CHAPTER 4

4.0 RESULTS

The whole study of insecticide resistance monitoring was done in laboratory conditions, maintaining range of temperature 27ºC to 29ºC. Humidity conditions were maintained at 65 RH to 70 RH. The collection of pests of *Spodoptera litura* was done from the fields of Vadodara during the year 2016 to 2017 mainly during Kharif seasons. During my field survey, it was observed that the major crops cultivated in the district were Rice, Wheat, Sorghum (Juwar), Yellow peas, Grams, Oil seeds, Groundnut, Tobacco, Cotton and Sugar cane.

The interviews of farmers were taken to know the current and past status of crops. During these interviews, it was noted that the main crops grown in Vadodara were groundnut, tobacco and cotton. Hence the selection of pest was based on my field surveys conducted in first year of the study. It came to my knowledge that Castor and cotton fields were damaged to a large extent by *Spodoptera litura*. Hence based on the results of this field surveys, I decided to take up the studies of resistance developed in the havoc creating pest, predominantly present on economically important crops. The next agenda was selection of insecticides. Based on the market survey and some local pesticide shops, it was observed that many newer insecticides were regularly consumed by farmers. The farmer used to take those insecticides to spray in fields, as a preventive measure. But this was not the end, some farmers used to spray without having the knowledge around dosage to be used during the sprays. Lack of acquaintance around the insecticides, created a spark in my mind to take up the work of resistance studies on *Spodoptera litura* in laboratory conditions. Thus, it became very vital to have a study on all these factors like pest rearing, insecticide bioassays, developing mother culture, understanding the pest life cycle, knowing the effects of insecticides in-vitro conditions, generation-wise study. Day by day newer insectides are coming to the market and farmers who do not educate themselves, come in trap of these insectides and the whole

pesticide luring business. Without knowing the long-term and even short-term effects, the sprays are done in the field for the control of destruction creating pests like *Spodoptera litura.* The outcome of all these qualitative surveys lead to the selection of insecticides belonging to different groups according to IRAC (Insecticide Resistance Action Committee). The four insecticides selected were

1. Cypermethrin 25EC, synthetic pyrethroid belonging to group 3A
2. Chlorpyriphos 20EC, belonging to Organophosphate group 1B
3. Spinosad 45SC, belonging to Spinosyn, group 5
4. Coragen 20SC, belonging to Diamides, group 28

Hence in this manner, the selection of pest and insectides was done in the initial year of research. Rearing of *Spodoptera litura* was initiated in 2017 and continued till one year. The initial phase of rearing was very difficult, as there were many challenges like fluctuating temperatures during summers, humidity conditions during winters, diet conditions, natural mortality found in culture, development of infections in larvae during rainy seasons. But all these challenges were overcome by slowly and patiently handling the cultures especially during the rainy seasons. After two to three months of initial rearing, it was found out that the survival of culture was maximum during the rainy season i.e June to August. Hence these months were selected for rearing as well as performing bioassays as they ruled out the possibilities of mortality caused due to external factors like temperature and humidity. Subsequently, the mother culture of *Spodoptera litura*, was developed and reared successfully on artificial diet as described the methodology. The bioassays were kept using the traditional leaf dip bioassay as described in the experimental portion of methodology. Healthy cotton leaves were brought from the fields, taking care of previous unsprayed conditions. The next challenge was performing the bioassays, in controlled conditions of laboratory. The results of these bioassays were continuously monitored and the dose range was every time cross checked so as to ensure the quality of experiments. These bioassays, resulted in development of another resistant culture of Spodoptera litura (RR), while the mother culture was concluded to be the susceptible culture (SS). The resistant and susceptible cultures were reared independently on artificial diet. The diet as described in methodology proved to be the ideal one for successful rearing. Hence all the

selections like selection of pest, selection of insecticides and selection of diet played a very important role in the experimental results as described in details in the results section.

4.1 Insecticide resistance developed in *Spodoptera litura* -The generations study

The methodology of traditional leaf-dip bioassay was performed in stringent laboratory conditions. All the factors which could affect the optimum conditions were strictly followed under supervision (Temperature, Humidity, Light: Dark hours). As the pest *Spodoptera litura* was exposed to different concentrations of four insecticides, Cypermethrin 25EC, Chlorpyriphos 20EC, Coragen 20SC and Spinosad 45SC. The mother culture was free of any kind of infection and utmost care was taken to avoid any kind of variations, which may influence the results of the experiments.

