STUDIES ON THE DEVELOPMENT OF INSECTICIDE RESISTANCE IN SPODOPTERA LITURA FABRICIUS, 1775 (LEPIDOPTERA: NOCTUIDAE)

Synopsis of Ph.D. Thesis
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1 INTRODUCTION

Indiscriminate pesticide use is detrimental to the environment and human health and increases insects' resistance to pesticides (Srinivasan Ramasamy, 2012). With the positive impacts of all types of agriculture revolution, there were negative impacts too. They included, the slow buildup of pest pressure, control of crops by all types of insect pests, greed of farming leading to introduction of pesticides. The usage of pesticides started way back in 1948-49 with introducing BHC and DDT. After this the pesticides consumption started increasing by leaps and bounds. When there was heavy pest pressure, the pesticides were applied for getting a quick control. In this process, it is forgotten that the effect of pesticide usage is much more as compared to the quick solution provided. Hence there was introduction of various pesticides for controlling a wide range of insect pests. As the farmers think "If little is good, a lot more would be better".

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 (Mushtaq Ahmad, Iqbal Arif, & Ahmad, 2007). It is recognized as a serious cosmopolitan pest with considerable host range of economically agricultural crops such as cotton, groundnut, soybean, tomato and many other crops (Uematsu, 1992). The presence of this pest on different crops throughout the year has widely exposed it to insecticides and resulted in the rapid development of resistance to a range of insecticides (Munir Ahmad, Sayyed, Saleem, & Ahmad, 2008). The tobacco caterpillar, *Spodoptera litura* is an agriculturally important pest species. The management of the pest has therefore become increasingly difficult all over the world and the most commonly used insecticides are ineffective in controlling it (Tong, Su, Zhou, & Bai, 2013). The insecticide resistance is a serious problem which has gained impetus because of extensive use of insecticides. Baseline data on the susceptibility of the target pest to the toxicant is the most important factor for insecticide use especially for monitoring the development of resistance (Kaur, P and Kang, 2015).

Resistance to insecticides is a major problem associated with the chemical control of insect pests (Munir Ahmad et al., 2008) Previous exposure and selection with insecticides can confer cross resistance to newly introduced insecticides. The presence of pests on different crops throughout the year has widely exposed it to insecticides and resulted in the rapid development of resistance to a range of these insecticides (Bisset, Rodriguez, Soca, Pasteur, & Raymond, 1997)

The insecticides are classified into various groups according to the toxicity levels i.e extremely toxic, highly toxic, moderately toxic and slightly toxic. Hence keeping in mind, the indiscriminate use of various types of insecticides and toxicity levels conferred by insecticides, this study has been planned to observe the effect of various insecticides. Commercial formulations of insecticides used in this experiment were: Auzar® (Cypermethrin 25% EC, Biostadt) and Dursban® (Chlorpyriphos 20%EC, Dow Agrochemicals). Both these insecticides have greater effectiveness against all lepidopteran pests. *Spodoptera litura* is an indigenous pest of a variety of crops in South Asia and was found to cause 26–100% yield loss in groundnut (Dhir, B. C., Mohapatra, H. K., & Senapati, 1992) Following the reports of various insecticides which are sprayed by farmers in fields, this work was planned as a laboratory study in controlled environment. This study is expected to be fruitful in implementing integrated pest management strategies. It will prove to be a helpful data for the agriculture professionals as well as scientists working towards a common cause of resistance development in pests like *Spodoptera litura.* The purpose of this study is to take into account the development of resistance of *Spodoptera litura* a major pest of agricultural crops.

According to Wang, Lou, & Su (2019) army worm *Spodoptera litura* when exposed to 21 insecticides (traditional and modern) showed resistance in most of the insecticides. High resistance was found in Metaflumizone and Emamectin benzoate, moderate resistance in Chlorantraniliprole and low resistance in Spinosad. . Another study shows the mechanism of underlying resistance in *S. litura*, through RNA-Seq approach. It concludes the involvement of TOM , a tetra saccharide along with other upregulated genes responsible for resistance (Li et al., 2019).. The pest selected for this study causes much damage to agricultural crops like cotton, groundnut, soybean, tomato etc. at their vegetative stages along with the stages of blossoming. It has high ability to migrate large distances. Thus, it becomes important to continuously monitor the level of resistance developed.

