

Studies on the development of insecticide  
resistance in *Spodoptera litura* Fabricius, 1775  
(Lepidoptera: Noctuidae)



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Executive Summary of Ph.D Thesis in the Field of  
Zoology for the Degree of Doctor of Philosophy

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## 1 INTRODUCTION

Agriculture is one of the strongest sectors of the Indian economy. The major agricultural produce includes rice, wheat, cotton, jute, tea, and many others. The reasons for good agricultural practices in India are suitable and favorable climate, strong and well extensive research.

There was a gradual development of resistance developing to these insecticides. Resistance to Pyrethroids and DDT was first reported in houseflies, *Musa domestica* which was termed as knockdown resistance (Harrison, 1951). Later on, mosquito *Aedes aegyptii* demonstrated the ability to develop resistance to a wide variety of Organophosphates, Carbamates, and Pyrethroids (Mazzarri & Georghiou, 1995). In 1967, the resistance populations were distributed among 14 orders of Arthropoda, with 91% of all of these appearing in 6 of the 14 (i.e., Diptera, Lepidoptera, Coleoptera, Arachnida, Hemiptera/Homoptera, and Hemiptera/Heteroptera); this has remained fairly constant through 1980 (Forgash, 1984). After this, neonicotinoid resistance was observed in pests like Aphids, Whiteflies, Beetles, Planthoppers, Bugs, *Thrips* sp. and lepidopteran pests like Tobacco Budworm (*Heliothis virescens*) (Nauen & Denholm, 2005).

Previous exposure with insecticides can confer resistance to newly introduced insecticides through cross-resistance reducing the effectiveness of new insecticides (Rehan, Saleem, & Freed, 2011). The problem of development of resistance to insecticides is more acute in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) because of its polyphagous nature and rapid multiplication (Ramakrishnan, Saxena, & Dhingra, 1984). The current study is carried out on laboratory culture of *Spodoptera litura*. This pest was brought from the nearby fields of Vadodara, Gujarat. *S. litura* has been shown to be resistant to a wide range of insecticides, which has led to sporadic outbreaks of the pest and failure of crops (Ahmad, Iqbal Arif, & Ahmad, 2007). Previous exposure with insecticides can confer resistance to newly introduced insecticides through cross-resistance reducing the effectiveness of new insecticides (Rehan et al., 2011). The problem of development of resistance to

insecticides is more acute in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) because of its polyphagous nature and rapid multiplication (Ramakrishnan et al., 1984). I was particularly interested in this pest, as it was common to almost all different crop fields like Castor, Cotton, Cabbage, Maize. The tobacco caterpillar, *Spodoptera litura* F. (Lepidoptera, Noctuidae) is recognized as a serious cosmopolitan pest with a considerable host range of economically important crops such as cotton, groundnut, soybean, tomato and many other crops (Uematsu, 1992). It has caused higher economic losses to crops at their blossoming and vegetative stages with 70 to 100% yield loss (Ahmad et al., 2007). Insecticide resistance must be continuously monitored and must form an integral part of chemical control to enable the detection of resistance as early as possible and to take necessary measures (Kaur, P and Kang, 2015). Hence considering all the facts and figures, I decided to take up the work of insecticide resistance developed in *Spodoptera litura* from generation to generation. The agricultural fields surveyed were very helpful for creating an insight into the true scenario of the damage caused by this pest. With all this background in my mind, a thought process slowly built up to perform a deep study in this area, which was less explored.

**To fulfill the above aim, the following objectives were undertaken:**

1. To study percentage hatchability and mortality rate of *Spodoptera litura* exposed to commonly used insecticides namely Cypermethrin, Chlorpyrifos, Spinosad and Coragen
2. Repeat the study over generation to find the development of insecticide resistance in terms of increase in survival and percentage hatchability to the doses of insecticides which were found effective in eliminating the *Spodoptera litura* population in the previous generation
3. To ascertain the relationship, if any, between generation turnover and the onset of insecticide resistance through a carefully controlled laboratory study

## 2 METHODOLOGY

### 2.1 Collection and Preservation

A site survey was done in some parts of Vadodara, Gujarat and populations of *Spodoptera litura* were collected from fields of nearby regions. The information on sprays occurring in these fields were recorded beforehand, taking help of the local farmers at the time of collecting populations of pest. The castor fields which were infested by this pest, were visited for collection. A mixed culture containing mostly smaller instars like second and third instars were collected in separate bowls along with healthy leaves of cotton and castor for survival.