The bioassays were performed in the year 2017 (March to August) and 2018 (June to September). The insecticides solutions were made considering the field rates. Commercially available Cypermethrin 25EC was diluted to make different concentrations in ppm and was exposed to 3rd instar larvae of Spodoptera litura. In the first generation, the larvae showed expected results (Mortalities in concentrations starting from 0.125, 0.25 and 0.5 ppm. The second, third and fourth generation also showed the mortalities in these concentrations. But in fifth generations, there was a decrease in mortalities, which indicated a low level of resistance. After completion of one insecticide, the culture was not exposed to any other insecticide to ensure its good health. A close watch was kept on the mother culture after the completion of insecticide resistance experiments. Moving forward in experimental procedures, the pest was exposed to Chlorpyriphos 20EC at different concentrations. In the fifth generation, there was a very low level of resistance observed at 0.5 ppm. This indicated the onset of resistance. But the resistance developed against Chlorpyriphos 20EC was low as compared to that developed by Cypermethrin 25 EC. The third insecticide was Spinosad (Tracer®) 45SC. Insecticide resistance was developed at 1 ppm showing mortality of 50 percent. But Spinosad being a biopesticide was considered to be an insectides with low

toxicity values. The last insecticide tested was Coragen 20SC. This insecticide showed alarming results. The data indicated the onset of resistance from the third generation itself.

4.2 Health parameters of *Spodoptera litura*

The initiation of the experimentation work was confirmed by inspection of health parameters of the mother culture of *Spodoptera litura*.

Table 1 Health Parameters for rearing of *Spodoptera litura*

Larvae (Total 10 nos)	Days for completion of life cycle	Larval weight (g) Early instar	Larval weight(g) Late instar	Weight of food consumed (g)	Adult longevity (days)
Set-1	27	0.12	0.42	1.88	4
Set-2	25	0.13	0.58	1.96	5
Set-3	26	0.14	0.65	1.85	5
Set-4	27	0.11	0.55	2.10	4
Set-5	28	0.15	0.49	1.95	5
Set-6	27	0.16	0.47	1.98	5
Set-7	26	0.13	0.50	1.95	4
Set-8	27	0.16	0.65	2.00	4
Set-9	28	0.15	0.57	2.10	5
Set-10	27	0.13	0.43	1.86	4
Set-11	28	0.16	0.47	2.05	5
Set-12	29	0.15	0.49	1.85	6
Set-13	27	0.12	0.48	2.01	4
Set-14	26	0.14	0.50	1.87	4
Set-15	28	0.17	0.47	1.95	5
Set-16	25	0.16	0.48	1.98	4

This was crucial as the results of these bioassays were dependant on it. This means it was not exposed to its natural conditions. So, it became very important to check all the minor parameters like larval weight of different instars, adult

longevity, weight of food consumed and days taken to complete life cycle (Table 1).

4.3 Observation of Mortalities

The bioassays performed in laboratory conditions on the pest *Spodoptera litura* indicated a decline in mortality values on some of the insecticides. Four insecticides selected were Cypermethrin 25EC, Chlorpyriphos 20EC, Coragen 20SC and Spinosad 45SC. Initially pilot tests were run to make sure that the concentrations (ppm) selected for the bioassays were of a particular range. All the four insecticides were individually run for assessing mortality values obtained in this pest. The mother culture was divided in two parts, one for experiment and another for backup. Initially, bioassays were kept for Cypermethrin 25EC in the experimental culture. The pest was exposed to different concentrations of Cypermethrin. After 72 hours, the test units were taken out of the incubation chamber and brought to laboratory conditions. Anything unusual was captured in data sheet. At 96 hours, the observation was taken using camel hair brush, which was pre-sterilized. The observations of mortalities in various generations for insecticides are mentioned in the below tables. Separate brushes were used for untreated check unit and treated units so as to avoid contamination. The test units were first checked for any kind of fungal/bacterial infections. Larvae were considered to be dead, if there was no movement after contact with brush. Larvae was considered to be moribund, if it showed less and uncoordinated movement as compared to untreated check. Larvae were considered live if it showed normal movement when compared to untreated check.

Live larvae

Moribund larvae

Dead larvae

Figure 1 Symptomatology parameters in larvae after exposure insecticides

4.4 Data analysis

Larval mortalities were recorded at 96 hours. The larvae were considered dead if they failed to make a coordinated movement when prodded with probe. Data was analyzed for control mortalities using Abbott's (1925) formula. The data was further analyzed by the probit analysis method through POLO-PC Program of LeOra, 2003. The resistance developed in the pest was concluded by decreasing mortalities and further from lethal concentration estimated like LC50, LC90 and other important statistical parameters. The larvae which survived to the different concentrations of testing insecticide were then mixed and continued to fourth generation for testing on the same concentrations. The whole process was repeated for five generations.

4.5 Observations recorded

After 96 hours of observation, the mortality observed in each set was noted down. In every experiment, the live larvae were continued to be added to experimental culture. The initial phase of the trials was carried on for five generations (G-1, G-2, G-3, G-4 and G-5). Mortality observed in all the generations were noted in record books. In this manner, all the observations were recorded according to the setups of experiments kept at different intervals. The stringent conditions of temperature and humidity were maintained, so as to obtain the actual results, without effect of external factors.