Hence the **aim** of the current study is to:

Investigate generation turnover and time taken to develop insecticide resistance in Oriental leaf-worm the *Spodoptera litura* subjected to repeated application of various classes of insecticides.

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, Chlorpyriphos, 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

"Repeated use of the same class of pesticides to control a pest can cause undesirable changes in the gene pool of a pest leading to a form of artificial selection named as pesticide resistance"

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. Mostly 2^{nd}, 3^{rd} and 4^{th} instars were dominant. To prevent shock condition, the larvae were kept on natural diet for 2 weeks after which it was slowly transferred on artificial diet.

Figure 1: Egg mass of *Spodoptera litura* collected from Vadodara agricultural fields

Figure 2: Castor leaf completely infested with *Spodoptera litura*

Tissue papers were kept in these containers and moist conditions were maintained so that the collected culture did not desiccate due to dry conditions. The collection was done in the early morning time. The pest was then reared in laboratory conditions by keeping stringent conditions of temperature and humidity. Incubators were also used, if required to maintain constant conditions for the survival of test insect before testing. The culture was reared for at least three generations so as to ensure the health and infection free nature. After successful rearing, next generation was selected for testing of insecticide monitoring.

2.2 Rearing in laboratory conditions

Larvae of *Spodoptera litura* were reared in controlled laboratory conditions i.e. 25±2°C, 65-70% relative humidity and a photoperiod of L: D, 14:10. It was reared on artificial diet (Siddiqui, K. H., & Debjani, 2002). The diet was poured in a plastic container which had partitions in it. The larvae were carefully transferred on diet by using brush. The diet was changed at regular intervals. All the lab paraphernalia used for the whole process was pre-sterilized to avoid fungal and bacterial infections. 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. Another method was used for rearing i.e. rearing on natural diet. For this, collection was done for the second time, from the same fields on different days. The freshly laid yellow coloured eggs, covered with brown hairs were collected along with the leaves. Adults and larvae of *Spodoptera litura* were also collected and were bought to the laboratory in perforated polythene bags along with infested leaves.

Figure 3: Insect culture of *Spodoptera litura* reared in laboratory conditions

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 × 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 × 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. The Petri dishes having *Spodoptera litura* eggs and rearing jars containing larvae and adults were kept in B.O.D. incubator maintained at 27 ± 2ºC temperature and 78 ± 2 % relative humidity. Both types of rearing i.e natural diet and artificial diet was done,

to ensure the survival of larvae for the testing against insecticide. The larvae reared on artificial diet was then selected for testing in subsequent generation.

Figure 4: Rearing of larvae on artificial diet pieces kept in plastic container

Figure 5: Selection of 3rd instar larvae for bioassay

2.3 Insecticide selection

The insecticides which are selected for this study of Insecticide resistance are Spinosad, Chlorpyriphos, Cypermethrin & Coragen because these are widely used insecticide for lepidopterans. All the four insecticides belong to different class of insecticides. The data for two insecticides i.e Chlorpyriphos 20EC and Cypermethrin 25EC has been included. Date related to rest of the two insecticides would be included in thesis.

1. Spinosad: Spinosad is a novel mode-of-action insecticide derived from a family of natural products obtained by fermentation of S. spinosa. Spinosyns occur in over 20 natural forms, and over 200 synthetic forms (Spinosoids) have been produced in the lab (Watson, 2001)
2. Chlorpyriphos: Chlorpyrifos is active by contact, ingestion, and vapor action. It inhibits an enzyme the nervous system (acetylcholine esterase); this causes convulsions and paralysis
3. Cypermethrin: Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting neurotoxin in insects
4. Coragen: It binds to the ryanodine receptors located in the insect's muscle, and activates them. As a result, calcium floods out of the open receptors. As stored calcium is needed for contraction, muscles become paralyzed

2.4 Selection of larvae for bioassay

As described in the rearing procedure, the larvae of fourth generation were selected for resistance monitoring bioassay. The larvae selected for the experiment were pre-checked for any type of infections. After ensuring the healthy nature of larvae, third instar was selected for the bioassay. These were separated from mother culture and kept for starvation (1 hour) before initiating the experiment. The larvae were checked for even a minute type of infection. It was regularly checked under microscope for observing the growth.