### 2.2 Rearing in laboratory conditions

Larvae of *Spodoptera litura* were reared in controlled laboratory conditions i.e.  $25\pm 2^{\circ}\text{C}$ , 65-70% relative humidity and a photoperiod of L: D, 14:10. It was reared on artificial diet (Siddiqui, K. H., & Debjani, 2002). Until pupation, the larvae were kept on artificial diet. Rearing in container was feasible as there was no cannibalism observed in *Spodoptera litura*. After complete formation of pupae, they were transferred to bowls. Pupae were also sterilized by using traditional sterilization methods. The completion of pupal stage lead to the beginning of adult emergence. As soon as adult emergence started, healthy male and female adults were released in oviposition pots in the ratio of 2:2. Adult diet was also provided by using honey solution. Moths emerged from the pupae were shifted into glass jars with 1:1 male and female ratio. The moths were provided with water and honey solution. The eggs were kept in Petri dishes (11 cm dia.) and were covered with fine muslin cloth and secured with rubber bands. The larvae were kept in rearing jars (15 cm  $\times$  13 cm) covered with muslin cloth and secured with rubber bands. They were daily supplied with fresh. cabbage leaves for feeding. The adults were also kept in rearing jars (15 cm  $\times$  13 cm), supplied with a piece of folded paper for oviposition and a cotton swab dipped in 50 % honey solution was hanged from the top in order to provide feeding material for adults. The honey solution was renewed after every 48 hours.



Figure 1: Insect culture of *Spodoptera litura* reared in laboratory conditions

The Petri dishes having *Spodoptera litura* eggs and rearing jars containing larvae and adults were kept in B.O.D. incubator maintained at  $27 \pm 2^\circ\text{C}$  temperature and  $78 \pm 2$  % relative humidity.



Figure 2 : Rearing of larvae on artificial diet pieces kept in plastic container

### 2.3 Selection of Insecticides

The insecticides were selected based on the market survey as well the field survey done. In the surrounding fields of Vadodara, the farmers were interviewed for the type of sprays, irrigation provided to the fields, maintenance chemicals used if any and also the pests attacking the crops. The farmers used Pyrethroids, Organophosphates, Diamides and Spinosyns were used extensively in some areas. The insecticides which are selected for this study of Insecticide resistance are Spinosad, Chlorpyrifos, Cypermethrin & Coragen because these are widely used insecticide for lepidopteran. All the four insecticides belong to different class of insecticides.

### 2.4 Leaf Dip Bioassay

The traditional leaf dip bioassay was conducted in laboratory conditions. Primary stock solutions of insecticides were calculated and bracketing was done to arrive the different concentration on third instar larvae of *S. litura*. Different ppm concentrations were made, using serial dilution process. Leaf discs of five centimeters were cut. These leaf discs were dipped in the test solutions for ten seconds with gentle agitation and were placed on tissue papers for drying with adaxial surface. Natural drying was performed by giving enough time. After ensuring, the leaf discs were placed in petri plates having moist filter paper to avoid desiccation of leaves in ten replicates. The larvae were kept for starvation for one hour before exposing it to testing. On each leaf disc, three 3rd instar larvae (F1 generation) were released, using fine camel hair brush. All the test units were kept in controlled environmental conditions, humidity chamber ( $25\pm 2^{\circ}\text{C}$ , 65-70%). The humidity chamber was properly checked to ensure the correct working according to the parameters set inside. Untreated check was also kept in which the leaf discs were treated with distilled water. After 72 hours, the test units were taken out of the chamber and brought to laboratory conditions. These test units were then carefully opened, and the larvae were checked for mortality. Before keeping the actual experiment, health parameters were checked and taken into consideration. These health parameters like Larval weight, pupal weight, adult longevity, number of pupae etc. were recorded on daily basis and were critically checked for any kind of slightest infections in the mother culture. After ensuring the health, further experiments were planned.

