in Animal Sciences

March 25th - 27th, 2022

Organized by: Ecophysiology Thrust Area, Centre of Advanced Study, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi - 221005, India

Certificate

This is to certify that **Ms. Harshita Sharma** participated in the conference as an oral presenter under the theme of **Entomology**.

The title of her presentation was **"Evaluating artificial diets for the laboratory rearing of insect *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae)".**

**Prof. M. Singaravel**
Convener

**Prof. S. Mittal**
Organizing Secretary

**International Seminar, Gorakhpur (U.P.)**



THREE DAY'S
INTERNATIONAL SEMINAR

CERTIFICATE

On the occasion of 106th Birth Anniversary of
Pt. Deen Dayal Upadhyaya
Rashtra Chetna Utsav
Vision-2047

This is to certify that Prof./Dr./Mr./Ms. Harshita Sharma
of M.S. University Vadodara has participated in the Three Day's International Seminar.
He/she has chaired a Session/Participated/Presented his/her paper on
"Insecticide Usage and Water Pollution"

Organising Secretary

Vice-Chancellor

24th-26th September, 2022

Organizer
DEEN DAYAL UPADHYAYA
GORAKHPUR UNIVERSITY
GORAKHPUR

www.ddugu.ac.i

**REFERENCES**


Aarthi, H., Tamboli, N., & More, S. (2022). Bioefficacy of bio-control agents against eggs , larvae and pupa of fall armyworm Spodoptera frugiperda (J . E . Smith) on maize under laboratory conditions. *The Pharma Innovation Journal*, *11*(4), 461–464.

Agarwal, M., & Garg, S. (2015). Study on Sub-Standard, Spurious / Counterfeit Pesticides in India. In *FICCI*.

Babu, R. S., Kalyan, R., Joshi, S., Balai, C., Mahla, M., & Rokadia, P. (2019). Report of an exotic invasive pest the fall armyworm, Spodoptera frugiperda. *Journal of Entomology and Zoology Studies*, *7*(3), 1296–1300.

Bhut, J., Khanpara, D., Bharadiya, A., & Madariya, R. (2022). Bio-Efficacy of Chemical Insecticides Against Defoliators Spodoptera litura and Achaea janata in Castor. *The Journal of Phytopharmacology*, *11*(5), 368–370.

Boaventura, D., Ulrich, J., Lueke, B., Bolzan, A., Okuma, D., Gutbrod, O., Geibel, S., Zeng, Q., Dourado, P. M., Martinelli, S., Flagel, L., Head, G., & Nauen, R. (2020). Molecular characterization of Cry1F resistance in fall armyworm , Spodoptera frugiperda from Brazil. *Insect Biochemistry and Molecular Biology*, *116*(2020), 103280.

Chormule, A., Shejawal, N., Deshmukh, S., Kalleshwaraswamy, C., Asokan, R., & Mahadeva Swamy, H. (2019). First report of the fall Armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera, Noctuidae) on sugarcane and other crops from Maharashtra, India. *Journal of Entomology and Zoology Studies*, *7*(1), 114–117.

Damasia, D. M., Pastagia, J. J., & Kachela, H. R. (2021). First report of the occurrence of fall armyworm , Spodoptera frugiperda (J E Smith) on finger millet (Eleusine coracana Gaertn) in Gujarat, India. *Indian Journal of Plant Protection*, *48*(4), 368–371.

Deshmukh, S., Kalleshwaraswamy, C. M., Asokan, R., & Maruthi, M. S. (2018). First report of the Fall armyworm, Spodoptera frugiperda (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on

maize in India. *Pest Management in Horticultural Ecosystems*, *24*(1), 23–29.

Devashrayee, V., Patel, D. R., & Sankhla, P. M. (2022). Efficacy of insecticides against pod borers of Indian bean. *Indian Journal of Entomology*, *2022*, 1–4. https://doi.org/10.55446/ije.2021.353

Dhobi, C. B., Zala, M. B., Verma, H. S., Sisodiya, D. B., Thumar, R. K., Patel, M. B., Patel, J. K., & Borad, P. K. (2020). Evaluation of Bio-pesticides against Fall Armyworm , Spodoptera frugiperda (J . E . Smith) in Maize. *International Journal of Current Microbiology and Applied Sciences*, *9*(8), 1150–1160.

Dobariya, U. R., & Sisodiya, D. B. (2022). Evaluation of insecticides as seed treatment against fall armyworm , Spodoptera frugiperda ( J . E . Smith ). *The Pharma Innovation Journal*, *11*(9), 1144–1148.

Fernandes, F. O., Abreu, J. A., Christ, L. M., & Rosa, A. P. S. A. (2018). Efficacy of Insecticides Against Spodoptera frugiperda (Smith, 1797). *Journal of Agricultural Science*, *11*(1), 494.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of the fall armyworm Spodoptera frugiperda (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE*, *11*(10), 1–9.