4.5.1 Insecticide Cypermethrin 25EC

The mortality observed in fifth generation for Cypermethrin 25EC @ 2, 1, 0.5, 0.25, 0.125 and 0.0625 ppm were 100.00, 93.33, 46.67, 13.33 and 0.00 % respectively. There is an onset of resistance developed in one of the concentrations i.e. 0.5 ppm (Table 2, Table 3, Table 4 ,Table 6)of *S.litura* against Cypermethrin 25EC, indicated a low level of resistance being developed in the fifth generation (Table 7)

The mortalities obtained in fifth generation were 100, 93.33, 96.67, 46.67 and 20.00% respectively. The LC 50 and LC 90 values indicated less amount of resistance being developed in the pest. In the fifth generation, LC50 value and LC 90 values were 0.05 and 0.34 (Table 5)

Table 2: Mortality obtained in 1st generation of *S.litura* against Cypermethrin

Sr. no	Concentration(ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	2	30	30	0	30	100
2	1	30	29	1	30	100
3	0.5	30	15	1	16	53.33
4	0.25	30	3	1	4	13.33
5	0.125	30	1	0	1	3.33
6	0.0625	30	0	0	0	0.00
7	Untreated Check	30	0	0	0	0.00

Table 3: Mortality obtained in 2nd generation of *S.litura* against Cypermethrin

Sr. No	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	2	30	30	0	30	100
2	1	30	28	1	29	96.66
3	0.5	30	13	3	16	53.33
4	0.25	30	3	1	4	13.33
5	0.125	30	0	0	0	0.00
6	0.0625	30	0	0	0	0.00
7	Untreated Check	30	0	0	0	0.00

Table 4: Mortality obtained in 3rd generation of *S.litura* against Cypermethrin

Sr. No	Concentration(ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	2	30	30	0	30	100
2	1	30	27	1	28	93.33
3	0.5	30	14	2	16	53.33
4	0.25	30	4	1	5	16.66
5	0.125	30	0	0	0	0.00
6	0.0625	30	0	0	0	0.00
7	Untreated Check	30	0	0	0	0.00

Table 5: Mortality obtained in 4th generation of *S.litura* against Cypermethrin

Sr. no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	2	30	30	0	30	100.00
2	1	30	27	1	28	93.33
3	0.5	30	13	2	15	50.00
4	0.25	30	3	1	4	13.33
5	0.125	30	0	0	0	0.00
6	0.0625	30	0	0	0	0.00
7	Untreated Check	30	0	0	0	0.00

Table 6 Mortality obtained in 5th generation of *S.litura* against Cypermethrin

Sr. no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	2	30	30	0	30	100
2	1	30	27	1	28	93.33
3	0.5	30	13	1	14	46.66
4	0.25	30	3	1	4	13.33
5	0.125	30	0	0	0	0.00
6	0.0625	30	0	0	0	0.00
7	Untreated Check	30	0	0	0	0.00

Table 7: Mortality values of *S.litura* over generations

Sr.no	Concentration (ppm)	Percent Mortality (%)				
		G-1	G-2	G-3	G-4	G-5
1	2	100.00	100.00	100.00	100.00	100.00
2	1	100.00	96.67	93.33	93.33	93.33
3	0.5	53.33	53.33	53.33	50.00	46.67
4	0.25	13.33	13.33	16.67	13.33	13.33
5	0.125	3.33	0.00	0.00	0.00	0.00
6	0.0625	0.00	0.00	0.00	0.00	0.00
7	Untreated check	0.00	0.00	0.00	0.00	0.00

Table 8: LC estimates of Cypermethrin 25EC over generations

LC estimates	*G-1	G-2	G-3	G-4	G-5
LC50	0.43	0.45	0.45	0.48	0.49
LC90	0.82	0.83	0.90	0.92	0.94
Slope+- Std Error	4.51±0.64	4.86±0.72	4.31±0.64	4.50±0.64	4.48±0.60
Chi square	4.99	0.82	0.83	0.68	1.08
Significance	0.28	0.93	0.93	0.95	0.89