Table 1: Health Parameters of *S.litura*

| Larvae (10 nos./set) | Days for completion of life cycle | Larval weight (g) | Larval weight(g) | Weight of food consumed (g) | Adult longevity (days) |
|----------------------|-----------------------------------|-------------------|------------------|-----------------------------|------------------------|
|                      |                                   | Early instar      | Late instar      |                             |                        |
| 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                                | 0.12              | 0.50             | 2.00                        | 4                      |
| Set-7                | 28                                | 0.14              | 0.60             | 1.97                        | 4                      |

## 2.5 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.

## 3 RESULTS

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.

### 3.1 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 in Cypermethrin 25EC, indicated a low level of resistance being developed in the fifth generation (Table 2). The LC 50 and

LC 90 values indicated less amount of resistance developed in the pest. In the fifth generation, LC50 value and LC 90 values were 0.05 and 0.34 (Table 3)

Table 2: Mortality values of *S.litura* against Cypermethrin 25EC- 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 3: LC estimates of Cypermethrin 25EC against *S.litura* -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      |

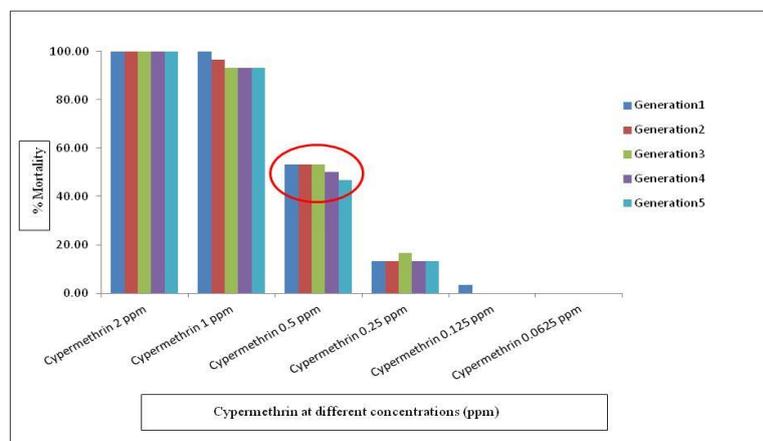


Figure 3 Graph representing resistance developed in Cypermethrin

### 3.2 Insecticide Chlorpyrifos 20EC

Similarly, the mortality bioassays were kept on the third instar larvae and it was exposed to different concentrations of Chlorpyrifos 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 4). These rates were in accordance with the field rates. All the concentrations of the solution were made by serial dilution. The observations recorded at 96 hours were recorded in lab notebook. All these were made by serial dilutions as described in methodology.

Table 4: Mortality values of Chlorpyrifos 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   |

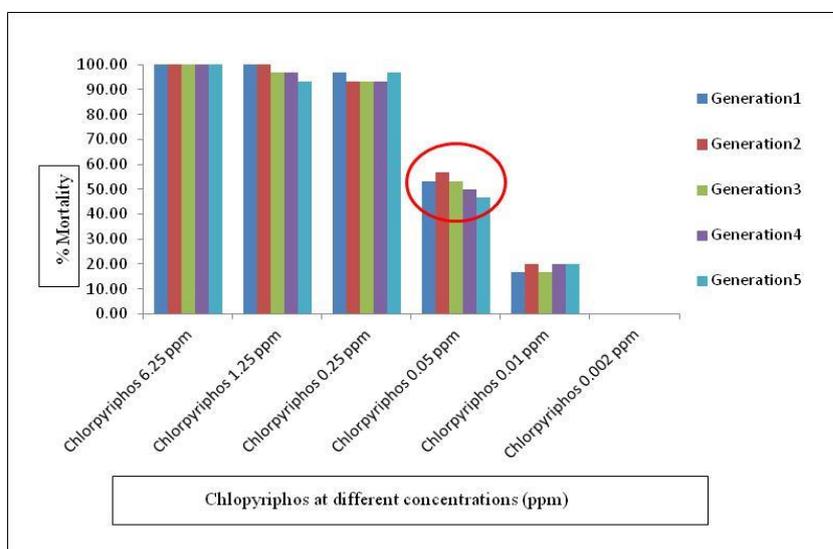


Figure 4 Graph indicating resistance in *S. litura* Chlorpyrifos at 0.05 ppm

### 3.3 Insecticide Spinosad 45SC(Tracer®)

The resistance studies were performed on the third instar larvae of *S. litura* exposed to different concentrations of Spinosad 45SC at concentrations like 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 ppm.