Groote, H. De, Kimenju, S. C., Munyua, B., Palmas, S., Kassie, M., & Bruce, A. (2020). Agriculture , Ecosystems and Environment Spread and impact of fall armyworm ( Spodoptera frugiperda J . E . Smith ) in maize production areas of Kenya. *Agriculture, Ecosystems and Environment*, *292*(July 2020), 106804.

Haenniger, S., Goergen, G., Akinbuluma, M. D., Kunert, M., Heckel, D. G., & Unbehend, M. (2020). Sexual communication of Spodoptera frugiperda from West Africa : Adaptation of an invasive species and implications for pest management. *Scientific Reports*, *10*(1), 1–9.

Harrison, R. D., Thierfelder, C., Baudron, F., Chinwada, P., Midega, C., Scha, U., & Berg, J. Van Den. (2019). Agro-ecological options for fall armyworm ( Spodoptera frugiperda JE Smith ) management : Providing low-cost , smallholder friendly solutions to an invasive pest. *Journal of*

*Enviroment Management*, *243*(May), 318–330.

Jing, D. P., Guo, J. F., Jiang, Y. Y., Zhao, J. Z., Sethi, A., He, K. L., & Wang, Z. Y. (2019). Initial detections and spread of invasive Spodoptera frugiperda in China and comparisons with other noctuid larvae in cornfields using molecular techniques. *Insect Science*, *2019*, 1–11.

Kaduskar, B., Kushwah, R. B. S., Auradkar, A., Guichard, A., Li, M., Bennett, J. B., Julio, A. H. F., Marshall, J. M., Montell, C., & Bier, E. (2022). Reversing insecticide resistance with allelic-drive in Drosophila melanogaster. *Nature Communications*, *13*(1), 1–8.

Kamaraju, R., Pant, C. S., Uragayala, S., Baharia, R. K., Srivastava, H. C., & Yadav, R. S. (2021). Small-scale field evaluation of the entomological efficacy and the residual activity of Fludora® Fusion WP-SB indoor residual spraying against Anopheles culicifacies s.l. in Gujarat, India. *Tropical Medicine and International Health*, *26*(4), 469–477.

Kumar, S., Naveen, S. B. S., Sekhar, K. J. C., & Nebapure, S. (2022). Insecticide susceptibility vis - à - vis molecular variations in geographical populations of fall armyworm , Spodoptera frugiperda (J . E . smith) in India. *3 Biotech*, *12*(9), 1–13.

Lad, D., & Pawar, G. (2022). Efficacy of different insecticides against larval population of Spodoptera frugiperda on rabi Jowar. *The Pharma Innovation Journal*, *11*(7), 1820–1822.

Li, D., Xu, L., Liu, H., Chen, X., & Zhou, L. (2022). Metabolism and antioxidant activity of SlGSTD1 in Spodoptera litura as a detoxification enzyme to pyrethroids. *Scientific Reports*, *12*(1), 1–9.

Liu, Y., & Tabashnik, B. E. (1997). Inheritance of Resistance to the Bacillus thuringiensis Toxin Cry1C in the Diamondback Moth. *Applied and Environmental Microbiology*, *63*(6), 2218–2223.

Lunagariya, M., Zala, M., Varma, H., Suthar, M., Patel, M., Patel, B., & Borad, P. (2020). Efficacy of poison baits against fall armyworm , Spodoptera frugiperda (J . E . Smith) infesting maize. *Journal of Entomology and Zoology Studies*, *8*(4), 2251–2256.

Montezano, D. G., Specht, A., Sosa-Gomez, D. G., Roque-Specth, V. F., & Sousa-Silva, J. C. (2018). Host Plants of Spodoptera frugiperda (Lepidoptera : Noctuidae) in the Americas. *African*

*Entomology*, *26*(2), 286–300.

Naganna, R., DM, J., JB, B., Wadaskar S, P., & Kachot, A. (2020). Present status of new invasive pest fall armyworm , Spodoptera frugiperda in India : A review. *Journal of Entomology and Zoology Studies*, *8*(2), 150–156.

Nagaratna, W., Kalleshwaraswamy, C. M., Dhananjaya, B. C., & Prakash, N. B. (2022). Effect of Silicon and Plant Growth Regulators on the Biology and Fitness of Fall Armyworm , Spodoptera frugiperda , a Recently Invaded Pest of Maize in India. *Silicon*, *14*(2022), 783–793.

Nauen, R., & Denholm, I. (2005). Resistance of Insect Pests to Neonicotinoid Insecticides : Current Status and Future Prospects. *Archives of Insect Biochemistry and Physiology*, *215*(58), 200–215.

Padhee, A., & Prasanna, B. (2019). The emerging threat of Fall Armyworm in India. *Indian Farming*, *69*(1), 51–54.

Paramasivam, M., Karthik, P., & Muralitharan, V. (2022). Dissipation, decontamination, dietary, and ecological risk assessment of chlorantraniliprole in chilli fields. *Toxicological and Environmental Chemistry*, *1*(1), 1–14.

Patel, P., Desai, C., & Usdadia, V. (2020). Correlation and regression of mango thrips (Scirtothrips dorsalis Hood ) in high-density mango plantation under South Gujarat conditions. *International Research Journal of Chemistry*, *2845*(2020), 1–7.

