Chapter 4

4. RESULTS

The increasing devastation caused by the new invasive insect species in India, *Spodoptera frugiperda* Smith, necessitates the search for reliable control methods in Vadodara's agricultural fields. Such a task can be done only after observing and knowing about the pest in its natural habitat. This was accomplished by going into fields where fall armyworm is a problem. Further, their natural behaviours in the field, like feeding, excreting, and crawling, were seen on the plants in the agricultural fields. After that, I collected the insects from the locations and brought them into the lab. The effective rearing method needs to be standardized to have a supply of the test insect constantly, for conducting various experiments to determine the control. Temperature, humidity, and diet must be optimal for insect growth and reproduction to have enough for testing. While abiotic factors such as temperature and humidity remained constant, diet evaluation was required. For that purpose, some diets were tested, and a good one from them was taken for further rearing and testing. A diet incorporation assay was used to test various concentrations of the two insecticides in the lab, and observations were taken at 24 and 72 hours. The testing was repeated until observations revealed a significant decrease in the expected mortality of the insect pest.

Meanwhile, the increased use of Emamectin Benzoate in the fields has raised concerns about pest resistance shortly. To that end, we attempted to identify the source of the resistance at the molecular level. The RNA sequencing was conducted by taking the midgut tissue of a control insect and a lab-tested Emamectin Benzoate-resistant insect. Differential gene expression was done to compare the two. The genes playing roles in this process of creating resistance can be used for further designing methods that target the culprits of the resistance mechanism.

4.1. RESULTS FROM THE FIELD SURVEY

Crop fields of Vadodara district were explored. Many crops of importance are grown in the around Vadodara's agricultural fields. The fields with crops grown there are mentioned (**Table 4.1**). Fields in various directions from the city were tried to be explored. Important pests included insect pests from various orders like Lepidoptera, Hemiptera, Coleoptera, and Orthoptera. The primary focus was, however, to find the damage by the pest *Spodoptera frugiperda* in the field. Damage was observed in the field. The field surveyed is shown **(Figure 4.1)**.

Table 4.1: Agriculture fields of Vadodara with location and crops are grown there

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

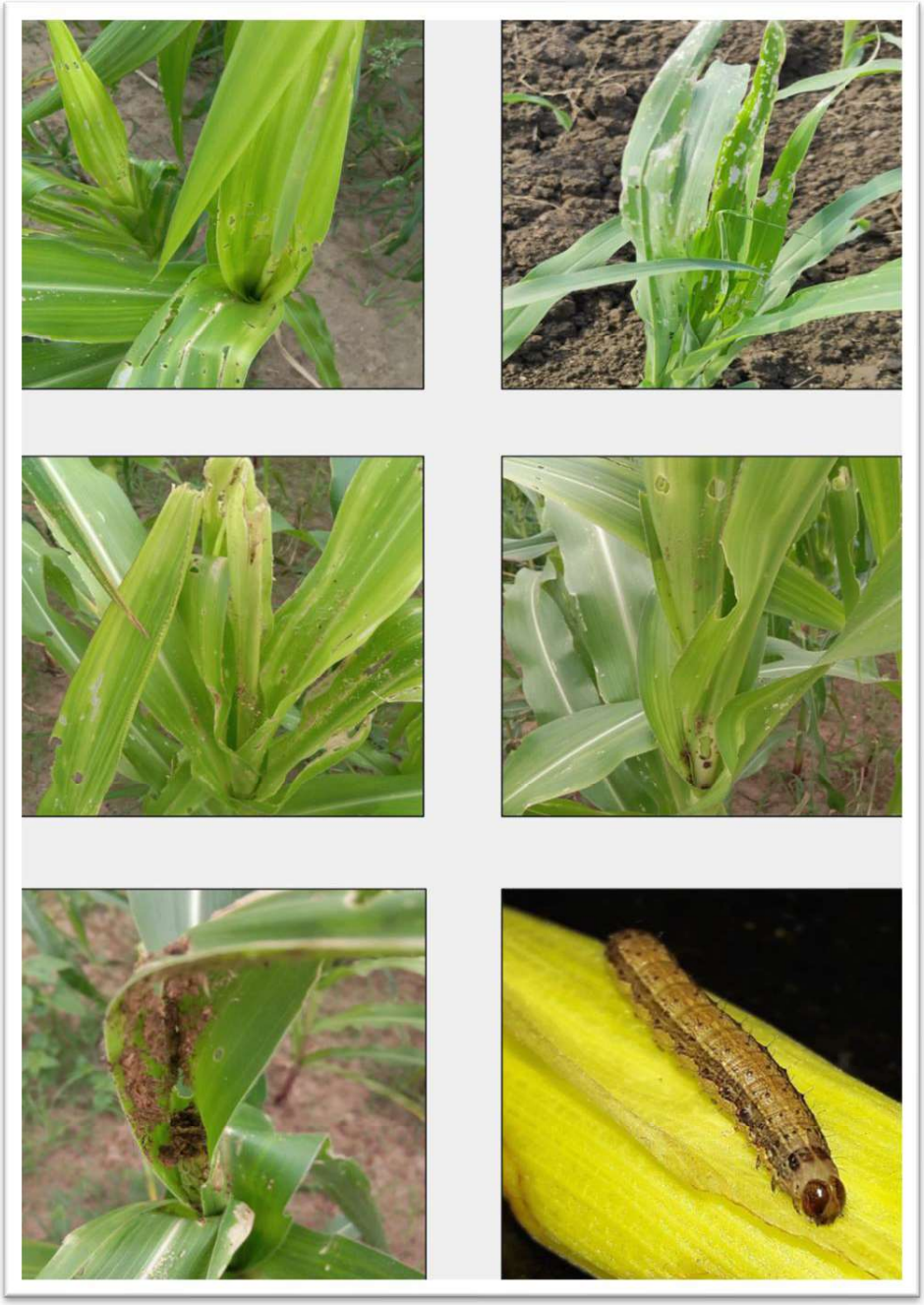


Figure 4.1: *S. frugiperda* infestation in maize fields (creating windows), Damage by FAW in Maize (complete holes), Excreta deposition and FAW on maize

The caterpillars from the agricultural fields were checked for their complete life cycle. Different stages and their duration were noted. Photography of different stages was done **(Figure 4.2)**. This was done to elicit information on the insect and check for its rearing inside lab conditions. The eggs were laid in masses multiple times, with each egg mass covered by scales in the early stage **(Figure 4.3)** and not in the later stage **(Figure 4.4)**. Immediately after hatching out of the egg, the neonate was fed on a diet where it grew till the last instar **(Figure 4.5)**, and then the larva converted into pupa and adult, as shown **(Figure 4.6)**.

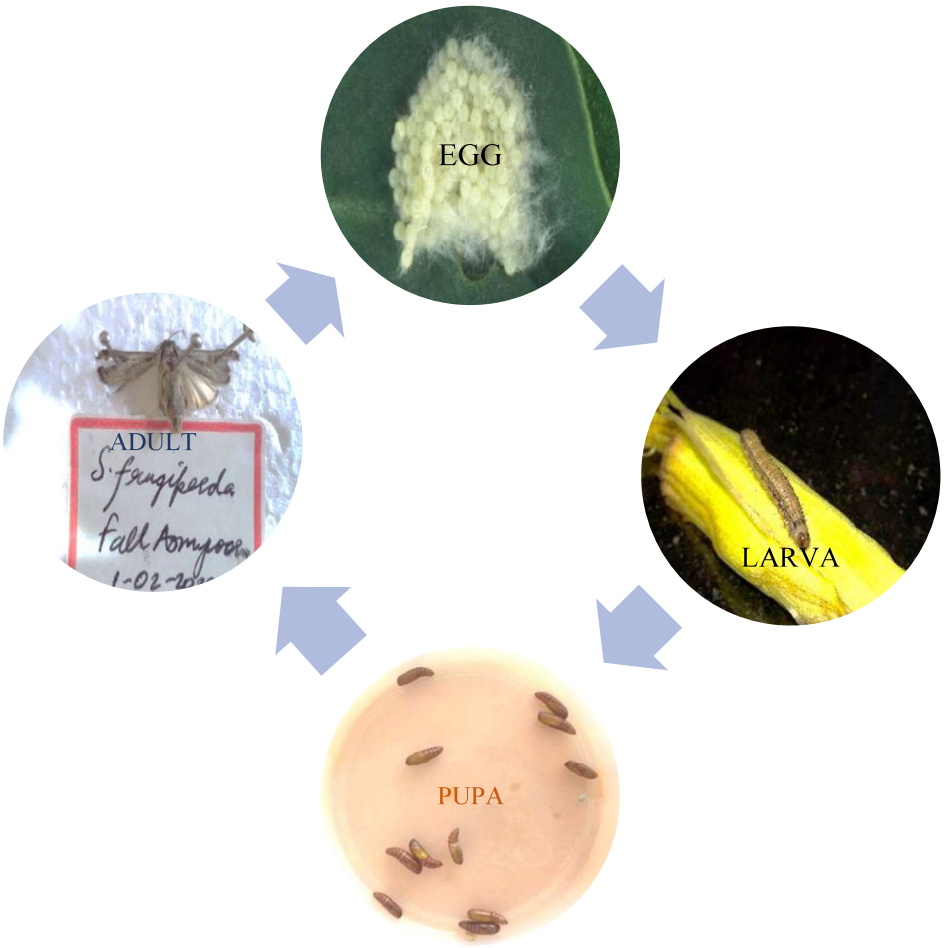


Figure 4.2: Life cycle of Fall Armyworm (*Spodoptera frugiperda*)

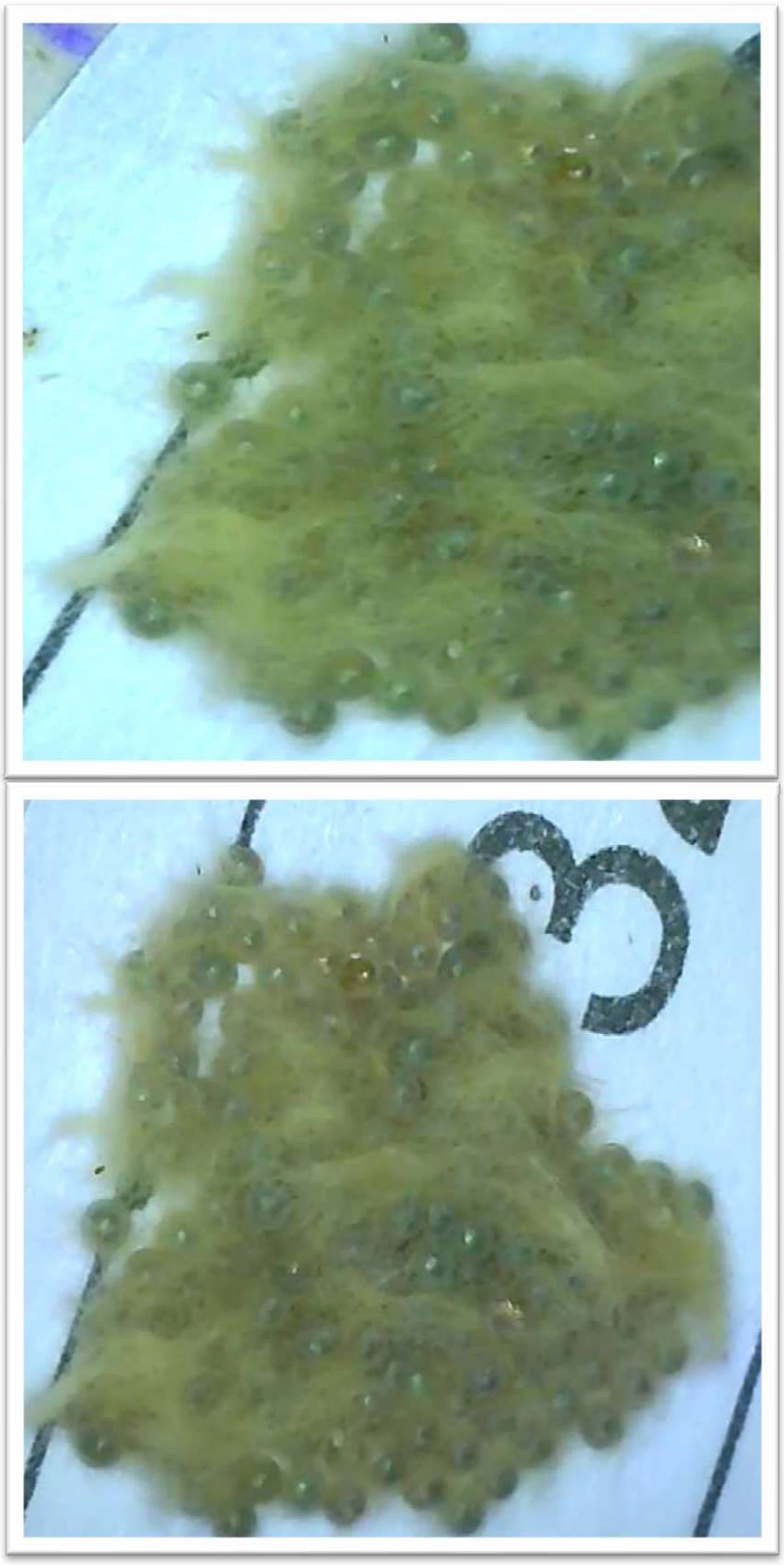


Figure 4.3: Egg mass with scales of *Spodoptera frugiperda* S. (4X)

