CHAPTER 3

3.0 MATERIALS AND METHODS

India which is the seventh largest country in the world in known for its rich heritage and unity in diversity. It has 29 states and 6 union territories. Gujarat, which is a state of western coast of India is the fifth largest Indian state in terms of area (Figure 1).

It is bordered by Rajasthan to the northeast, Daman and Diu on the south and Dadra- Nagar haveli and Maharashtra to the southeast, Madhya Pradesh to the east. Vadodara is in the eastern part of the state of Gujarat in western India (Figure 2). It is located at 22º11' N latitude and 73º 07' E longitude. Vadodara District covers an area of 36818.7 acres.

Historically several dynasties have ruled over this township like the Guptas, Chalukyas and Rashtrakutas and Solankis to name a few. After 1298 AD, during the Moghul rule in Gujarat, Vadodara became a district town continued to be an important centre for trade, commerce and even military settlements (Rajyagor and Tripathy, 1979: 1-2). However, the history of Vadodara completely transformed after defeating Moghuls by Pilajirao Gaikwad in 1732 AD and re-capturing the territory by Damajirao Gaikwad II in 1734. After which, continuous wars and quarrels for expansion of the reign by other Gaikwad rulers like Sayajirao II, Ganpatrao and Khanderao resulted in establishment of Baroda state with a vast area to its credit and Vadodara city being the capital of their rule. Gaikwad rule changed the legacy of Vadodara during their rule from 1734-1949 (215 years) till its merger in the state of Gujarat making it a 'cultural capital' (sanskaar nagari) of Gujarat. Famous Gujarati poet Premanand has described it as the 'brave yard'. The state of Baroda at that time comprised of five districts i.e. Baroda, Kadi, Navsari, Amreli and Okhamandal to enable smooth and efficient functioning of the state affairs by Gaikwad rulers (Kantawala, 1992: 138).

Figure 1 The satellite image of the state of Gujarat indicating different districts

(Source: https://www.google.com/maps/place/Gujarat/)

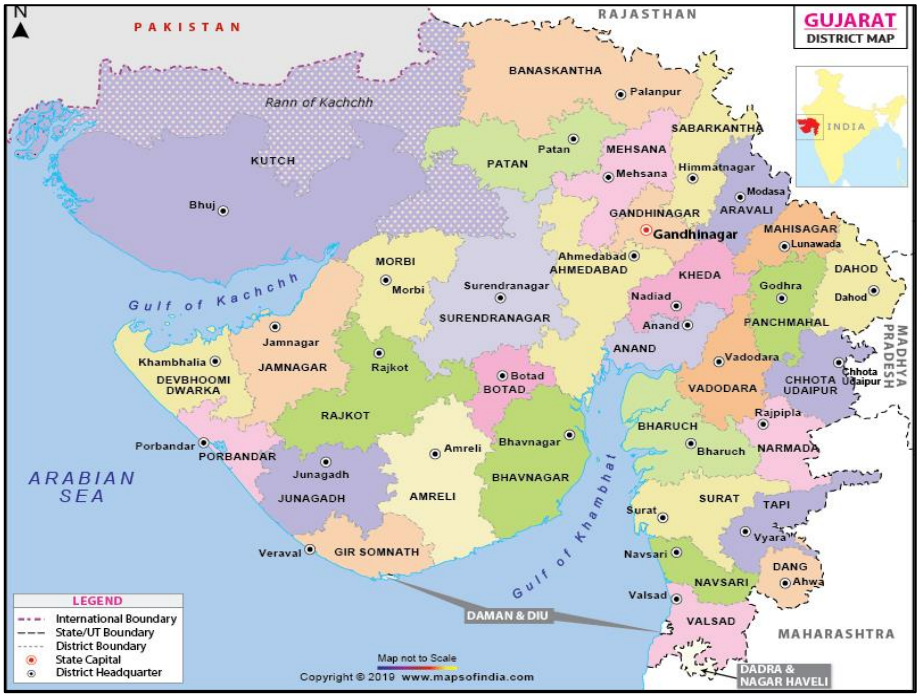

Figure 2 The district map of Gujarat indicating the study location of research

(Source: https://www.mapsofindia.com/maps/gujarat/gujarat.htm)

Figure 3 District-wise distribution of agricultural crops in Gujarat

(Source: https://www.mapsofindia.com/maps/gujarat/gujarat.htm)