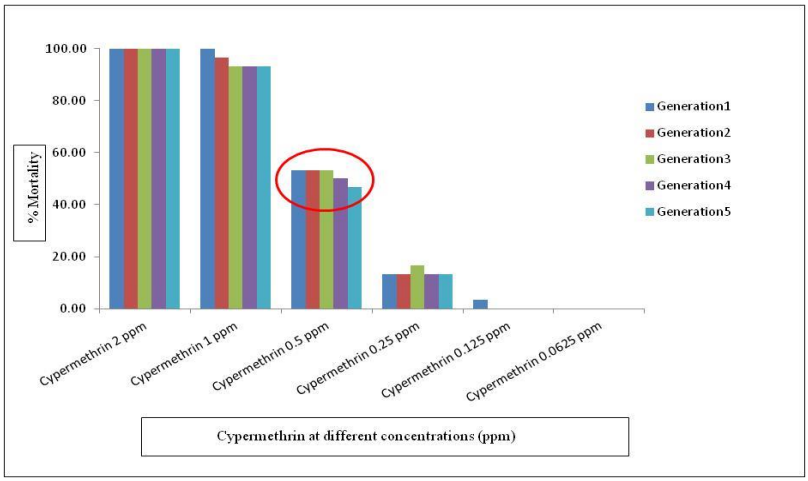

Figure 2 Graph representing resistance developed in Cypermethrin

The LC values observed in all the generations show a gradual development of resistance LC 50 and LC 90 in first generation for Cypermentrhin 25EC @ 0.5 ppm were 0.43 and 0.82 respectively. In the second generation, these values show a slight increase i.e 0.45 and 0.83 respectively. While in third and fourth generation,these attain a value of 0.45, 0.90 and 0.48, 0.92 respectively. When the bioassay was repeated in fifth generation, the LC50 and LC90 values indicate 0.49 and 0.94 respectively (Figure 2)

4.5.2 Insecticide Chlorpyriphos 20EC

Similarly, the mortality bioassays were kept on the third instar larvae and it was exposed to different concentrations of Chlorpyriphos 20EC. Different rates used were 6.25 ppm, 1.25 ppm, 0.25 ppm, 0.05 ppm, 0.01 ppm and 0.002 ppm (Table 9)

Table 9 Mortality obtained in 1st generation of *S.litura* against Chlorpyriphos

Sr. no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	6.25	30	29	1	30	100.00
2	1.25	30	29	1	30	100.00
3	0.25	30	28	1	29	96.67
4	0.05	30	10	6	16	53.33
5	0.01	30	4	1	5	16.67
6	0.002	30	0	0	0	0.00
7	Untreated check	30	0	0	0	0.00

These rates were in accordance with the field rates. All the concentrations of the solution were made by serial dilution. Utmost care was taken while mixing the solutions, the use of lab apparatus was also done very carefully. All the equipment used during the dilution process, were thoroughly cleaned with acetone and water. The observations recorded at 96 hours were recorded in lab notebook. All these were made by serial dilutions as described in methodology.

Table 10: Mortality obtained in 2nd generation against Chlorpyriphos*

Sr. no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	6.25	30	29	1	30	100.00
2	1.25	30	29	1	30	100.00
3	0.25	30	27	1	28	93.33
4	0.05	30	12	5	17	56.67
5	0.01	30	5	1	6	20.00
6	0.002	30	0	0	0	0.00
7	Untreated check	30	0	0	0	0.00

Table 11: Mortality obtained in 3rd generation of *S.litura* against Chlorpyriphos

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	6.25	30	29	1	30	100.00
2	1.25	30	28	1	29	96.67
3	0.25	30	27	1	28	93.33
4	0.05	30	12	4	16	53.33
5	0.01	30	4	1	5	16.67
6	0.002	30	0	0	0	0.00
7	Untreated check	30	0	0	0	0.00

Table 12: Mortality obtained in 4th generation of *S.litura* against Chlorpyriphos

Sr. no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	6.25	30	29	1	30	100.00
2	1.25	30	28	1	29	96.67
3	0.25	30	27	1	28	93.33
4	0.05	30	11	4	15	50.00
5	0.01	30	5	1	6	20.00
6	0.002	30	0	0	0	0.00
7	Untreated check	30	0	0	0	0.00

Table 13: Mortality obtained in 5th generation of *S.litura* against Chlorpyriphos

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	6.25	30	29	1	30	100.00
2	1.25	30	27	1	28	93.33
3	0.25	30	28	1	29	96.67
4	0.05	30	10	4	14	46.67
5	0.01	30	5	1	6	20.00
6	0.002	30	0	0	0	0.00
7	Untreated check	30	0	0	0	0.00

Table 14: Mortality values of *S.litura* against Chlorpyriphos 2EC over generations

Concentration (ppm)	G*-1	G-2	G-3	G-4	G-5
6.25	100.00	100.00	100.00	100.00	100.00
1.25	100.00	100.00	96.67	96.67	93.33
0.25	96.67	93.33	93.33	93.33	96.67
0.05	53.33	56.67	53.33	50.00	46.67
0.01	16.67	20.00	16.67	20.00	20.00
0.002	0.00	0.00	0.00	0.00	0.00