Figure 4.4: Egg mass of *Spodoptera frugiperda* S (4X)

Figure 4.5: Magnified neonate/first instar (4X) and fully grown caterpillar (normal view)

Figure 4.6: Pupa and Adult of *S. frugiperda* (male)

Although various pests were found in the maize fields, the major damage caused in the maize fields was mainly by the fall armyworms. It was creating holes in the leaves during the early instar stage and making holes in the later stage. There was a deposition of excreta on the plant, mainly on the leaves and in the whorl. As the plant grows, the excreta deposition comes on the outer side. The excreta and the hole are the initial damage symptoms that can be used to identify fall armyworm infestation. The morphological features of the insect are the second stage of identification in the field; only after which can we confirm the presence of the pest. White colour Y-shaped front at the anterior end and four dots forming a square and a crescent shape at the posterior end are features unique to FAW caterpillars.

The life cycle of the fall armyworm inside the laboratory at maintained temperature and humidity lasted between 30 to 40 days, depending on the season. Around 100-200 eggs are present in an egg mass with scales. The neonate emerges from the egg in 3-5 days, initiating a larval period of six instars and every instar lasting about 2-3 days. The larva then converts into a pupa which remains in that stage for a week or so. Pupa converts into an adult, which can be male or female. Gender can be easily identified with the males having a prominent design of dark brown-black colour while females being plain greyish-brown.

Cannibalism: A major issue in the rearing and culturing of the *Spodoptera frugiperda,* is the cannibalistic behaviour of the pest. The behaviour is present in the larval stage of the insects. In the life cycle study, cannibalism was observed by the early larva on the eggs. Also, late larvae instars were observed eating smaller larvae when kept together.

Prevention from cannibalism: The cannibalistic behaviour hinders the experiment and affects the observation negatively. So, to prevent it, some measures were taken. Trays were ordered to be manufactured where different cells are present in each cell so that every cell can be used to inhabit a single larva. Stickers were made to cover the tray, which prevented the insect from escaping. Diet was also changed regularly.

4.2. RESULTS FROM THE DIET STUDIES

The per cent pupation of the two diets is shown in **Table 4.2**. The larval growth index is shown in **Table 4.3**. The **Table 4.4** shows descriptive statistics of diets that reveal average number of 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 economical for easy laboratory rearing of the pest inside the lab.

The table displays the observations for different diets on *Spodoptera frugiperda* survival. **(Table 4.5)**. The larval growth index was calculated **(Table 4.6)**. A graphical comparison between survival and completion of the life cycle between natural (red bars) and artificial diets (yellow bars) with various life stage duration **(Graph 4.1)** and various artificial diets has been made **(Graph 4.2)**

Table 4.2: Pupal percentage emerged from natural and artificial diet

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

Table 4.3: Larval Growth Index from natural and artificial diet

Diet	Per cent Pupation	Larval period	Larval Growth Index
1 (Natural)	85%	19.6	4.34
2 (Artificial)	95%	17.85	5.32

Larval Growth Index was calculated using the following formula: Percent pupation/ Larval period (days)

Table 4.4: Statistical analysis showing survival rate of different stages on two diets: natural & artificial

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

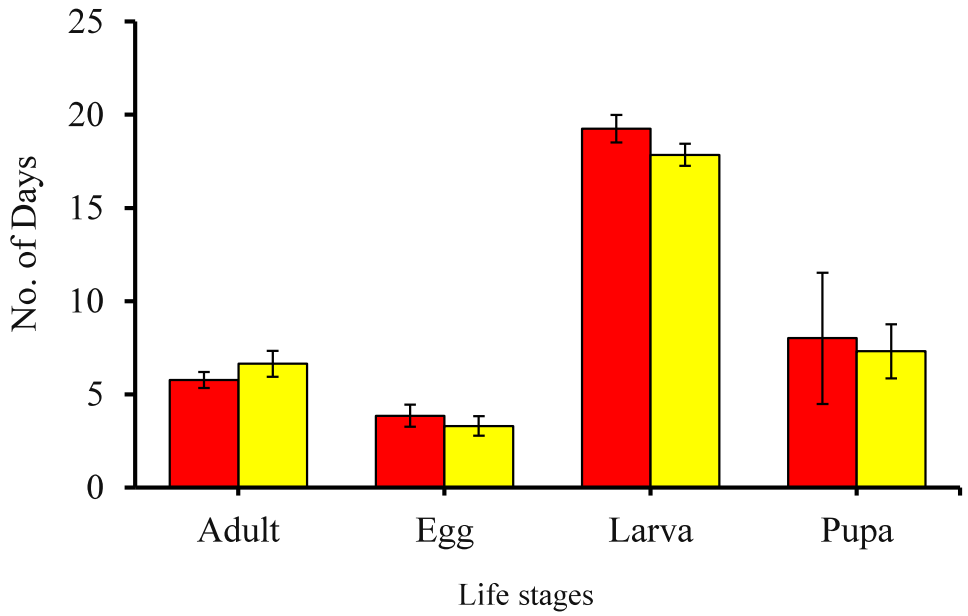
N= Number of individuals survived; N*= Number of individuals died

Table 4.5: Percent survival of *Spodoptera frugiperda* larva on diets

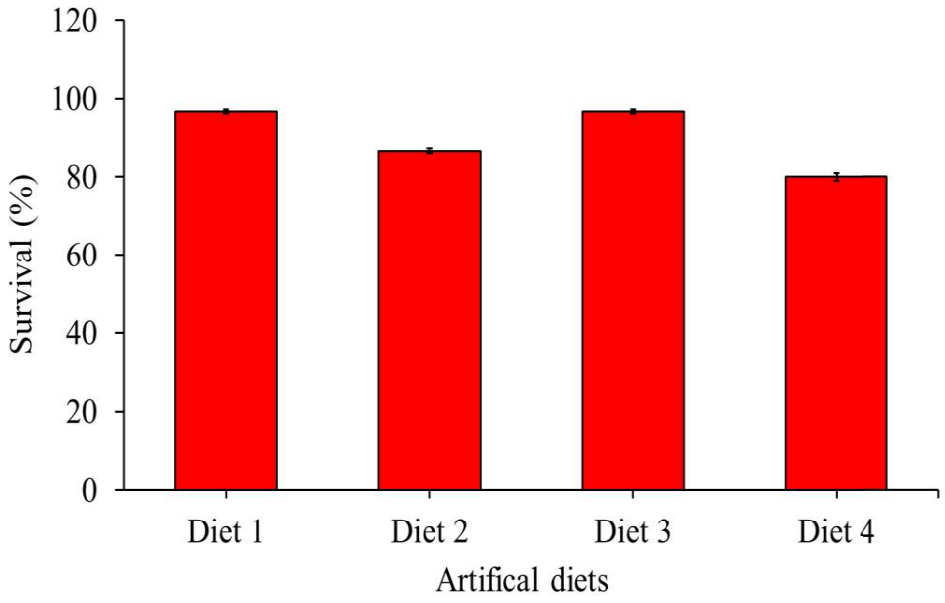
Diets	2nd instar larvae/ cell	Total larvae released	Total pupa formed	Per cent survival
1	1	30	29	96.66%
2	1	30	26	86.66%
3	1	30	29	96.66%
4	1	30	24	80.00%

Table 4.6: Assessment of larval growth index (in FAW) using four artificial diets

Diet	Larval period	Per cent survival	Larval Growth Index
1	14.61	96.66%	6.61
2	15.67	86.66%	5.53
3	14.52	96.66%	6.65
4	16.15	80.00%	4.95



Graph 4.1: Survival on natural & artificial diet



Graph 4.2: Survival percentage of larva on different artificial diets

Natural and artificial diets help in insect growth and life cycle completion. It is easy to rear plants on the natural diet (here, maize leaves/ plants) while doing field assays. However, it becomes difficult to have a natural diet in lab studies. A continuous natural food supply (here, fresh maize leaves are difficult). Also, keeping the insect on the natural diet would require changing and adding the diet multiple times daily. Even after that, the biggest challenge is ensuring a chemical-free natural diet, as most agricultural fields are spraying pesticides to prevent the crop from pests. Using the maize leaves coming directly from the field can give deceitful results as we cannot know the specific result of our test insecticides. Therefore, we tested artificial diets for the rearing of fall armyworms.

The criteria for the comparison of the diets were:

1. Percentage survival of larvae and pupae
2. Larval Growth Index

The per cent survival on the natural and artificial diet was close to being 85% and 95%, respectively. The larval growth index depends on persistence of larvae and the number of days it continued as larval instar. It came out to be 4.34 and 5.32 for natural and artificial diets, respectively. A higher value of LGI depicts an artificial diet suitable for rearing fall armyworm larvae.

A comparison of the four different artificial diets was made- 1, 2, 3, and 4 containing maize flour, soya flour, chickpea flour and jowar (sorghum) flour, respectively. The per cent survival was 96.66%, 86.66%, 96.66%, and 80.00% for diets 1, 2, 3, and 4. The LGI for diets 1, 2, 3, and 4 was 6.61, 5.53, 6.65, and 4.95, respectively. This suggests diets 1 and 3 give equal survival of the insects on them, whereas the LGI is similar or slightly more in the case of diet 3, followed by diet 1. Both diets 1 and 3 can be used for the rearing of the fall armyworm in the lab. However, having a supply of maize flour throughout the year is more convenient. A chickpea flour-based diet also acts as a good medium for rearing other Lepidoptera species, as done in the past. Throughout the study, from then on was done rearing the insect pest on a chickpea-based artificial diet.

4.3. RESULTS FROM THE INSECTICIDE EFFICACY

The bioassays performed on the pest *Spodoptera frugiperda* indicated efficacy in both the insecticides to control fall armyworm. The insecticides selected were Chlorantraniliprole and Emamectin Benzoate. Both insecticides were individually checked to assess 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. In order to prevent contamination, different instruments were utilised for treated and untreated insects. Any bacterial or fungal infections were examined in them. If a larva didn't move after coming into touch with the brush, it was thought to be dead. If a larva moved less erratically than an untreated larva, it was deemed to be moribund. A larva was deemed to be alive if, following exposure to a stimulus from the brush, it moved normally in comparison to an untreated larva (or any physical stimulus). To get the real data without outside interference, the appropriate temperature and humidity levels were maintained.
Data evaluation: LD_{50} was the concentration value where 50% of the population died. LD_{50} value for Chlorantraniliprole and Emamectin Benzoate was checked over generation and calculated with Probit analysis of SPSS software.

Insecticide Chlorantraniliprole (First generation):

The pest *Spodoptera frugiperda* was treated with Chlorantraniliprole at different concentrations. The concentrations, i.e., 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm, gave % mortalities as 100.00, 100.00, 100.00, 93.33, 60.00, 53.33, 46.66, and 20.00, respectively. No mortality in control was observed **(Table 4.7)**.