Along with the crops, the pest distribution was also closely observed in these fields, as it was very important to know these parameters before conducting the research study in laboratory conditions. The field information plays a very important role in knowing the actual scenario of application of pesticide and its effect on crops and pests. Thus, at the beginning of the research survey, wide-ranging exploratory work was carried out on the pest occurrence, the climatic conditions of all the main seasons during which the pest is seen, the irrigation facility provided by the farmers, the insecticide sprays done by the farmers before and after the field preparation. Though the research work was carried out in laboratory conditions, the field scenario was very crucial for the understanding of pest occurrence, pesticide assessment, the effect of pesticide on the pest in the actual field condition and the problem of resistance development in pest.

The information on sprays occurring in these fields was recorded beforehand, taking help of the local farmers at the time of collecting the populations of the pest. Hence this entire exercise was carried out in the first year of research i.e. 2016 and the initial years of 2017 (March 2016 to February 2017).

3.1 The statistical reports related to agriculture

The statistical reports were required for the knowing the complete background of the studies before undertaking it as research. This exercise was done for the purpose of getting the complete information around numbers and statistical data of crops sown, area wise. The agricultural fields of Gujarat are divided in many important areas. These reports were taken from the official websites of Gujarat government. These values were taken for consideration while conducting the research work. All the reports indicated the crops sown; yield obtained area wise. These data were supporting framework as the results of this research can be extrapolated to field conditions. The reports referred were of two seasons, Summer and winter. The seasonal distribution of crops has always been a region's strength of agricultural farming. Agriculture plays an important role in any country's economy. The main crops like Paddy, Bajra, Maize, Cotton, Castor, Moong, Udad, Sugarcane, Onion etc are distributed in a precise manner and these reports are readily available on the government online sites.

Before moving on to the actual research study, the statistical reports related to agriculture were studied thoroughly to have an insight of the main crops, season patterns, crop wise distribution in the state of Gujarat. The official websites of Gujarat government were mainly considered to ascertain the information for the same.

Gujarat State: Area Covered During Summer 2019 As on Date: 06-05-2019 (Area in Ha.) (FINAL)					
Sr. No.	**Crops**	**Normal Area (Last Three year Avg.)**	**Area of Previous Summer Season**	**Progressive Area of Summer 2019**	**% Over Normal**
1	**Paddy**	48,435	55,504	29,494	60.89%
2	**Bajara**	249,652	245,556	228,049	91.35%
3	**Maize**	10,094	11,481	3,101	30.72%
Total Cereal Crops		**308,181**	**312,541**	**260,644**	**84.57%**
4	**Mung**	32,089	30,067	21,211	66.10%
5	**Udad**	8,510	8,130	4,890	57.46%
Total Pulse Crops		**40,599**	**38,197**	**26,101**	**64.29%**
6	**Groundnut**	60,450	52,349	28,060	46.42%
7	**Seasamum**	16,789	15,967	18,887	112.50%
Total Oilseed Crops		**77,239**	**68,316**	**46,947**	**60.78%**
8	**Onion**	11,375	9,124	1,814	15.95%
9	**Sugarcane**	10,552	14,656	5,395	51.13%
10	**Vegetables**	86,649	75,148	72,249	83.38%
11	**Fodder**	236,043	232,229	268,450	113.73%
12	**Guar gum**	7,512	5,337	3,177	42.29%
13	**Others Crop**	4,655	3,664	1,417	30.44%
State Total		**782,805**	**759,212**	**686,194**	**87.66%**

Figure 4 Area covered by agricultural crops in summers (2019)-Gujarat

(Source: https://dag.gujarat.gov.in/sowing-report-2019-20.htm)

This gave a complete and holistic picture of the background details like the overall cropping pattern, and area covered by each cop in tonnes/hectare (Figure 4). Such reports and data formed a very important basis for all types of selection, i.e. selection of pest from the crop which is damaged to a larger extent. As the area covered by the important crops were known beforehand, it was possible to select the right pest for the research study.

Frist Advance Estimates of Area, Production and Yield of Major Kharif/Rabi crops of Gujarat State for the year 2019-20.