*- Generation

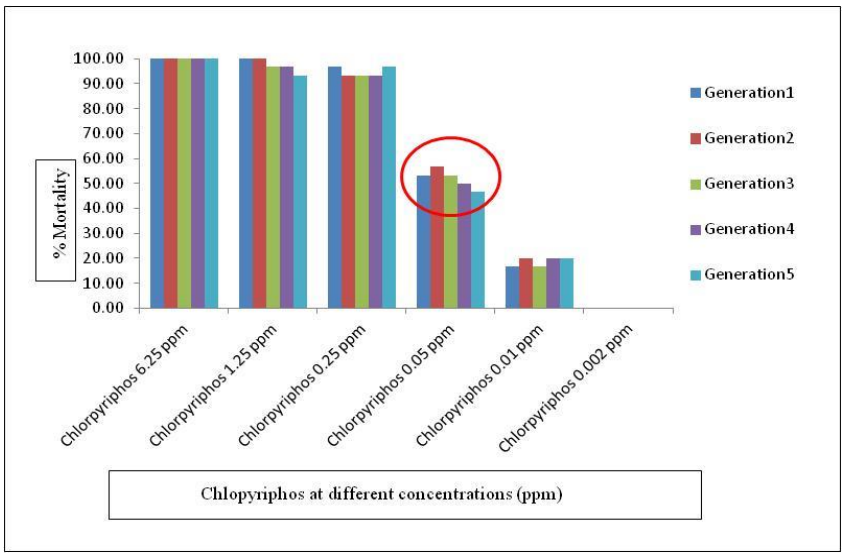

Figure 3 Graph indicating resistance in *S.litura* Chlorpyriphos at 0.05 ppm

Chlorpyriphos 20EC at different concentrations i.e 6.25, 1.25, 0.25, 0.05, 0.01 and 0.002 ppm was exposed for five generations. The mortalities obtained in fifth generation were 100, 93.33, 96.67, 46.67 and 20.00% respectively. The LC 50 and LC 90 values indicated less amount of resistance being developed in the pest. In the fifth generation, LC50 value and LC 90 values were 0.05 and 0.34. There was an onset of resistance observed in Chlorpyriphos 20EC @ 0.05 (Figure 3). All these values indicate onset of resistance in both the insecticides in laboratory conditions.

As compared to Cypermethrin 25EC, the resistance level developed in this insecticide was low. But this does not confirm the low development in coming generations of the pest. Hence these studies presented the important

phenomenon of resistance being developed in *Spodoptera litura* in the laboratory population in controlled conditions. The field scenario may be different as it is affected by other external factors like crop vigour, soil properties, temperature, humidity, weather conditions etc.

4.5.3. Insecticide Spinosad 45SC(Tracer®)

The resistance studies were performed on the third insecticide Spinosad 45SC at different concentrations like 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 ppm.

Table 15 Mortality (%) obtained in 1st generation of *S.litura* against Spinosad

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	30	30	27	3	30	100.00
2	10	30	22	3	25	83.33
3	3	30	14	5	19	63.33
4	1	30	10	4	14	46.67
5	0.3	30	0	3	3	6.67
6	0.1	30	0	1	1	3.33
7	0.03	30	0	0	0	0.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

The mortalities observed in different concentrations of Spinosad were 100.00%, 83.33%, 63.33%, 46.67%, 6.67%, 3.33%, 0.00% and 0.00% for 30 ppm, 10ppm, 3 ppm, 1 ppm, 0.3 ppm, 0.03 ppm, 0.01 ppm. In the first generation, the results were similar as expected in different concentrations. There were dead, moribund larvae found in the higher concentrations with no unusual characteristics. Hence the test units were discarded and the live larvae were retrieved and mixed with the testing culture. These were then multiplied to continue to the next generation for the bioassays at same concentrations. The generations study was inclusive of the additional observations of any unusual characteristics or any other thing noticed like size reduction, involuntary movement due to insecticide mode of action affecting nervous system etc.

Table 16 Mortality obtained in 2nd generation of *S.litura* against Spinosad

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	30	30	27	3	30	100.00
2	10	30	23	4	27	90.00
3	3	30	14	6	20	66.67
4	1	30	12	5	17	56.67
5	0.3	30	1	0	1	10.00
6	0.1	30	0	0	0	0.00
7	0.03	30	0	0	0	0.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

The mortalities obtained in first (Table 15) and second generation. The mortalities observed in different concentrations of Spinosad were 100.00%, 83.33%, 63.33%, 46.67%, 6.67%, 3.33%, 0.00% and 0.00% for 30 ppm, 10ppm, 3 ppm, 1 ppm, 0.3 ppm, 0.03 ppm, 0.01 ppm. In the first generation, the results were similar as expected in different concentrations. There were dead, moribund larvae found in the higher concentrations with no unusual characteristics. Hence the test units were discarded and the live larvae were retrieved and mixed with the testing culture. These were then multiplied to continue to the next generation for the bioassays at same concentrations(Table 16) of *S.litura* indicated no resistance developed at any of the concentrations . Any unusual thing was captured in record book. Slowly, the results began to differ from the expected ones. There was shift of mortality values and lowering down, which indicated the onset of resistance in one of the concentrations. Due to this, there was a gradual decrease in mortality from third generation onwards. The results obtained were recorded and cross-checked for any mistakes in visual observations of dead and moribund. Spinosad being a bio-pesticide had a less toxic effect on human health as well as less resistance development in pest.