Table 5: 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

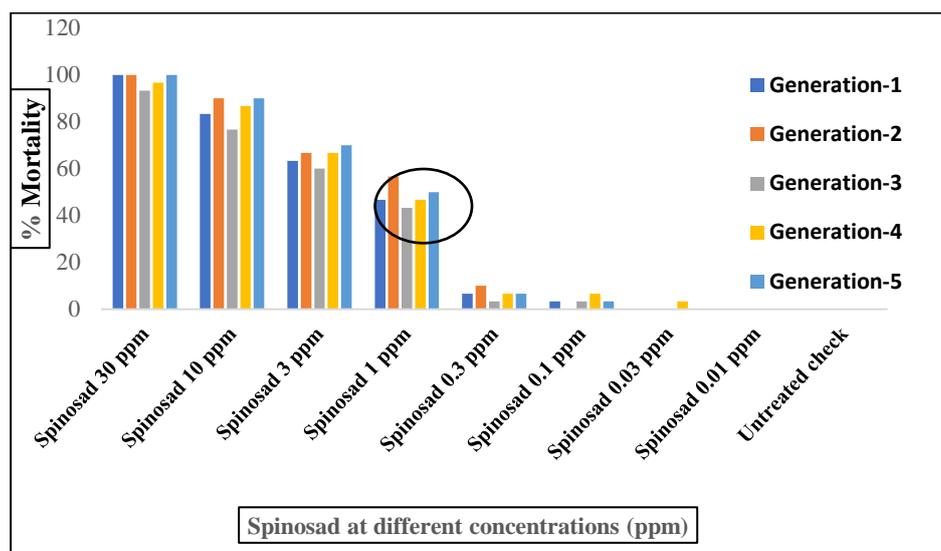


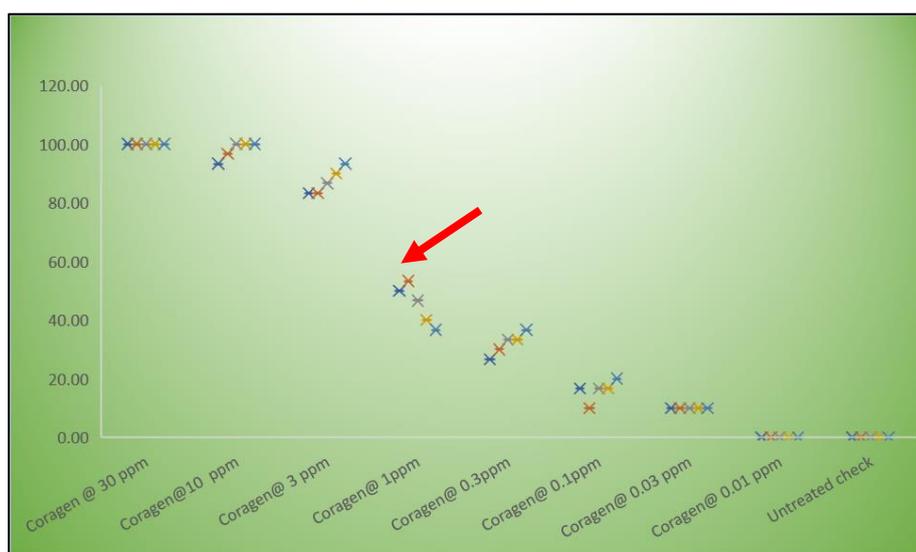
Figure 5 Graph representing resistance developed in Spinosad at 1 ppm

### 3.4 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. From third generation, the mortalities increased in all the concentrations except 1 ppm. 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 6).

Table 6 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  |
| 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   |

Figure 6 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 6). 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.