Insecticide Emamectin Benzoate (First generation):

The pest *Spodoptera frugiperda* was treated with Emamectin Benzoate at different concentrations. The concentrations, i.e., 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm, gave % mortalities as 100.00, 100.00, 93.33, 60.00, 46.66, 40.00, 10.00, and 0.00. No mortality in control was observed **(Table 4.8)**.

The surviving insects from the various dose ranges were taken to further generations and treated repeatedly on the third instar larvae until a prominent decline in expected mortality was observed. The statistics used was statistical software IBM SPSS software Version: 28.0.1.1(15)- Probit analysis, regression.

The surviving insects from the exposure to insecticide were cultured from further generations with testing and exposure to various doses on the third-fourth instar in every generation, similar to the first generation.

Insecticide Chlorantraniliprole (Second to Fourth generations):

The concentrations 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm gave % mortalities of 100, 100, 100, 93.33, 60.00, 50.00, 46.66, 16.67 in the second generation, 100.00, 93.33, 86.66, 60.00, 50.00, 46.66, 20.00, 10.00 in the third generation, and 100.00, 90.00, 60.00, 50.00, 46.66, 20.00, 0.00, 0.00 in the fourth generation.

Insecticide Emamectin Benzoate (Second to Fourth generations):

The concentrations 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm gave % mortalities of 100.00, 100.00, 90.00, 56.66, 50.00, 46.66, 33.33, 0.00 in the second generation, 100.00, 100.00, 86.00, 53.33, 43.33, 33.33, 20.00, 0.00 in the third generation, and 100.00, 86.00, 56.66, 46.66, 30.00, 16.66, 0.00, 0.00 in the fourth generation.

(Table 4.9-4.14).

Probit analysis using SPSS was done, with mortality values over the generations found.
In the case of Chlorantraniliprole, mortality values over the generation for 0.05 ppm concentration changed from 53.33% in G-1, 50.00% In G-2, 46.66% in G-3, to 20.00% in G-4 **(Table 4.15)**.
In the case of Emamectin Benzoate, mortality values over the generation for 0.1 ppm changed from 46.66% in G-1, 50.00% in G-2, 43.33% in G-3, to 30.00% in G-4 **(Table 4.16)**. The various results came from SPPS regression probit analysis.

Table 4.7: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (1st generation)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4.8: Larval (%) mortality obtained in *S. frugiperda* against Emamectin Benzoate (1st gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4.9: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (2nd gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	0.05	30	14	1	15	50.00
7	0.02	30	13	1	14	46.66
8	0.01	30	3	2	5	16.67
9	Control	30	0	0	0	0.00

Table 4.10: Larval (%) mortality obtained in *S. frugiperda* against Emamectin B. (2nd gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4.11: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (3rd gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4.12: Larval (%) mortality obtained in *S. frugiperda* against Emamectin B. (3rd gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4.13: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (4th gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4. 14: Larval (%) mortality obtained in *S. frugiperda* against Emamectin B. (4th gen)

Sets of conc.	Dose (ppm)	No. of larvae treated	No. of larvae succumbed to the dose	Defunct larvae	Total (Succumbed+ Defunct)	Larval percent 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	30	0	0	0	0.00

Table 4.15: Mortality of *S. frugiperda* larvae against Chlorantraniliprole over generations (G 1-4)

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

Table 4.16: Mortality values of *S. frugiperda* larvae against Emamectin Benzoate over generations (G1-4)

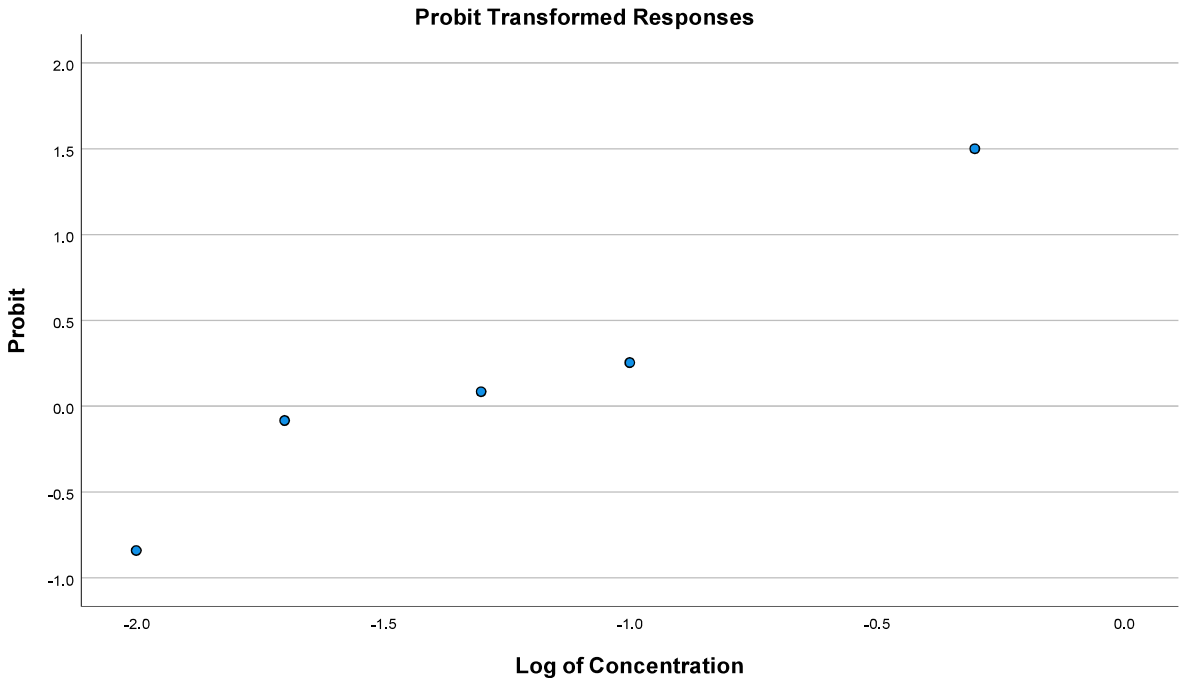
Concentration (ppm)	G-1	G-2	G-3	G-4
10	100.00	100.00	100.00	100.00
5	100.00	100.00	100.00	86.00
1	93.33	90.00	86.00	56.66
0.5	60.00	56.66	53.33	46.66
0.1	46.66	50.00	43.33	30.00
0.05	40.00	46.66	33.33	16.66
0.02	10.00	33.33	20.00	0.00
0.01	0.00	0.00	0.00	0.00
Control (or Untreated)	0.00	0.00	0.00	0.00

Parameter estimates and probit-transformed responses were noted. Estimates for Chlorantraniliprole generation 1st (C G-1) to Chlorantraniliprole generation 4th (C G-4) and Emamectin Benzoate generation 1st (EM G-1) to Emamectin Benzoate generation 4th (EM G-4) is shown **(Table 4.17-4.24)**. Probit-transformed responses in every generation for both insecticide is depicted graphically **(Graph 4.3-4.10)**.

Table 4.17: Probit analysis using software SPSS for chlorantraniliprole-generation 1

Parameter Estimate			Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.340	.182	7.370	<.001	.984	1.696
	Intercept	1.900	.256	7.422	<.001	1.644	2.156

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

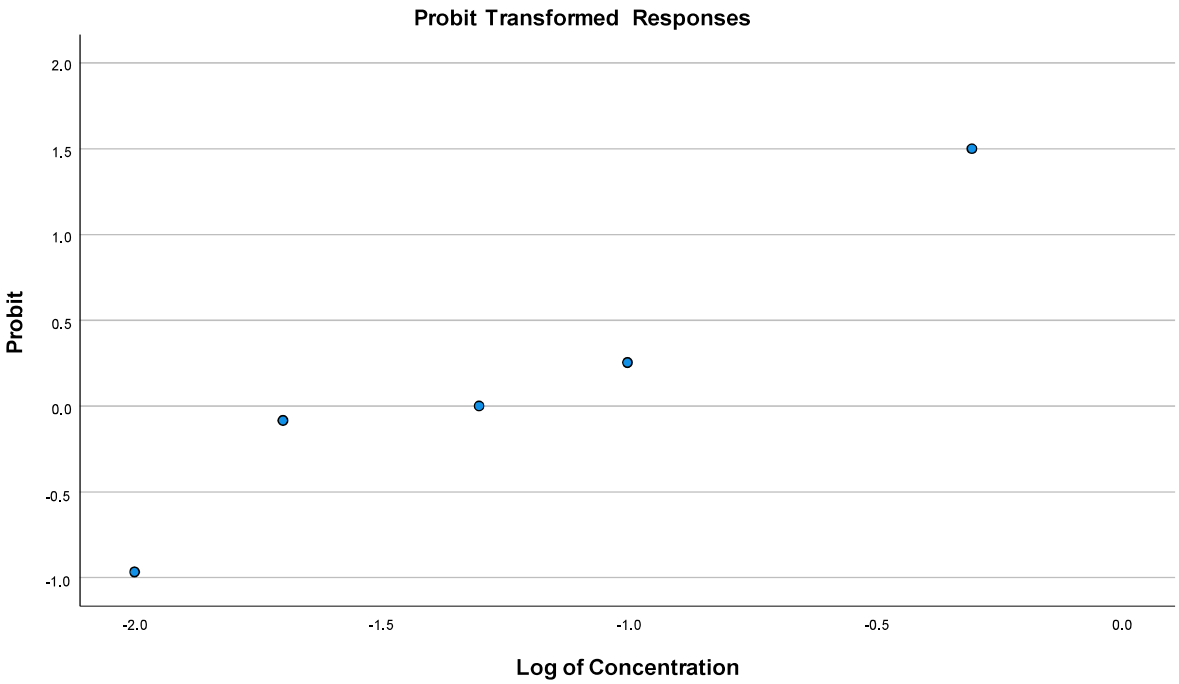


Graph 4.3: Probit response for Chlorantraniliprole (1st gen)

Table 4.18: Probit analysis using software SPSS for chlorantraniliprole-generation 2

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.384	.185	7.479	<.001	1.022	1.747
	Intercept	1.916	.259	7.386	<.001	1.656	2.175

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

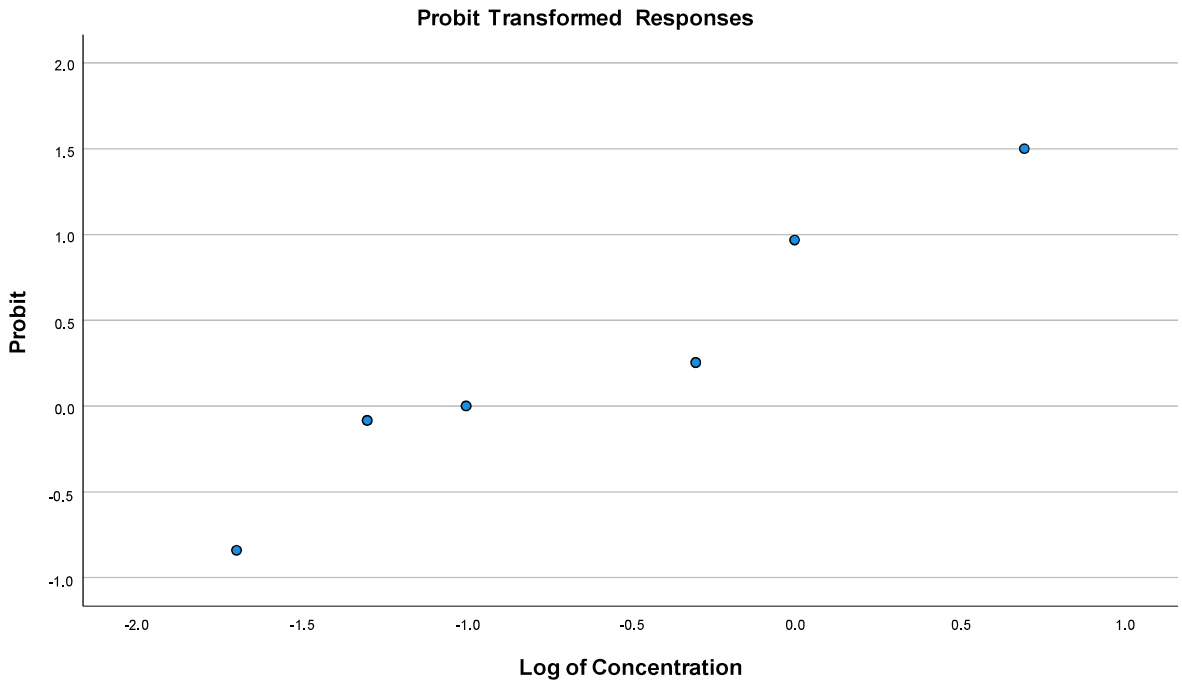


Graph 4.4: Probit response for Chlorantraniliprole (2nd gen)