2.5 Leaf Dip Bioassay

Table 1: Health Parameters of *S.litura*

Larvae (10 nos./set)	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	0.12	0.50	2.00	4
Set-7	28	0.14	0.60	1.97	4

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.* Test solution was prepared by using the commercial formulation of Spinosad (Tracer®, Dow Agro Sciences). Different ppm concentrations were made, using serial dilution process. Healthy and infestation free cotton leaves were collected from field and they were washed in laboratory using distilled water. 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±2°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. 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. Observations were recorded for mortality in each petri plate. 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. All these observations were recorded and if there were any special findings, there were also recorded. 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 seven generations and observations were recorded keeping all the parameters constant. Table 3 indicates the Lethal Concentration (LC) values for subsequent generations. The rearing process was done on artificial diet, while the testing was done by leaf dip. Data analysis

2.6 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.

Table 2: Mortality obtained in first generation of *S.litura* against Cypermethrin 25EC

Sr. no	Concentration (ppm)	Total live (TL)	Dead (D)	Moribund (M)	Total (D+M)	Percent 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 second generation of *S.litura* against Cypermethrin 25EC

Sr. No	Concentration (ppm)	Total live (TL)	Dead (D)	Moribund (M)	Total (D+M)	% 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 third generation of *S.litura* against Cypermethrin 25EC

Sr. No	Concentration (ppm)	Total live (TL)	Dead (D)	Moribund (M)	Total (D+M)	% 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 fourth generation of *S.litura* against Cypermethrin 25EC

Sr. no	Concentration (ppm)	Total live (TL)	Dead (D)	Moribund (M)	Total (D+M)	% 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 fifth generation of *S.*litura against Cypermethrin 25EC

Sr. no	Concentration (ppm)	Total live (TL)	Dead (D)	Moribund (M)	Total (D+M)	% 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* against Cypermethrin 25EC 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 against *S.litura* 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

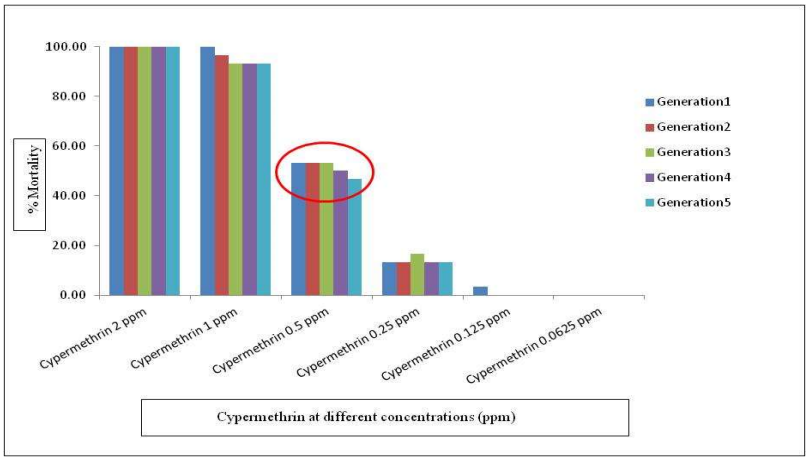

Figure 6: Graph representing resistance developed in Cypermethrin at 0.5 ppm

Table 9: Mortality obtained in first generation of *S.litura* against Chlorpyriphos 20EC

Sr. no	**Concentration (ppm)**	**Total live (TL)**	**Dead (D)**	**Moribund (M)**	**Total (D+M)**	**% 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

Table 10: Mortality obtained in second generation of *S.litura* against Chlorpyriphos 20EC

Sr. no	**Concentration (ppm)**	**Total live(TL)**	**Dead(D)**	**Moribund(M)**	**Total (D+M)**	**% 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 third generation of *S.litura* against Chlorpyriphos 20EC

Sr.no	Concentration (ppm)	Total live(TL)	Dead(D)	Moribund(M)	Total (D+M)	% 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 fourth generation of *S.litura* against Chlorpyriphos 20EC

Sr. no	Concentration (ppm)	Total live(TL)	Dead(D)	Moribund(M)	Total (D+M)	% 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 fifth generation of *S.litura* against Chlorpyriphos 20EC

Sr.no	Concentration (ppm)	Total live(TL)	Dead(D)	Moribund(M)	Total (D+M)	% 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: LC estimates of Chlorpyriphos 20EC against *S.litura* 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