Pinto, J. R. L., Torres, A. F., Truzi, C. C., Vieira, N. F., Vacari, A. M., & De Bortoli, S. A. (2019). Artificial Corn-Based Diet for Rearing Spodoptera frugiperda (Lepidoptera: Noctuidae). *Journal of Insect Science*, *19*(4), 1–8. https://doi.org/10.1093/jisesa/iez052

Rao, G. M. V. P. (2020). Indian scenario on the occurrence of a dreaded insect pest Pink bollworm, Pectinophora gossypiella on Bt cotton-A review. *Journal of Environment Biology*, *41*(July), 840–844.

Scott, J. G. (1999). Cytochromes P450 and insecticide resistance. *Insect Biochemistry and Molecular Biology*, *29*(9), 757–777.

Sharanabasappa, Kalleshwaraswamy, C. M., Maruthi, M. S., & Pavithra, H. B. (2018). Biology of invasive fall army worm Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) on maize. *Indian Journal of Entomology*, *80*(3), 540–543.

Singh, K., Raju, S. V. S., & Sharma, K. R. (2022). Field efficacy of novel insecticides emamectin benzoate and spinosad against fruit borer, Helicoverpa armigera (Hübner) on tomato. *Journal of Entomological Research*, *46*(1), 106–110.

Sisodiya, D., Raghunandan, B., Bhatt, N., Verma, H., Shewale, C., Timbadiya, B., & Borad, P. (2018). The fall armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae); first report of new invasive pest in maize fields of Gujarat, India. *Journal of Entomology and Zoology Studies*, *6*(5), 2089–2091.

Sparks, A. N. (1979). A Review of the Biology of the Fall Armyworm. *The Florida Entomologist*, *62*(2), 82–87.

Srikanth, J., Geetha, N., Singaravelu, B., Ramasubramanian, T., Mahesh, P., Saravanan, L., Salin, K. P., Chitra, N., & Muthukumar, M. (2018). First report of occurrence of fall armyworm Spodoptera frugiperda in sugarcane from Tamil Nadu , India. *Journal of Sugarcane Research*, *8*(2), 195–202.

Subbireddy, K. B., Patel, H. P., Patel, N. B., & Bharpoda, T. M. (2018). Screening of okra cultivars and genotypes for their resistance to fruit borers in middle Gujarat. *Pest Management in Horticultural Ecosystems*, *24*(1), 36–43.

Tambo, J. A., Day, R. K., Lamontagne-godwin, J., Silvestri, S., Beseh, P. K., Oppong-mensah, B., Phiri, N. A., Tambo, J. A., Day, R. K., Lamontagne-godwin, J., & Silvestri, S. (2020). Tackling fall armyworm (Spodoptera frugiperda) outbreak in Africa : an analysis of farmers' control actions. *International Journal of Pest Management*, *66*(4), 298–310.

Thumar, R., Zala, M., Varma, H., Dhobi, C., Patel, B., & Patel, M. (2020). Evaluation of insecticides against fall armyworm , Spodoptera frugiperda ( J . E . Smith ) infesting maize. *International Journal of Chemical Studies*, *8*(4), 100–104.

Verma, R. D., Patel, V. K., Vijay, T. M., & Tripathi, A. K. (2016). Eco friendly management tools for an invasive pest species maize fall armyworm (FAW) Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae). In *Agriallis* (Vol. 4, Issue 6).

Wang, H. H., Zhao, R., Gao, J., Zhang, L., Zhang, S., Liang, P., Gao, X. W., & Gu, S. H. (2022). Genetic architecture and insecticide resistance in Chinese populations of Spodoptera frugiperda. *Journal of Pest Science*, *2022*, 1–16.

Wei, Y., Yan, R., Zhou, Q., Qiao, L., Zhu, G., & Chen, M. (2019). Monitoring and Mechanisms of Chlorantraniliprole Resistance in Chilo suppressalis (Lepidoptera: Crambidae) in China. *Journal of Economic Entomology*, *112*(3), 1348–1353.

Yu, S. J. (1991). Insecticide resistance in the fall armyworm, Spodoptera frugiperda (J. E. Smith). *Pesticide Biochemistry and Physiology*, *39*(1), 84–91.

Zalucki, M. P., Shabbir, A., Silva, R., Adamson, D., Shu-, L., Furlong, M. J., Zalucki, M. P., Shabbir, A., Silva, R., Adamson, D., & Shu-sheng, L. I. U. (2012). Estimating the Economic Cost of One of the World's Major Insect Pests , Plutella xylostella (Lepidoptera : Plutellidae): Just How Long is a Piece of String? *Journal of Economic Entomology*, *105*((4)), 1115–1129.

Zhang, G., & Zhang, W. (2018). Protein – protein interaction network analysis of insecticide resistance molecular mechanism in Drosophila melanogaster. *Insect Biochemistry and Molecular Biology*, *October*, 1–24.

Harshita Sharma
Candidate

Dr Dolly Kumar
Guide