Table 4.19: Probit analysis using software SPSS for chlorantraniliprole-generation 3

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.088	.121	9.030	<.001	.852	1.324
	Intercept	.929	.139	6.700	<.001	.790	1.068

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

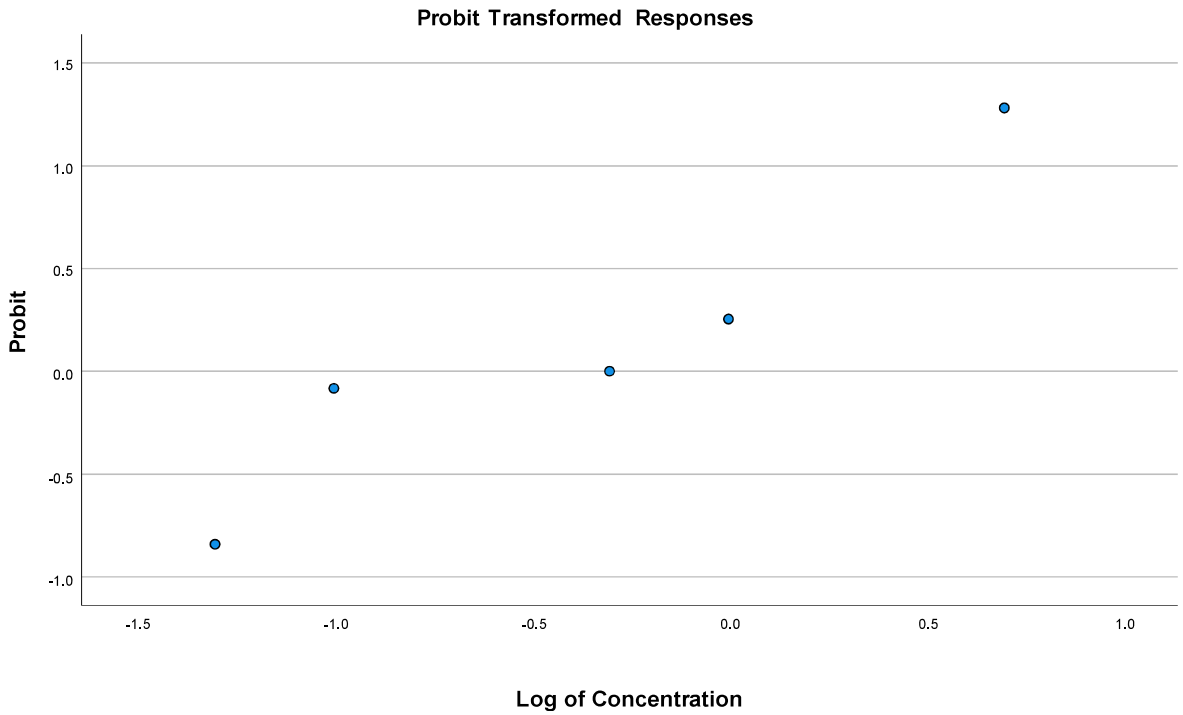


Graph 4.5: Probit response for Chlorantraniliprole (3rd gen)

Table 4.20: Probit analysis using software SPSS for chlorantraniliprole-generation 4

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.209	.127	9.527	<.001	.960	1.457
	Intercept	.545	.123	4.428	<.001	.422	.668

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

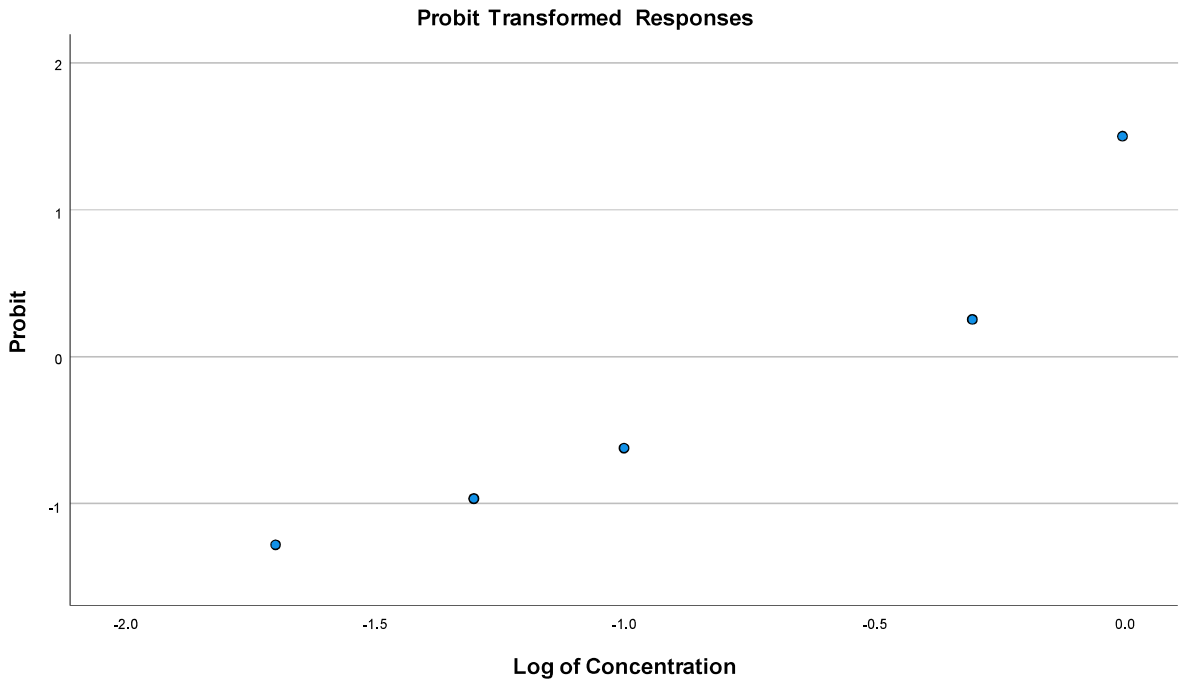


Graph 4.6: Probit response for Chlorantraniliprole (4th gen)

Table 4.21: Probit analysis using software SPSS for emamectin benzoate-generation 1

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.634	.175	9.346	<.001	1.291	1.976
	Intercept	1.104	.178	6.198	<.001	.925	1.282

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

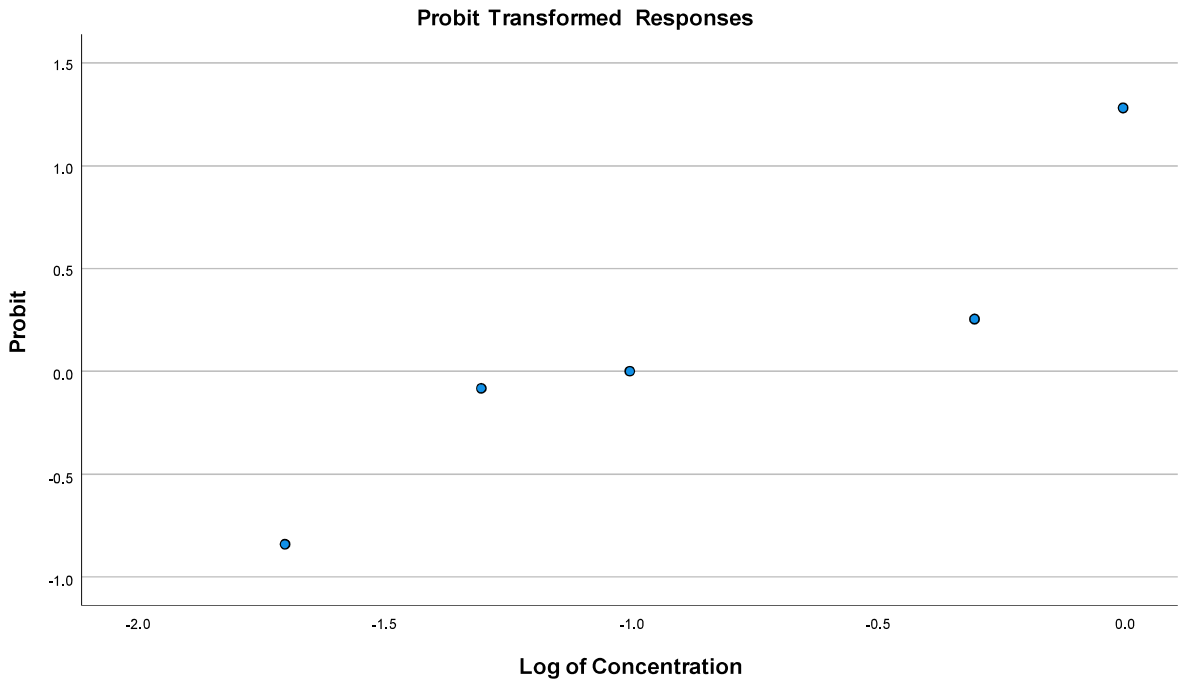


Graph 4.7: Probit response for Emamectin Benzoate (1st gen)

Table 4.22: Probit analysis using software SPSS for emamectin benzoate-generation 2

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.262	.141	8.968	<.001	.987	1.538
	Intercept	1.164	.165	7.055	<.001	.999	1.329

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

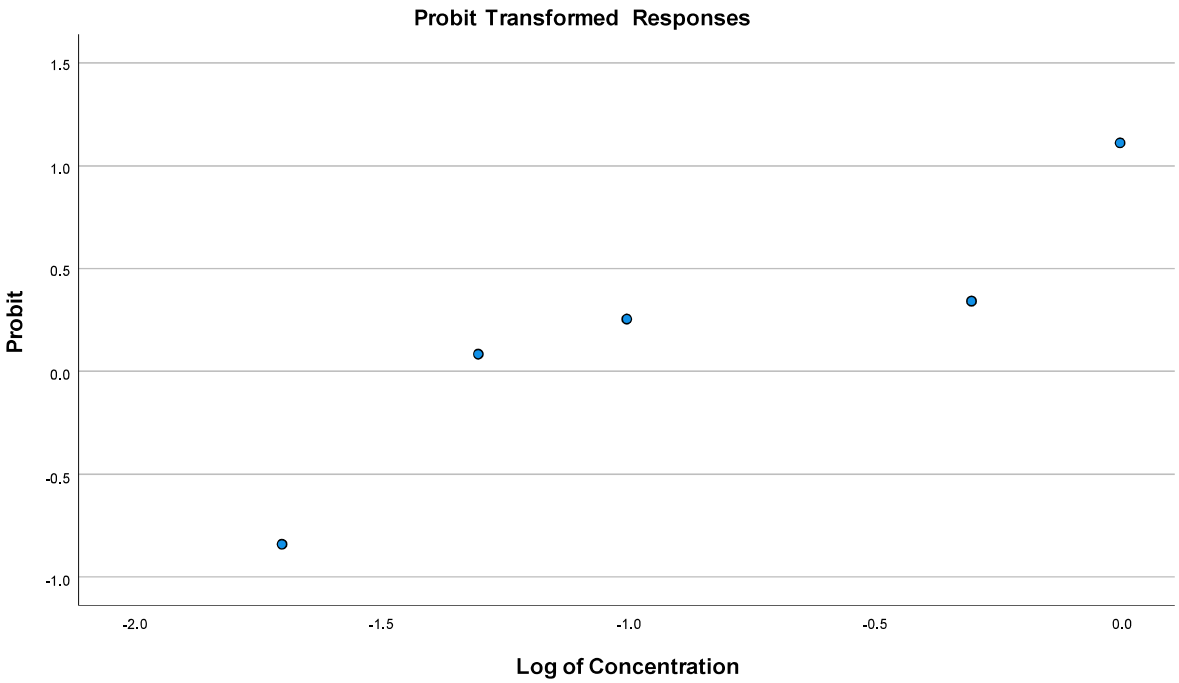


Graph 4.8: Probit response for Emamectin Benzoate (2nd gen)