#### 4 KEY FINDINGS

- Insecticides sprayed in the fields are used non-judiciously by the farmers. They are not having proper knowledge about the label of the particular insecticide and symbols placed on the labels, which indicate toxicity level of the same.
- Though it is difficult to make the farmer understand, it proves to be helpful to the scientists and agriculture professionals worldwide. They can slowly educate the farmer by showing the long-term effects of such insecticides used in extensive and haphazard manner.
- The current studies showed onset of resistance in Coragen®, Chlorpyrifos, Cypermethrin and Spinosad in different generation with varying mortality values. This must be considered as an alarming situation. Spinosad being a bio insecticide can be used in the rotation of insecticide programs.
- Farmer awareness training programmes, flash mobs, interactive sessions with farmer are the ways and means to educate the farmer to address their issues related to pest attack, development of resistance, application of pesticides, understanding the labels for dosage of insecticides.
- With reference to the studies done so far in the area of resistance development against various insecticides, safety practices, pesticide use regulations, the correct application technologies and integrated pest management form the basis of developmental strategy for lessening exposure to harmful pesticides.
- *Spodoptera litura* is one of the pests, which has caused detrimental damage to crops like Castor, Cotton, Maize and Cabbage in the fields of Vadodara.
- Other pests included *Helicoverpa armigera*, *Scirpophaga incertulas*, *Chilo partellus*, *Chilo infuscatellus*, *Leucinodes orbonalis*, *Plutella xylostella*, *Earias vitelli*, *Scirpophaga auriflua*, *Percallia ricini*, Aphids- *Aphis gossypii*, *Lypaphis erysimi*, *Aphis craccivora*, Hoppers- *Nilaparvata lugens*, *Sogatella furcifera* (Table 3).

- There were broadly four ways implemented by farmers for controlling the insect pests: Biological methods, Mechanical, Cultural and Chemical methods.
- Unfortunately, Chemical methods were followed by 80% of the farmers
- Major insecticides belonging to Organophosphates, Carbamates and Synthetic Pyrethroids were used in Savli, Chhani, Waghodiya, Dabhoi, Varnama and Timbi.
- The insecticide resistance studies were carried out in laboratory conditions using four insecticides: Spinosad 45SC, Cypermethrin 25EC, Chlorpyrifos 20EC and Chlorantraniliprole 18.5 SC
- Exposure to bio-pesticide, **Spinosad** (Tracer®) for five generations indicated there was lowering down of mortality in the concentration of 1 ppm. 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% This meant that the insecticide was a potent insecticide and along with that it had the power to make the pest resistant.
- As compared to Spinosad, the resistance level developed due to **Chlorpyrifos** was higher. But this does not confirm the higher 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. Chlorpyrifos @ 0.05 ppm had a potency to develop resistance in the fifth generation as indicated by drop in mortality values from 53.33% in third generation to 50.00% in fourth generation to 46.67% in fifth generation
- When the pest was exposed to **Cypemethrin** at various concentrations like 2 ppm, 1 ppm, 0.5 ppm, 0.25 ppm, 0.125 ppm and 0.0625 ppm, there was development of resistance in one of the concentrations i.e 0.5 ppm. The onset of resistance was observed in the fifth generation, where the drop-in mortality was recorded as 46.67%
- Lastly the testing of **Coragen®** was done against third instar larvae of *S.litura* 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. Hence this was the most potent insecticide having a strong tendency to develop resistance in the pest

- These results can be extrapolated to field conditions, as the whole life cycle of the pest completes in natural surroundings and there are full chances of development of resistance, if rotation of insecticides is not followed (Rotation of Organophosphates and Carbamates with biopesticides).

## 5 CONCLUSION

- Resistance and generation turnover hold a strong relation
- The resistance developed in *Spodoptera litura* for four insecticides were in the order: **Coragen® > Cypermethrin 25EC > Chlorpyrifos 20EC > Spinosad 45SC.**

## 6 RECOMMENDATIONS

- Rotation of less toxic insecticides with bio pesticides as well as usage of bio products must be adopted to get maximum control which will lead to high yield. This will cause less harm to the farmers as well as people consuming food ultimately.
- Scientists can slowly educate farmers by showing long term effects of insecticides
- Information regarding the correct application of pesticides and the use of advanced technologies for target delivery of pesticides
- Farmer awareness training programmes, flash mobs, interactive sessions with farmer are the ways and means to educate the farmer
- The practice of using insecticides to get control of havoc caused by this pest, must be continuously monitored. If this is done, there would be progress not just on the economic front but also on the public health front.
- The practice of using insecticides to get control of havoc caused by this pest, must be continuously monitored. If this is done, there would be progress not just on the economic front but also on the public health front

- It is strongly recommended to make judicious use of insecticides in combination with bio pesticides so that the toxic effects if insecticides in the form of resistance development will be reduced

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