Table 17 Mortality (%) obtained in 3rd generation against Spinosad

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	30	30	26	2	28	93.33
2	10	30	21	2	23	76.67
3	3	30	13	5	18	60.00
4	1	30	12	3	15	50.00
5	0.3	30	0	1	1	3.33
6	0.1	30	1	0	1	3.33
7	0.03	30	0	0	0	0.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

Table 18 Mortality (%) obtained in 4th generation against Spinosad

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	30	30	25	4	29	96.67
2	10	30	22	4	26	86.67
3	3	30	14	6	20	66.67
4	1	30	12	2	14	46.67
5	0.3	30	1	1	2	6.67
6	0.1	30	1	1	2	6.67
7	0.03	30	1	0	1	3.33
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

The mortalities obtained in third (Table 17) and fourth generation (Table **18**) of *S.litura* indicated some amount of resistance developed at one of the concentrations i.e. 1 ppm. To confirm this, the bioassay was continued for the next and the last generation i.e. fifth generation. The live larvae were taken for further multiplication. In the fifth generation, there was resistance observed, which indicated the development through a paradigm shift from normal

mortality to mortality due to resistance being lowered. Thus, in Spinosad, there were slightly different results than expected.

There was lowering down of mortality in the concentration of 1 ppm. The mortality observed in the earlier generation was the LC50 value of that generation. But when the live larvae were continued in the next generation, to keep the bioassay in fifth generation, mortality reduced from 46.67 to 43.33%. There was a swift decrease in the mortality in the otherwise susceptible. The results were a bit disturbing, as Spinosad was considered to be safe as it was a bio-pesticide.

Table 19 Mortality (%) obtained in 5th generation of *S.litura* against Spinosad

Sr.no	Concentration (ppm)	Total larvae	Dead	Moribund	Total	% Mortality
1	30	30	25	5	30	100.00
2	10	30	23	4	27	90.00
3	3	30	15	6	21	70.00
4	1	30	12	1	13	43.33
5	0.3	30	1	1	2	6.67
6	0.1	30	0	1	1	3.33
7	0.03	30	0	0	0	0.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

The LC estimates were also calculated to infer the relation between the LC values and mortality values. The LC 50 values indicated a decrease in values from 2.24 in third generation to 1.51 in fourth generation to 1.38 in fifth generation. Thus, as generations were passing, the mortality was showing a decrease in one of the concentrations i.e 1 ppm.

Table 20: Mortality values of *S.litura* against Spinosad 45SC over generations

Sr. No	Concentration (ppm)	G*-1	G-2	G-3	G-4	G-5
1	30	100.00	100.00	93.33	96.67	100.00
2	10	83.33	90.00	76.67	86.67	90.00
3	3	63.33	66.67	60.00	66.67	70.00
4	1	46.67	56.67	50.00	46.67	43.33
5	0.3	6.67	10.00	3.33	6.67	6.67
6	0.1	3.33	0.00	3.33	6.67	3.33
7	0.03	0.00	0.00	0.00	3.33	0.00
8	0.01	0.00	0.00	0.00	0.00	0.00
9	Untreated check	0.00	0.00	0.00	0.00	0.00

*- Generation

Table 21 LC estimates of Spinosad 45SC against *S.litura* over generations

Treatment (Spinosad)	Heterogeneity (Chi square)	LC 50	LC 90	Fiducial limit (Upper)	Fiducial limit (lower)	Slope± Error	Intercept ± Error
Generation 1	6.00	1.68	10.31	21.72	6.30	2.79±0.35	-0.63 ± 0.22
Generation 2	6.71	1.33	6.87	13.70	4.36	3.08±0.40	-0.38 ± 0.23
Generation 3	5.64	2.24	17.78	41.48	10.19	2.44±0.31	-0.85 ± 0.22
Generation 4	3.44	1.51	11.82	26.57	6.88	2.45±0.30	-0.44 ± 0.20
Generation 5	3.65	1.38	7.06	14.07	4.49	3.10±0.40	-0.43 ± 0.23

In the first generation, the LC 50 and LC 90 values were 1.68 and 10.31 respectively. The second generation indicated values 1.33 and 6.87 respectively. In the third and fourth generation 2.24, 3.34 and 1.51, 11.82 indicated low amount of resistance developing in the culture. In the fifth generation, 1.38 and 7.06 values indication onset on resistance. The upper and lower Fiducial limits were found to be 1.17 and 2.24 for first generation. Subsequently, the second third, fourth and fifth generation upper and lower fiducial limits.