Table 4.23: Probit analysis using software SPSS for emamectin benzoate-generation 3

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.218	.139	8.790	<.001	.946	1.489
	Intercept	1.207	.165	7.302	<.001	1.042	1.373

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)

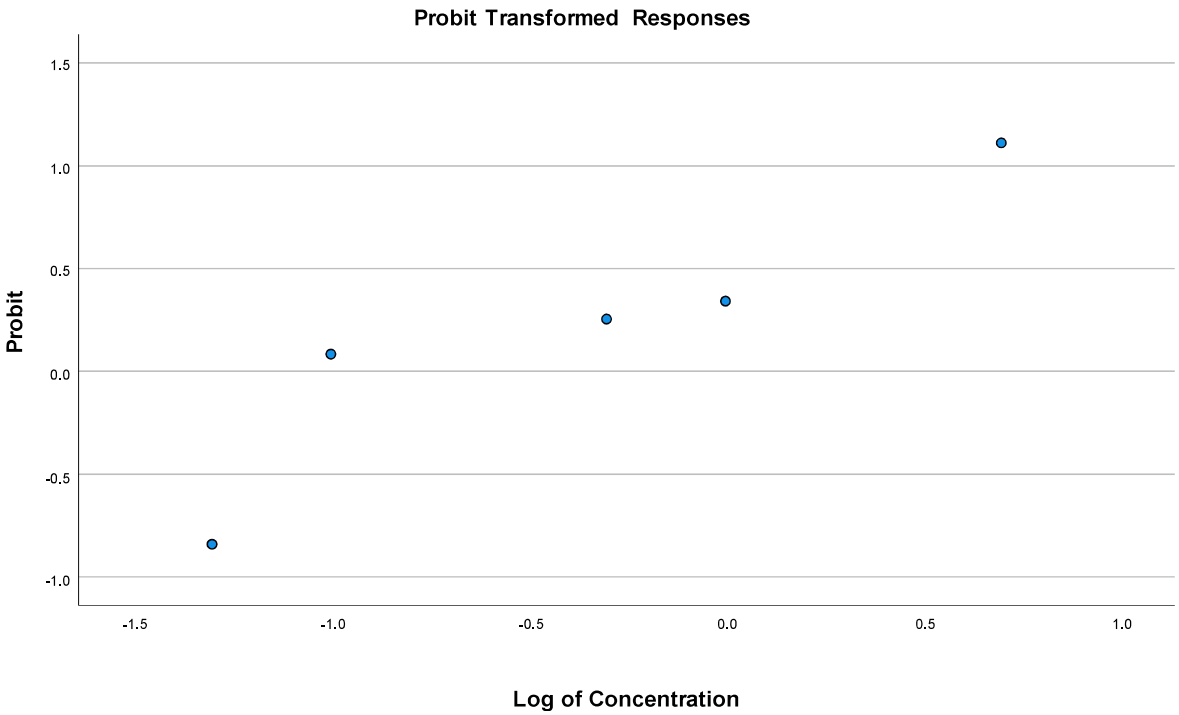


Graph 4.9: Probit response for Emamectin Benzoate (3rd gen)

Table 4.24: Probit analysis using software SPSS for emamectin benzoate-generation 4

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	1.178	.124	9.527	<.001	.936	1.421
	Intercept	.611	.124	4.939	<.001	.487	.734

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000logarithms.)



Graph 4.10: Probit response for Emamectin Benzoate (4th gen)

Behavioural Changes

The following change was observed in the 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, and 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

We observed that a low quantity of both insecticides is sufficient to cause death to the insects. It shows the effectiveness of the chemicals in controlling the pest. A very low dose of 0.05 ppm of Chlorantraniliprole caused the death of nearly fifty Percent of the test insect in the first generation. A dose as low as 0.1 ppm of Emamectin Benzoate caused mortality of about fifty Percent of test insects.

Although a lesser concentration of Chlorantraniliprole than Emamectin Benzoate was sufficient to kill the insect pest, Emamectin Benzoate was selected for the detailed resistance analysis. The reason behind the same is the enormous increment observed in the usage of Emamectin Benzoate in the field in recent years for controlling fall armyworm. This can have repercussions shortly as the pest might develop resistance against it.

4.4. RESULTS FROM THE HISTOLOGY

The histology of *Spodoptera frugiperda* midgut was observed in a brightfield microscope- DM 750, Leica. Photography was done for all three types of midguts- control (susceptible), treated/tested (dosing and observing after 72 hrs.) and resistant (Emamectin-resistant insect). Observations were taken at three magnifications, namely, 10X, 20X and 100X as 1, 2, and 3.

Control: *S. frugiperda*'s midgut had an epithelial layer, and the digestive cells' cytoplasm had 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 its basal surface **(Figure 4.7 A, B, C)**.

Resistant: The midgut region had fewer deformities observed than the treated ones. The structure seemed largely intact. The longer and more regular exposure to the insecticide might have played a role in keeping the structure more stable than the initial exposure. However, little vacuolization was observed with a slight deformation in shape **(Figure 4.7 D, E, F)**.

As the high visibility of various parts of the three types was seen at 100X magnification, these were analyzed and compared at this magnification. The images have been labelled as L for lumen, Ep for epithelium, P for peritrophic matrix, B for basal membrane, C for cytoplasm, N for nucleus, V for vacuole, L for loss of l

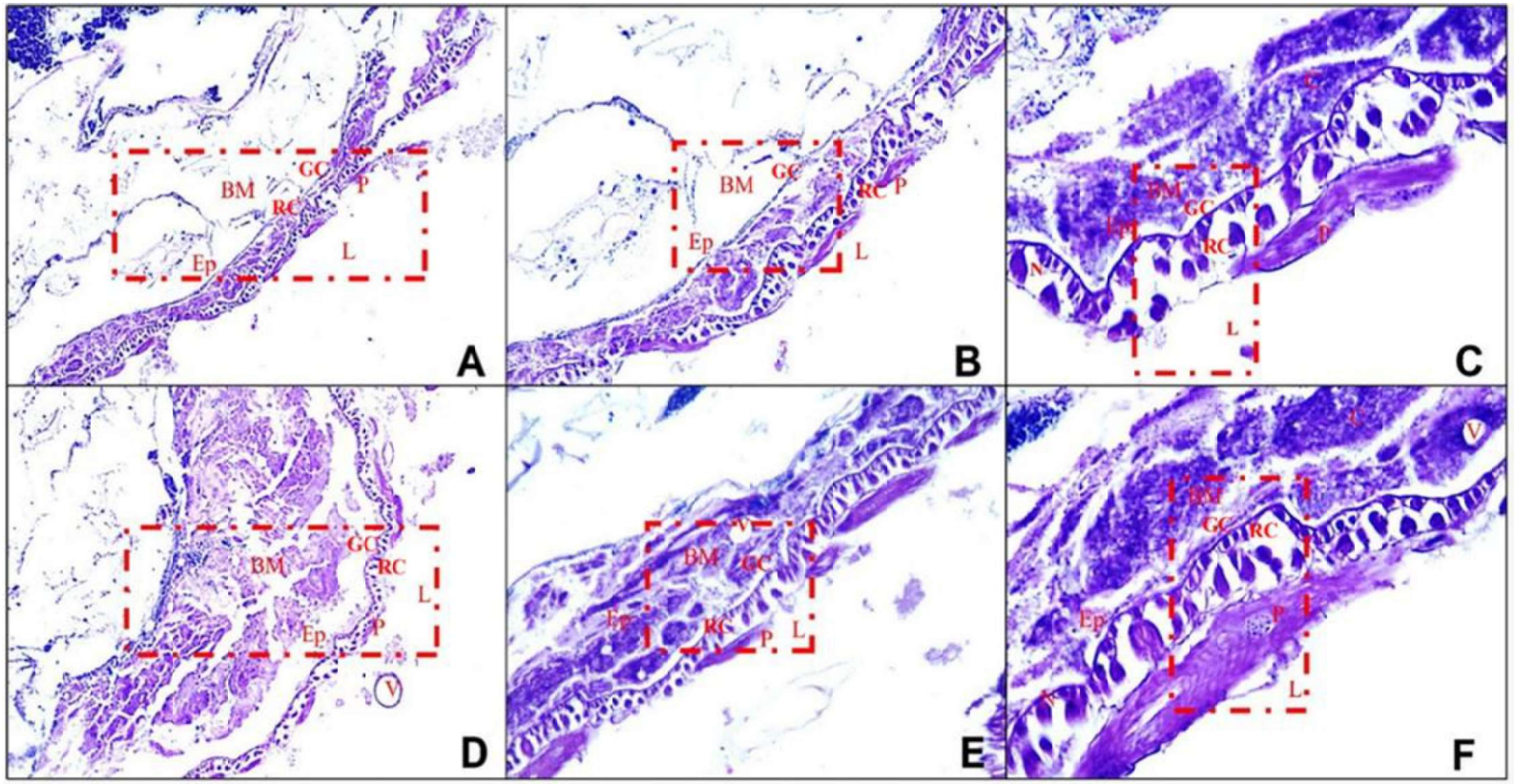


Figure 4.7: Histological slides of A: Control slide at 10X. Midgut section of 4th instar larva of susceptible/control population which were reared for more than three generation inside the lab without any exposure to insecticides); B: Control midgut sample at 20X. (4th instar larva of susceptible/control population which were reared for more than three generation inside the lab without any exposure to insecticides); C: Control midgut sample at 100X (4th instar larva of susceptible/control population which were reared for more than three generation inside the lab without any exposure to insecticides); D: Resistant slide at 10X. Midgut section of 4th instar larva exposed to 0.1 ppm concentration for four generations (Table 14); E: Resistant midgut sample at 20X (4th instar larva exposed to 0.1 ppm concentration for four generations); F: Resistant at 100X (4th instar larva exposed to 0.1 ppm concentration for four generations); [L=lumen, GC= goblet cell, RC= regenerative cell, Ep=epithelium, P=peritrophic matrix, B=basal membrane, C=cytoplasm, N=nucleus, V=vacuole]

4.5. RESULTS FROM THE TRANSCRIPTOME STUDIES

Midgut

There have been many studies from the past wherein midgut has been extracted from the caterpillars for conducting studies related to histology and transcriptomics.

A study on another noctuidae pest, *Spodoptera litura,* used midgut and fat bodies for RNA sequencing. They found that genes from these sites provide 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.

Transcriptome Analysis

We must clearly understand the transcriptome to elucidate the working components of the genetic data, expose the minute components of cells and tissues. The total set of transcripts present in a cell for a particular developmental stage or physiological condition is known as the transcriptome **(Wang et al., 2010)**.

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

- Differential gene expression (DGE) analysis compares control and treatment groups by sensitively quantifying transcriptional activity and gene expression levels. Wide-ranging analysis - identification of more differentially expressed genes with a larger fold change
- It may also reveal some uncharacterized, unidentified genes. Finding both well-known and new transcripts
- Almost any organism's RNA transcripts can be profiled. A comprehensive representation of the transcriptome, or every gene
- A complete set of genes working inside can be known at once

This study assembled the transcriptome using approximately 20-25 million paired-end reads. Sequencing of cDNA libraries in the Illumina Novaseq 6000. The process started with RNA isolation from the extracted tissue, which was done by the trizol method.

RNA was extracted, and its quality checked and its quality checked (**Table 4.25**). The difference between the gene expression patterns of the two samples was noted. One sample was from the susceptible population, made through several generations of rearing inside laboratories without insecticide exposure. Another sample was the fourth generation of Emamectin Benzoate, surviving even after insecticide exposure.

***Quality check*:** The RNA after extraction was checked for its quality. A quality check (QC) was done **(Table 4.25)**. Ratio A260/280 as well as the RIN values. The absorbance ratio of 260 by 280 was used to check the purity of RNA. All the samples gave 260/280 values around 2 which is satisfactory for RNA purity. A higher RIN value (score between 1 and 10) indicates higher RNA integrity. All the values of our samples were between 9 and 10 indicating an excellent-quality RNA sample. The samples were considered to be optimum for taking further for analysis.