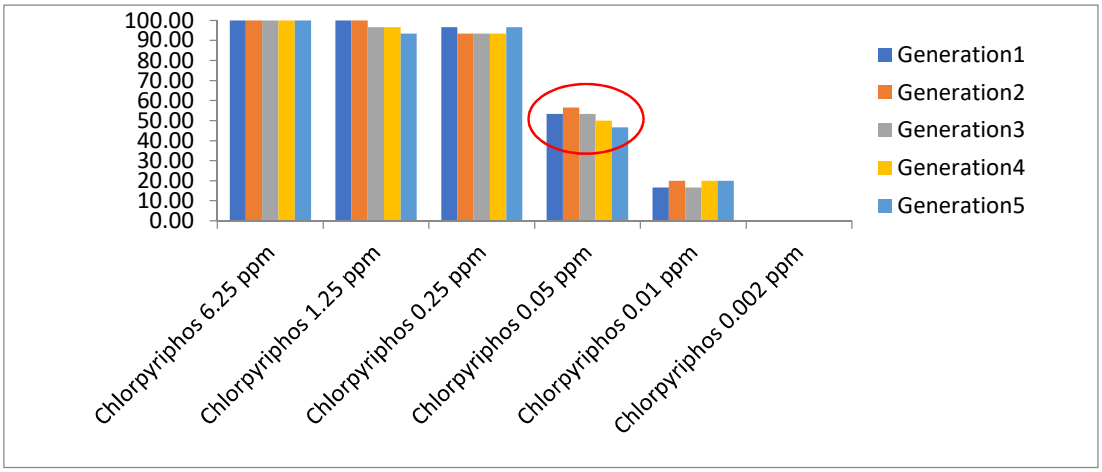

Figure 7: Graph representing resistance developed in Chlorpyriphos at 0.05 ppm

3 RESULTS

As Table 1 indicates, 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. If this is compared to the mortalities observed in previous generation , it shows that there is an onset of resistance developed in one of the concentrations i.e. 0.5 ppm as indicated by Figure 1. 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. Similarly another insecticide 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 as indicated in Figure 2. All these values indicate onset of resistance in both the insecticides in laboratory conditions. The experimental results indicate greater resistance developed in Cypermehtrin 25EC @ 0.5 ppm (LC 50: 0.49, LC 90: 0.94) as compared to Chlorpyriphos 20EC @ 0.05 ppm. (LC 50: 0.05, LC 90: 0.34). In today's scenario, the farmers are making extensive use of insecticides in lieu of getting fast control of pest attack. But in this process, they become unaware of the fact that resistance is developing slowly and the insecticides which are used extensively will slowly become ineffective.

4 DISCUSSION

This type of study shows that the insecticides if at all used, must be delivered in an effective rotation pattern based on their respective mode of actions. Though this 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 best alternative to this is switching to organic farming or using bio-pesticides which are having less adverse effect on environment and more positive and healthy effects on plants. The study accounts to discover the long-term effects of using the same insecticides on destructive pest like *Spodoptera litura.* It slowly shows onset of resistance in laboratory-maintained culture from generation to generation. If usage of insecticides is made, the insecticides must have less amount of stability and more reversion rate. Otherwise it will even cause more problems than providing right solution. This research shows that the insecticides belonging to two different groups must not be used in an uncontrolled manner. They have less stability as indicated by the mortality and LC values. The baseline values may be used for monitoring the resistance development for *Spodoptera litura*. Information regarding the correct application of pesticides and the use of advanced technologies for target delivery of pesticide, as well as intensive training on selective application of the correct pesticides at the correct time for the correct pests, should be disseminated to the user group. The current studies showed onset of resistance which may be considered as an alarming situation. At the same time, due to its less toxicity and biological properties, this type of bio insecticide can be used in the rotation of insecticide programs. Information regarding the correct application

of pesticides and the use of advanced technologies for target delivery of pesticide, as well as intensive training on selective application of the correct pesticides at the correct time for the correct pests, should be disseminated to the user group. The current studies showed onset of resistance which may be considered as an alarming situation. At the same time, due to its less toxicity and biological properties, bio insecticide can be used in the rotation of insecticide programmes. These were the results of resistance monitoring against two insecticides i.e. Cypermethrin 25 EC and Chlorpyriphos 20 EC. Rest of the two insectides i.e Coragen 20SC and Spinosad 45 SC were also tested for resistance. Its data will be included in the thesis.

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Insecticide resistance studies of Cypermenthrin 25EC and Chlorpyriphos 20EC against *Spodoptera litura* fabricius, 1775 (Lepidoptera: Noctuidae)

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