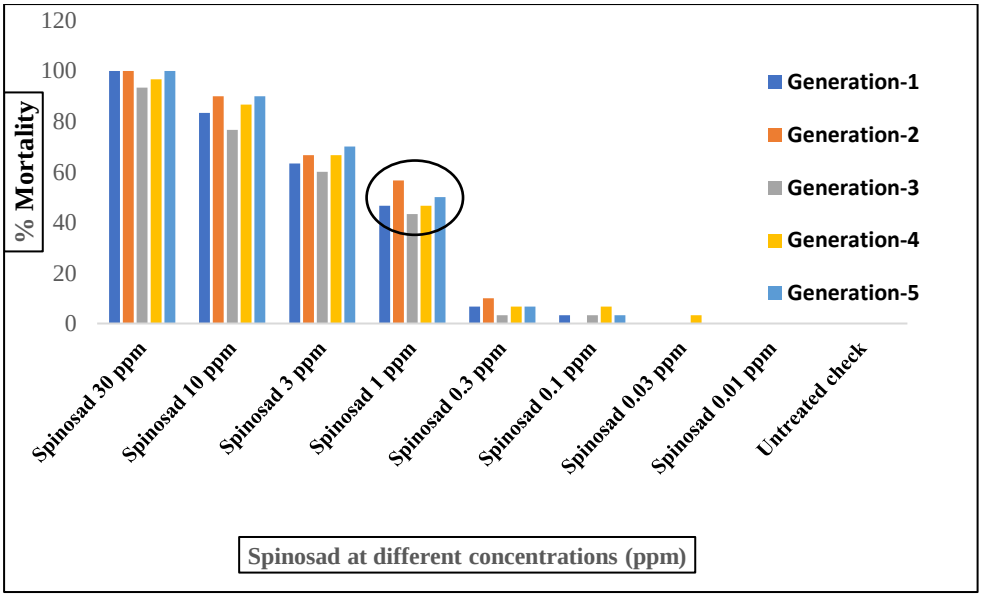

Figure 4 Graph representing resistance developed in Spinosad at 1 ppm

4.5.4. Insecticide Coragen® 20SC

The pest *Spodoptera litura* was treated with Coragen 20SC at difference concentrations in accordance to field rates i.e. 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 ppm. The mortalities obtained in generation 1 and 2 were as per the expectation i.e 0.00, 10.00, 16.67, 26.67, 50.00, 83.33, 93.33 and 100.00 percent respectively. In the second generation the same pest culture was exposed to same concentration of treatment. The mortality values obtained were 0.00, 10.00, 10.00, 30.00, 53.33, 83.33, 96.67 and 100.00 percent at various concentration. Coragen 20SC @ 0.1 ppm showed lowering efficacy from 16.67% in generation 1 to 10.00% generation 2. However, from third generation, the mortalities increased in all the concentrations except 1 ppm. The mortality obtained in *Spodoptera litura* for Coragen 20SC @ 1 ppm decreased from 53.33 in second generation to 46.67 in third generation. The fourth and fifth generation indicated again a decrease in mortalities attaining the values 40.00 and 36.67 in generation 4 and generation 5 respectively (Table 22)

This decrease in mortality values indicated the onset of resistance, which is an alarming situation for future.

Table 22 Mortality (%) obtained in 1st generation of *S.litura* against Coragen

Sr.no	Concentration (ppm)	Total Larvae	Dead	Moribund	Total (Dead +Moribund)	% Mortality
1	30	30	30	0	30	100.00
2	10	30	26	2	28	93.33
3	3	30	24	1	25	83.33
4	1	30	13	2	15	50.00
5	0.3	30	5	3	8	26.67
6	0.1	30	4	1	5	16.67
7	0.03	30	2	1	3	10.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

Table 23 Mortality obtained in 2nd generation of *S.litura* against Coragen

Sr.no	Concentration (ppm)	Total Larvae	Dead	Moribund	Total (Dead +Moribund)	% Mortality
1	30	30	30	0	30	100.00
2	10	30	27	2	29	96.67
3	3	30	23	2	25	83.33
4	1	30	14	2	16	53.33
5	0.3	30	5	4	9	30.00
6	0.1	30	3	0	3	10.00
7	0.03	30	2	1	3	10.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

Table 24 Mortality (%) obtained in 3rd generation of *S.litura* against Coragen

Sr.no	Concentration (ppm)	Total Larvae	Dead	Moribund	Total (Dead +Moribund)	% Mortality
1	30	30	30	0	30	100.00
2	10	30	28	2	30	100.00
3	3	30	24	2	26	86.67
4	1	30	13	1	14	46.67
5	0.3	30	5	5	10	33.33
6	0.1	30	3	2	5	16.67
7	0.03	30	2	1	3	10.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

From the second generation, the live larvae of the bioassay, were continued in the next generation. As the insecticide Coragen 20SC was treated at same concentrations i.e 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 ppm, the mortalities observed in second generation (Table 22) showed a slight difference as compared to first generation.10 ppm and 3 ppm indicated a slight increase in mortality values from generation 2 (Table 23)