There were two types of RNA samples **(Table 4.26)**. One being the midgut-control and the other being the midgut-treated (Emamectin Benzoate). These were taken for RNA-seq Analysis. The RNA is converted into cDNA by reverse transcription. Sample QC raw and trimmed data statistics were conducted **(Table 4.27)**. Alignment Statistics of Hisat2 gave a good quality alignment rate above 90% **(Table 4.28)**. Following that, libraries were prepared. Gene expression profiling by RNA-Seq was done. The upregulated and downregulated genes in the resistant population compared to the susceptible population were observed **(Table 4.29)**.

Table 4.25: RNA Quality check for the samples

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 4.26: Sample Overview

Sample Name	Library type	Number of samples	Approach used
MGC (Mid Gut Control) MGT (Mid Gut Treated)	Paired-end	2	RNA-Seq Analysis

Table 4.27: Sample QC Statistics of raw and trimmed data

Sample Name	Read Orientation	Raw Data			Trimmed Reads		
		Average GC (%)	Length (bp)	Total Sequences (Millions)	Average GC (%)	Length (bp)	Total Sequences (Millions)
MGC	**R1**	52	159	20.23	52	152	10.16
	R2	50	159	20.23	52	154	10.16
MGT	**R1**	49	159	24.22	49	152	11.80
	R2	48	159	24.22	49	152	11.80

Table 4.28: Hisat2 Alignment Statistics against *Spodoptera frugiperda*

Samples	Aligned concordantly exactly 1 time		Overall Alignment Rate
	Count	%	%
MGC	2835994	27.91	91.42
MGT	3577117	30.32	90.25

Table 4.29: Upregulated and downregulated count based on p-value (unadjusted value), FDR (adjusted p-value) and Log2FoldChange

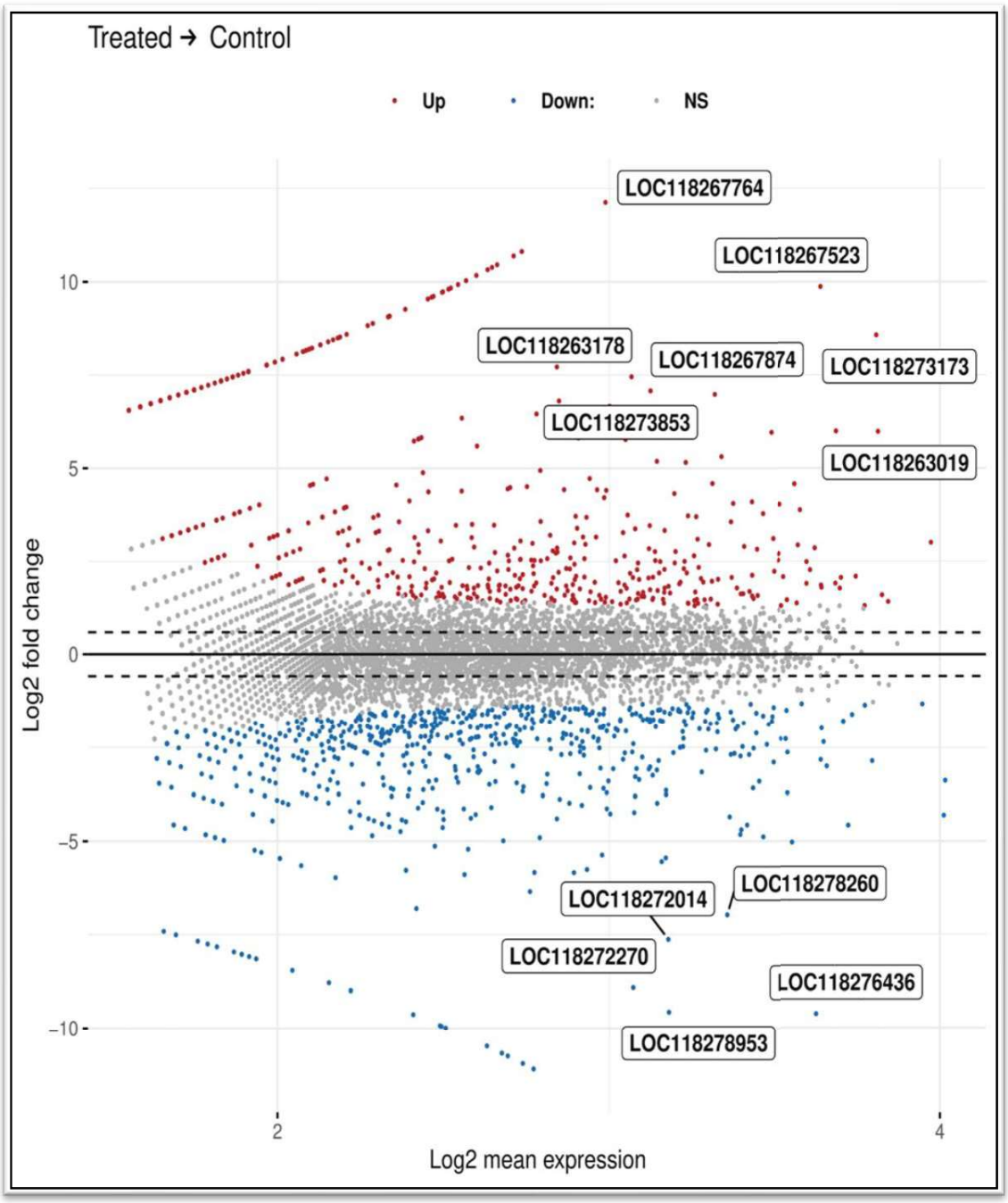
Filter Parameters	Treated vs Control	
	Up	**Down**
P Value <= 0.05 & log2FC ∓ 1.5	464	607

464 genes were upregulated and 607 were downregulated in treated (Emamectin) compared to control (susceptible) insects. It includes various categories of genes performing important functions inside the insect's body. This difference in the profile between the control and treated sample serves as a basis for comparison of the two. It helps to find out the reasons helping the insect to increase its tolerance to the lethal chemical. Further, these upregulated and downregulated genes are analyzed and discussed.

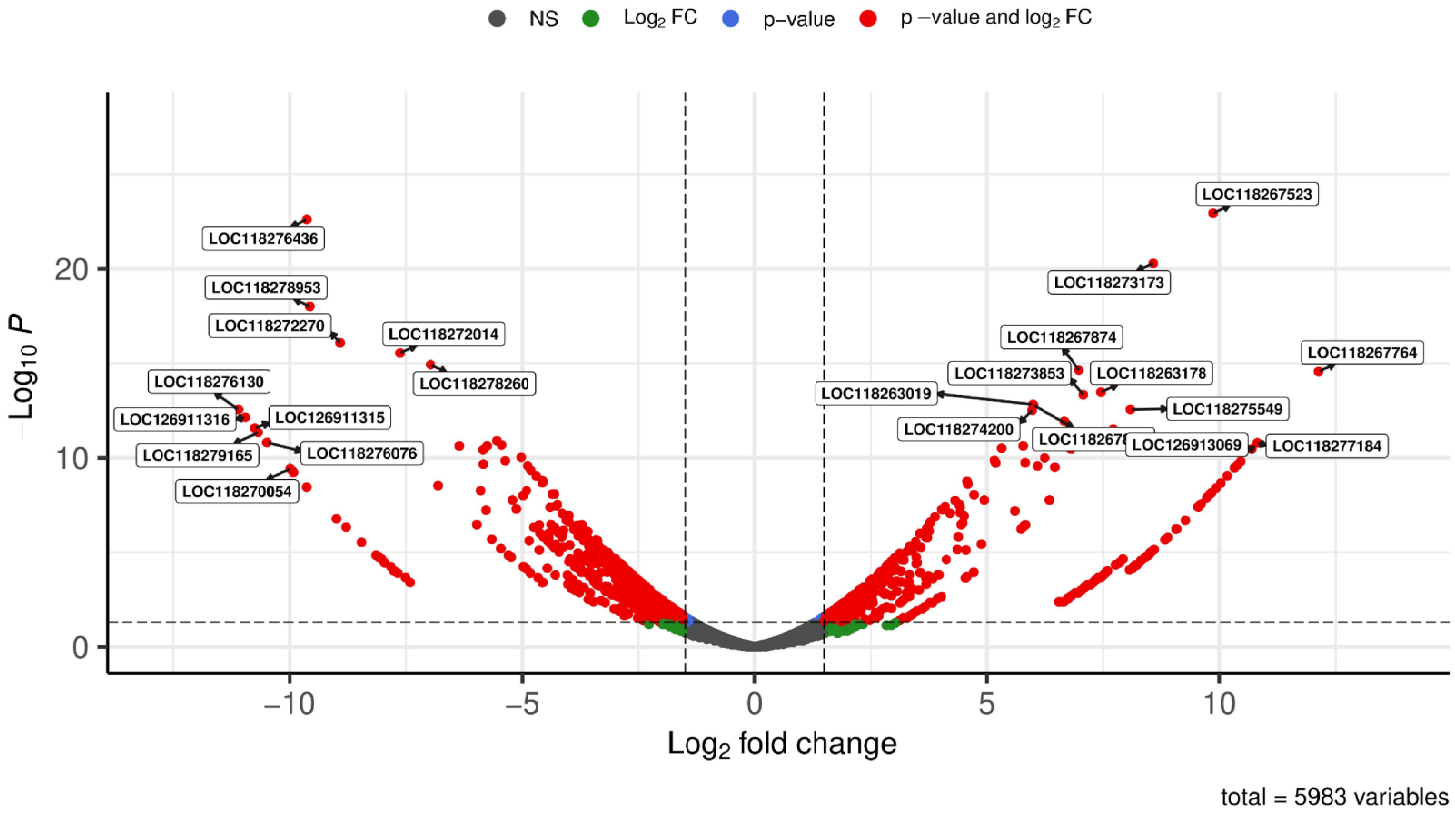
Some plots were made to analyze the differential gene expression. One such is the MA plot. MA-plot shows the distribution of the gene expression between the groups' MGT (treated) and MGC (control). The Y axis shows the Log2fold change (M) and the X axis represents the log of the mean of normalized expression counts (A) of the samples. Red dots correspond to genes up-regulated (>+1.5) and blue dots correspond to the down-regulated genes (<- 1.5) based on the p-value `0.05`. The grey dot corresponds to the non-significant genes where the p-value > 0.05. **(Graph 4.11)**

Another plot is the volcano plot. Volcano plot of MGT (treatment) and MC expressed genes (control). The x-axis represents a log 2-fold change in gene expression between the treated and control groups, and the y-axis displays log 10 p values. Significant genes are represented by the red points (p value 0.05). **(Graph 4.12)**

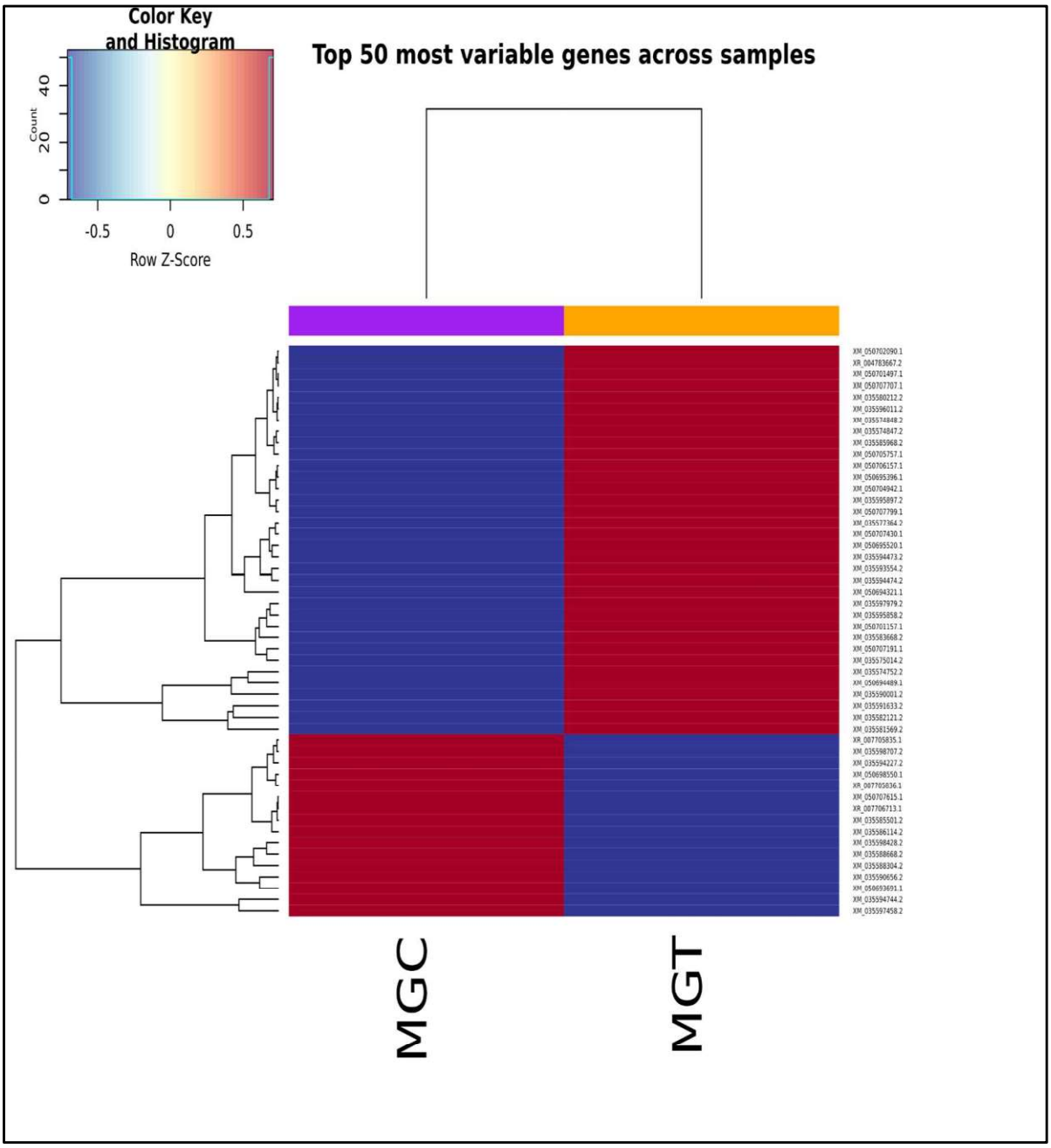
A heat map was also made. The top 50 most variable genes are shown in the heatmap across samples. Heatmap is showing the top 50 genes with the highest variance across samples between the group of the 2 samples **(Graph 4.13)**



Graph 4.11: MA Plot of Treated vs Control



Graph 4.12: Volcano Plot of Treated vs Control



Graph 4.13: Top 50 most variable genes heatmap across samples Heatmap showing the top 50 genes with the highest variance across samples between the group of the 2 samples.

The names of various genes mentioned in the plots are written below as tables. The name of genes with gene ids of the MA plot is mentioned **(Table 4.30)**. The name of genes with gene ids of the Volcano plot is mentioned **(Table 4.31)**. The function of various genes must be known to determine their role in the insect in general. Other than the function, whether it has gone upregulated or downregulated should be known. The genes of MA plot with their regulation (up/down) and functions are mentioned **(Table 4.32)**. Also, the genes of the Volcano plot with their regulation (up/down) and functions are mentioned **(Table 4.3)**.

Table 4.30: Naming of gene ids of MA plot

Sr no.	Gene id	Name of the gene
1	LOC118267764	uncharacterized LOC118267764
2	LOC118267523	collagenase
3	LOC118263178	cholinesterase 1-like
4	LOC118267874	brachyurin-like
5	LOC118273173	pancreatic triacylglycerol lipase-like
6	LOC118273853	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like
7	LOC118263019	high choriolytic enzyme 2
8	LOC118272014	basic juvenile hormone-suppressible protein 2
9	LOC118278260	keratin, type I cytoskeletal 9-like
10	LOC118272270	basic juvenile hormone-suppressible protein 2
11	LOC118278953	hemolin
12	LOC118276436	basic juvenile hormone-suppressible protein 1

Table 4.31: Naming gene ids of Volcano plot

Sr no.	Gene id	Name of the gene
1	LOC118267764	uncharacterized LOC118267764
2	LOC118267523	collagenase
3	LOC118263178	cholinesterase 1-like
4	LOC118267874	brachyurin-like
5	LOC118273173	pancreatic triacylglycerol lipase-like
6	LOC118273853	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like
7	LOC118263019	high choriolytic enzyme 2
8	LOC118275549	cholinesterase 2-like
9	LOC118274200	pancreatic triacylglycerol lipase-like
10	LOC118267328	uncharacterized LOC118267328
11	LOC126913069	peritrophin-1-like
12	LOC118277184	uncharacterized LOC118277184
13	LOC118272014	basic juvenile hormone-suppressible protein 2
14	LOC118278260	keratin, type I cytoskeletal 9-like
15	LOC118272270	basic juvenile hormone-suppressible protein 2
16	LOC118278953	hemolin
17	LOC118276436	basic juvenile hormone-suppressible protein 1
18	LOC118276130	neuropeptide-like protein 29
19	LOC126911316	uncharacterized LOC126911316
20	LOC126911315	uncharacterized LOC126911315
21	LOC118279165	uncharacterized LOC118279165
22	LOC118276076	arp2/3 complex-activating protein rickA-like
23	LOC118270054	uncharacterized LOC118270054

Table 4.32: Status and function of MA plot genes

Sr. No.	Gene id	Up/ Down	Name of the gene	Go function
1	LOC118267764	UP	uncharacterized LOC118267764	Peroxisome, FAD binding, 2 iron, 2 sulfur cluster binding, iron ion binding, oxidoreductase activity
2	LOC118267523	UP	collagenase	Serine type, endopeptidase activity, proteolysis
3	LOC118263178	UP	cholinesterase 1-like	Carboxylic ester hydrolase activity
4	LOC118267874	UP	brachyurin-like	Serine type, endopeptidase activity, proteolysis
5	LOC118273173	UP	pancreatic triacylglycerol lipase-like	Lipase activity, extracellular region, lipid metabolic processes, carboxyl ester hydrolase activity
6	LOC118273853	UP	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	Metalloendopeptidase activity, proteolysis, nucleus, zinc ion binding, intraceelular receptor signalling pathway, nuclear receptor activity, DNA binding
7	LOC118263019	UP	high choriolytic enzyme 2	Extracellular region, nutrient reservoir activity, transmembrane receptor protein serine/threonine kinase signalling pathway, innate immune response, modulation of process of other organism, proteolysis

8	LOC118272014	DOWN	basic juvenile hormone-suppressible protein 2	Nutrient reservoir activity, transmembrane receptor protein/ threonine kinase signalling pathway, membrane
9	LOC118278260	DOWN	keratin, type I cytoskeletal 9-like	Defense to gram negative bacterium, extracellular region, toxin activity, serine peptidase activity, innate immune response, modulation of process of other organism, proteolysis
10	LOC118272270	DOWN	basic juvenile hormone-suppressible protein 2	Extracellular region, nutrient reservoir activity, transmembrane receptor serine/threonine kinase signalling pathway, membrane
11	LOC118278953	DOWN	Hemolin	Defense to gram negative bacterium, extracellular region, toxin activity, serine peptidase activity, innate immune response, modulation of process of other organism, proteolysis
12	LOC118276436	DOWN	basic juvenile hormone-suppressible protein 1	Extracellular region, nutrient reservoir activity, transmembrane receptor serine/threonine kinase signalling pathway, membrane

Table 4.33: Status and function of MA plot genes

Sr. No.	Gene id	Up/down	Name of the gene	Function
1	LOC118267764	UP	uncharacterized LOC118267764	Peroxisome, FAD binding, 2 iron, 2 sulfur cluster binding, iron ion binding, oxidoreductase activity
2	LOC118267523	UP	Collagenase	Serine type, endopeptidase activity, proteolysis
3	LOC118263178	UP	cholinesterase 1-like	Carboxylic ester hydrolase activity
4	LOC118267874	UP	brachyurin-like	Serine type, endopeptidase activity, proteolysis
5	LOC118273173	UP	pancreatic triacylglycerol lipase-like	Lipase activity, extracellular region, lipid metabolic processes, carboxyl ester hydrolase activity
6	LOC118273853	UP	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	Metalloendopeptidase activity, proteolysis, nucleus, zinc ion binding, intraceelular receptor signalling pathway, nuclear receptor activity, DNA binding
7	LOC118263019	UP	high choriolytic enzyme 2	Extracellular region, nutrient reservoir activity, transmembrane receptor protein serine/threonine kinase signalling pathway, innate immune response, modulation of process of other organism, proteolysis
8	LOC118275549	UP	cholinesterase 2-like	Carboxylic ester hydrolase activity, neurotransmitter catabolic process, synpase
9	LOC118274200	UP	pancreatic triacylglycerol lipase-like	Lipase activity, extracellular region, metabolic process, carboxylic ester hydrolase activity
10	LOC118267328	UP	uncharacterized LOC118267328	-
11	LOC126913069	UP	peritrophin-1-like	Chitin binding, extracellular region
12	LOC118277184	UP	uncharacterized LOC118277184	-
13	LOC118272014	DOWN	basic juvenile hormone-suppressible protein 2	Nutrient reservoir activity, transmembrane receptor protein/ threonine kinase signalling pathway, membrane

14	LOC118278260	DOWN	keratin, type I cytoskeletal 9-like	Defense to gram negative bacterium, extracellular region, toxin activity, serine peptidase activity, innate immune response, modulation of process of other organism, proteolysis
15	LOC118272270	DOWN	basic juvenile hormone-suppressible protein 2	Extracellular region, nutrient reservoir activity, transmembrane receptor serine/threonine kinase signalling pathway, membrane
16	LOC118278953	DOWN	Hemolin	Defense to gram negative bacterium, extracellular region, toxin activity, serine peptidase activity, innate immune response, modulation of process of other organism, proteolysis
17	LOC118276436	DOWN	basic juvenile hormone-suppressible protein 1	Extracellular region, nutrient reservoir activity, transmembrane receptor serine/threonine kinase signalling pathway, membrane
18	LOC118276130	DOWN	neuropeptide-like protein 29	Neuropeptide signalling pathway, ribonucleoprotein complex
19	LOC126911316	DOWN	uncharacterized LOC126911316	-
20	LOC126911315	DOWN	uncharacterized LOC126911315	-
21	LOC118279165	DOWN	uncharacterized LOC118279165	-
22	LOC118276076	DOWN	arp2/3 complex-activating protein rickA-like	-
23	LOC118270054	DOWN	uncharacterized LOC118270054	-

A heat map is a type of data visualization that displays aggregated information visually appealingly. Here, the heat map showed the top fifty differentially expressed genes between the control and treatment or resistant samples (MGC and MGT). The name of genes with gene ids of the heat map is mentioned. The top 25 upregulated and the top 25 downregulated genes have been identified and mentioned **(Table 4.34)**.