Table 25 Mortality obtained in 4th generation of *S.litura* against Coragen

Sr.no	Concentration (ppm)	Total Larvae	Dead	Moribund	Total (Dead +Moribund)	% Mortality
1	30	30	30	0	30	100.00
2	10	30	28	2	30	100.00
3	3	30	25	2	27	90.00
4	1	30	11	1	12	40.00
5	0.3	30	4	6	10	33.33
6	0.1	30	3	2	5	16.67
7	0.03	30	2	1	3	10.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

Hence the mortality values were obtained all generations and there was gradual development of resistance observed against Coragen 20SC. The fourth-generation values were indicative of decrease in mortality. The larvae which were live and were continued for later generation, were active, with no signs of mortality.

Table 26 Mortality obtained in 5th generation of *S.litura* against Coragen

Sr.no	Concentration (ppm)	Total Larvae	Dead	Moribund	Total (Dead +Moribund)	% Mortality
1	30	30	30	0	30	100.00
2	10	30	28	2	30	100.00
3	3	30	25	3	28	93.33
4	1	30	10	1	11	36.67
5	0.3	30	5	6	11	36.67
6	0.1	30	4	2	6	20.00
7	0.03	30	2	1	3	10.00
8	0.01	30	0	0	0	0.00
9	Untreated check	30	0	0	0	0.00

The bioassay kept in fourth generation clearly indicated resistance developed in one of the concentrations i.e Coragen @ 20 SC at 1 ppm (Table 26). This trend was continued from generation three onwards. Hence there was swift development of resistance observed in Spinosad over five generations.

Table 27 Mortality values of *S.litura* against Coragen 20SC over generations

Concentration (ppm)	G*-1	G-2	G-3	G-4	G-5
30	100.00	100.00	100.00	100.00	100.00
10	93.33	96.67	100.00	100.00	100.00
3	83.33	83.33	86.67	90.00	93.33
1	50.00	53.33	46.67	40.00	36.67
0.3	26.67	30.00	33.33	33.33	36.67
0.1	16.67	10.00	16.67	16.67	20.00

Concentration (ppm)	G*-1	G-2	G-3	G-4	G-5
0.03	10.00	10.00	10.00	10.00	10.00
0.01	0.00	0.00	0.00	0.00	0.00
Untreated check	0.00	0.00	0.00	0.00	0.00

*- Generation

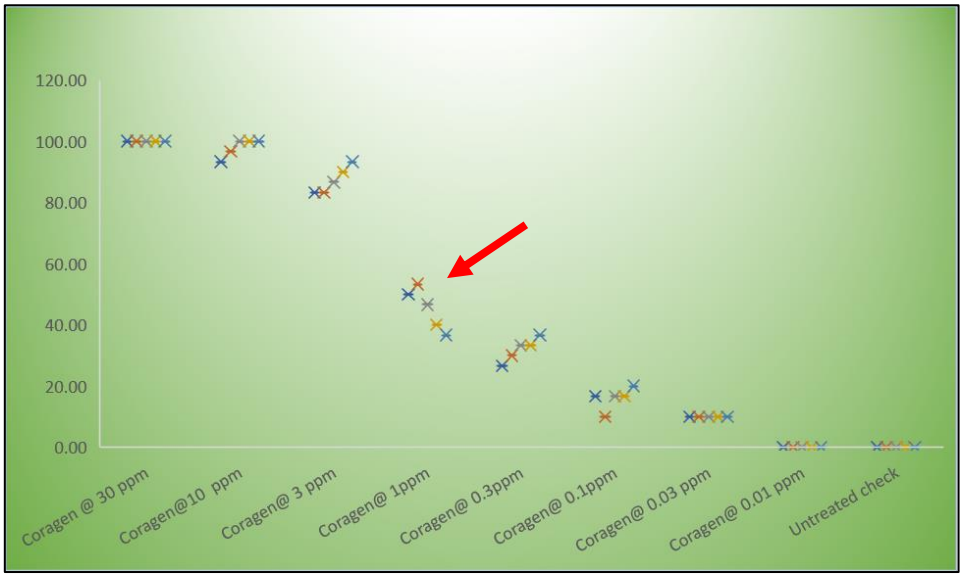

Figure 5 Graph representing resistance developed in *S.litura* against Coragen

The fifth generation indicates a constant decline in mortality values. The pest *Spodoptera litura* showed resistance developed in fifth generation (Figure 5) The mortality values were 50.00 %, 53.33% in generation 1 and 2. From third generation onwards, there was decline in the mortality values 46.67 % in generation 3, 40.00 % in generation 4 and 36.67% in fifth generation. Thus, there was development of resistance seen in one of the concentrations i.e 1 ppm.