Table 4.34: Top fifty differentially expressed genes

Sr no.	Transcript_id	Start	End	Strand	Gene	Product	Gene id	Protein_id	Pvalue
Upregulated									
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	+	LOC118272758	pyrimidodiazepine synthase	118272758	XP_035445314.2	0.049454694
5	XM_035583751.2	8991096	8993996	-	LOC118268908	keratin, type I cytoskeletal 10	118268908	XP_035439644.1	0.049439689
6	XM_035597498.2	6235517	6236370	+	LOC118278278	coiled-coil domain-containing protein 115	118278278	XP_035453391.1	0.049271322
7	XM_035593058.2	8845256	8850469	+	LOC118275188	fatty acid-binding protein 1-like	118275188	XP_035448951.1	0.049115466

8	XM_035585375.2	12219871	12235415	-	LOC118269971	putative carbonic anhydrase 3	118269971	XP_035441268.1	0.048036813
9	XM_050694368.1	3430357	3433237	+	LOC126910766	uncharacterized LOC126910766, transcript variant X3	126910766	XP_050550325.1	0.047581525
10	XM_035575232.2	7764744	7771243	+	LOC118263317	ethanolamine kinase	118263317	XP_035431125.1	0.047387679
11	XM_050696551.1	8266742	8283690	-	LOC118277316	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 LOC12	126912277		0.0447917

						6912277			
18	XM_035602697.2	6123378	6124516	-	LOC11828190 4	mitochondrial import inner membrane translocase subunit Tim9	118281904	XP_035458590.1	0.044178093
19	XM_035573163.2	6364368	6365184	-	LOC11826205 8	39S ribosomal protein L20, mitochondrial	118262058	XP_035429056.1	0.043613955
20	XM_035589784.2	370418	379004	-	LOC11827303 6	CKLF-like MARVEL transmembrane domain-containing protein 7	118273036	XP_035445677.1	0.043613955
21	XM_035580523.2	8640739	8642732	-	LOC11826690 1	39S ribosomal protein L16, mitochondrial	118266901	XP_035436416.1	0.043367409
22	XM_035587689.2	10893159	10894295	+	LOC11827159 9	fumarylacetoacetate hydrolase domain-containing protein 2-like	118271599	XP_035443582.2	0.042727823
23	XM_050702463.1	3887662	3893065	+	LOC11827954 4	uncharacterized LOC11	118279544	XP_050558420.1	0.042699475

						8279544			
24	XR_007706807.1	12655258	12657023	+	LOC118266924	MICOS complex subunit Mic10, transcript variant X2	118266924		0.042250704
25	XM_050706611.1	11821569	11833859	+	LOC118273915	cytochrome P450 6B7, transcript variant X15	118273915	XP_050562568.1	0.041837243

Sr no.	Transcript_id	Start	End	Strand	Gene	Product	Gene id	Protein_id	Pvalue
Downregulated									
26	XM_050698476.1	6673669	6716728	-	LOC118279255	anoctamin-8-like	118279255	XP_050554433.1	0.049705260
27	XM_050695383.1	4588693	4685711	+	LOC118275576	hemicentin-1-like	118275576	XP_050551340.1	0.049631622
28	XR_007705838.1	7724788	7726803	-	LOC126911321	uncharacterized LOC126911321	126911321		0.049612969
29	XM_035595884.2	4971093	4974773	-	LOC118277170	uncharacterized LOC118277170	118277170	XP_035451777.2	0.049110684
30	XM_035586999.2	5048448	5052815	+	LOC118271102	piggyBac transposable element-derived protein 4-like	118271102	XP_035442892.2	0.048770873

31	XM_035591056.2	671221	673654	+	LOC118273879	dnaJ protein homolog 1	118273879	XP_035446949.1	0.048172076
32	XM_035578157.2	4713848	4719545	+	LOC118265333	plasminogen activator inhibitor 1	118265333	XP_035434050.2	0.046559831
33	XM_035583503.2	3220598	3228530	+	LOC118268821	protein henna	118268821	XP_035439396.1	0.046313622
34	XM_035576392.2	3884901	3895012	-	LOC118264052	mitochondrial glycine transporter, transcript variant X3	118264052	XP_035432285.1	0.046289229
35	XM_050703618.1	5176204	5188665	-	LOC118278849	UDP-glucosyltransferase 2-like	118278849	XP_050559575.1	0.04578509
36	XM_050706927.1	12525277	12582543	-	LOC118269960	cadherin-87A	118269960	XP_050562884.1	0.04578509
37	XM_035580179.2	7406095	7408813	-	LOC118266693	uncharacterized LOC118266693	118266693	XP_035436072.2	0.045651874
38	XM_035581819.2	12615701	12621598	+	LOC118267693	uncharacterized LOC118267693	118267693	XP_035437712.1	0.045651874
39	XM_035596082.2	3853545	3881117	-	LOC118277333	transmembrane protein 68, transcript variant X1	118277333	XP_035451975.2	0.045651874
40	XM_050698379.1	8300406	8326649	+	LOC118279068	ribosomal protein	118279068	XP_050554336.1	0.045651874

						S6 kinase delta-1, transcript variant X1			
41	XM_035597708.2	9115082	9115898	-	LOC11827849 2	nuclear protein 1	118278492	XP_035453601.1	0.045523716
42	XM_035585904.2	8970723	9008122	+	LOC11827034 3	homeotic protein empty spiracles	118270343	XP_035441797.1	0.045254176
43	XM_035586554.2	10326912	10338632	-	LOC11827078 0	protein Skeletor, isoforms D/E	118270780	XP_035442447.2	0.045254176
44	XM_050697007.1	2748697	2767742	+	LOC11826512 2	fatty acid synthase-like	118265122	XP_050552964.1	0.045137961
45	XM_035583108.2	1432471	1434637	-	LOC11826857 1	carcinine transporter-like	118268571	XP_035439001.2	0.045127361
46	XM_050695657.1	5871796	5875680	-	LOC12691097 6	uncharacterized LOC126910976	126910976	XP_050551614.1	0.044888643
47	XM_035580164.2	7798124	7801117	+	LOC11826668 3	argininosuccinate lyase	118266683	XP_035436057.2	0.044497416
48	XM_035593168.2	9206589	9225703	-	LOC11827526 8	uncharacterized LOC118275268	118275268	XP_035449061.2	0.044472514
49	XM_035574957.2	3607782	3628031	+	LOC11826314 4	phytanoyl-CoA dioxygenase domain-contain	118263144	XP_035430850.1	0.044176692

						ing protein 1 homolog			
50	XM_035591965.2	4293304	4302521	+	LOC118274476	peptidoglycan-recognition protein LB	118274476	XP_035447858.2	0.044167711

Cytochrome P450: The cytochrome P450-dependent monooxygenases are a very important metabolic pathway involved in the catabolism and anabolism of xenobiotics and endogenous compounds**.** It is clear from the numerous insect species and pesticides involved that monooxygenase-mediated metabolism is a typical method by which insects acquire resistance to insecticides **(Scott, 1999)**. In our study, around 78 cytochromes have been upregulated and 66 are downregulated. Some of the up and downregulated cytochromes are mentioned **(Table 4.35)**. The cytochrome P450 along with other genes are known to help in providing resistance in insects against insecticides or any other xenobiotic components. Here, we observed differential expression of various genes in treatment compared to the control insects. Hence, we can say that these genes showing upregulation or downregulation are responsible for providing resistance.

Table 4.35: Top differentially expressed cytochromes

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 cytochromes, various other genes also participate in the metabolic detoxification processes in the insect pest. The other top upregulated detoxification genes are mentioned. Other than them, cuticle protein genes and DNA binding genes which were found to be upregulated in the resistant midgut sample as listed **(Table 4.36)**.

Table 4.36: Top upregulated detoxification and cuticle genes

Sr. No.	Gene	Function
1	glutathione S-transferase 1	metabolic processes
2	acetylcholinesterase	catabolic processes
3	cuticle protein 3	structural constituent of cuticle
4	skin secretory protein xP2	structural constituent of chitin-based cuticle
5	alpha-amylase 2	carbohydrate metabolic processes
6	oxidoreductase TM_0325-like	steroid metabolic processes
7	glyoxylate/hydroxypyruvate reductase	glyoxylate metabolic processes
8	lipase 3-like	lipid metabolic processes
9	lipase member H	extracellular regions
10	pancreatic triacylglycerol lipase-like	extracellular regions
11	lipase member H-like	extracellular regions
12	pancreatic lipase-related protein 2-like	extracellular regions
13	myrosinase 1-like	carbohydrate metabolic processes
14	pancreatic triacylglycerol lipase-like	lipid metabolic processes
15	uricase	urate metabolic processes
16	pancreatic lipase-related protein 2-like	lipid metabolic processes
17	juvenile hormone esterase	neurotransmitter catabolic processes
18	esterase FE4-like	neurotransmitter catabolic processes
19	apyrase-like, transcript variant X1	nucleotide catabolic processes
20	tubulin beta chain	structural constituent of cuticle
21	beta-1,4-galactosyltransferase 4	carbohydrate metabolic processes
22	alpha-amylase 4N	carbohydrate metabolic processes
23	venom carboxylesterase-6-like, transcript variant X1	neurotransmitter metabolic processes
24	venom carboxylesterase-6-like, transcript variant X3	neurotransmitter metabolic processes
25	prostaglandin reductase 1-like, transcript variant X2	metabolic processes
26	homeobox protein araucan, transcript variant X2	DNA-binding genes
27	lipase 3-like	DNA-binding genes

28	3-oxoacyl-[acyl-carrier-protein] reductase FabG-like	DNA-binding genes
29	peptidyl-prolyl cis-trans isomerase, transcript variant X3	DNA-binding genes
30	high choriolytic enzyme 2	DNA-binding genes

The continual exposure of a population to the selective pressure indicated by the careless use of insecticides which results in the evolution of insect resistance. Since insect resistance is biologically complicated phenomena linked to adaptive mechanisms like mutations and metabolic processes essential for organism maintenance, this information is extremely important **(Perry et al., 2011)**. Pesticide target site mutations, detoxification enzymes' mediation of insecticide metabolism, and tegumental alterations that reduce insecticide penetration are now the key processes linked to resistance development **(Georghiou, 1972)**.

NGS technology has made significant advancements in genomic research in non-model organisms. These methods offer a lot of data at a low cost, increasing the likelihood that crucial biological facts can be gleaned from transcriptomes (**Zhao et al., 2011)**.

Some studies have claimed that resistance could result from increased chitin processing enzyme activity, and alterations in the expression of chitin synthases, which are engaged in either chitin production or chitin breakdown (chitinases). Others have suggested that cuticle protein synthesis may be influenced by resistance **(Merzendorfer et al., 2012)**. In our results, endocuticle proteins were upregulated by several chitin proteins and larval cuticle proteins. These form the structural constituent of the cuticle and were found differentially expressed between the two types.

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

According to **(Brun-barale et al., 2010), (Carvalho et al., 2013)** and others, P450 monooxygenases are one of the key groups of enzymes connected to lepidopteran resistance to the pesticides like pyrethroids, and organophosphates. Also, it is similar in case of diamides **(Lin et al., 2013)**.

Our findings revealed that a significant subset of DEGs was associated with detoxification, including genes encoding cytochrome P450 monooxygenases (P450s), glutathione S-transferases (GSTs), UDP glucosyltransferases (UGTs), carboxylesterases (COEs), and ATP-binding cassette transporters (ABCs). The other important group of DEGs included cuticle proteins (CPs), which are crucial structural components of insect tissues and influence how efficiently pesticides penetrate insect bodies. According to our findings, the pathways that were most significantly enriched in whole larval body and midgut samples were "Metabolism of xenobiotics", "Structural component" and "DNA-binding". The focus was on the P450 superfamily and the CPs family, both related to insecticide metabolic resistance. P450-encoding genes are present in the reference FAW genome used in this work. Cuticle proteins provide structural and mechanical support. Biological roles in DNA replication, repair, storage, and modification, such as methylation, are played by DNA-binding domains with DNA structure-related functions.

4.4. RESULTS FROM THE HISTOLOGY

The histology of *Spodoptera frugiperda* midgut was observed in a brightfield microscope- DM 750, Leica. Photography was done for all three types of midguts- control (susceptible), treated/tested (dosing and observing after 72 hrs.) and resistant (Emamectin-resistant insect). Observations were taken at three magnifications, namely, 10X, 20X and 100X as 1, 2, and 3.

Control: *S. frugiperda*'s midgut had an epithelial layer, and the digestive cells' cytoplasm had 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 its basal surface **(Figure 4.7 A, B, C)**.

Resistant: The midgut region had fewer deformities observed than the treated ones. The structure seemed largely intact. The longer and more regular exposure to the insecticide might have played a role in keeping the structure more stable than the initial exposure. However, little vacuolization was observed with a slight deformation in shape **(Figure 4.7 D, E, F)**.

As the high visibility of various parts of the three types was seen at 100X magnification, these were analyzed and compared at this magnification. The images have been labelled as L for lumen, Ep for epithelium, P for peritrophic matrix, B for basal membrane, C for cytoplasm, N for nucleus, V for vacuole.