A :- Area in "000" hectares; P :- Production in "000" Tonnes; Y :- Yield in kgs./Hectare

No.	Crop	Kharif		
		Area	Prod.	Yield
A	Foodgrains			
1	Rice	834.49	2068.29	2478.51
2	Jowar	33.72	40.67	1205.89
3	Bajra	173.74	278.89	1605.23
4	Maize	302.95	517.76	1709.08
5	Ragi	10.48	8.43	803.72
6	Small Millets	6.64	7.86	1183.43
	Total Cereals	**1362.02**	**2921.90**	**2145.26**
7	Tur (Red Gram)	210.28	277.38	1319.10
8	Udad	89.77	60.36	672.40
9	Mung (Green Gram)	93.59	54.63	583.70
10	Math	11.29	6.37	564.36
11	Other Pulses	1.13	0.58	516.56
	Total Pulses	**406.05**	**399.32**	**983.42**
	Total Food Grians	**1768.08**	**3321.22**	**1878.43**
B	Oil Seeds			
13	Groundnut	1550.38	3188.97	2056.90
14	Castor seed	647.93	1456.66	2248.17
15	Sesamum	116.19	71.65	616.65
16	Rapeseed & Mustard	0.00	0.00	0.00
17	Soyabean	100.18	120.33	1201.12
18	Other Oilseeds	3.00	1.28	425.00
	Total Oil Seeds	**2417.68**	**4838.88**	**2001.46**
C	Cash crops, Vegetables, Spices & Other			
19	#Cotton	2666.40	9272.63	591.19
20	Sugarcane	0.07	4.20	60000.00
21	Tobacco	56.23	125.70	2235.48
22	Guar Seeds	141.23	105.64	747.96

 # Production of cotton in '000' bales each of 170 kgs in unit & productivity of cotton is in lint.
Other Oilseeds mainly includes Niger & Sunflower
Production & productivity of Paddy in terms of Rice.

Directorate of Agriculture
G.S.Gandhinagar

Figure 5 Advance estimate of Area crops in winter season in Gujarat

(Source: https://dag.gujarat.gov.in/images/directorofagriculture/pdf/Frist-Estimate-2019-20.pdf)

Thus, all these statistics indicate the importance of crops like cotton, castor, maize, sugarcane for the state of Gujarat. The district of Vadodara, where the laboratory research was carried out, also has a rich history of agriculture. The same exercise was done for knowing the crop pattern, information on the area covered by economically important crops of Vadodara, the irrigation pattern etc. The official websites of Vadodara agriculture were referred during the entire study(Figure 5)They played a very crucial role in understanding the crop pattern and hence co-related with the pest pattern, which was the main concern of this entire research plan.

Table 1 The Agro-Ecological situation (AES) of Vadodara district

(Source: http://kvkvadodara.org/district-profile/, Krishi Vigyan Kendra, Vadodara)

Sr. no.	Name of AES	Situation	Crop grown	Cropping pattern	Taluka/Mandal covered
1	AES-I	Sandy Loam soil with high rainfall	Predominately Maize, Cotton, Tur, Tobacco, Vegetables & Horticulture crops	Cotton based Paddy-Wheat Pigeonpea based Tobacco-Bajra Vegetable based Sesamum-Jowar	Vadodara, Savli, Padara, Vaghodia, Part of Dabhoi
2	AES-V	Medium Black soil with high rainfall	Predominately Maize Pulses, Drilled Paddy, Hill millets	Drilled Paddy-Groundnut Maize – Pulse Hill millets-fallow	Pavi Jetpur, Naswadi, Part of Chhota Udaipur
3	AES-IX	Deep Black soil with high rainfall	Major Banana, Cotton, Vegetables, Sugarcane	Cotton based Banana based Sugarcane based Paddy-Wheat	Karjan, Part of Dabhoi, Sankheda, Shinor
4	AES-XII	Hilly Light soil with high rainfall	Drilled Paddy, Maize, Pulses	Drilled Paddy- - Groundnut Maize – Pulse Hill millets- - fallow	Part of Chhota Udaipur & Kavant

During the survey, it was found out that there were different pests observed in different areas of Vadodara, depending on the predominant crops in those areas. As discussed, in the above introductory session, the crops found in various areas were listed down and distributed area wise. The main crops found were cotton, tobacco, maize, vegetables and other horticultural crops.

3.2 The selection of pest

After collecting all the background information required for the research study, the selection of pest was done on the basis of severity of damage, its occurrence and response to insecticidal sprays. During the field visits, it in the agricultural fields of surrounding the district of Vadodara, it was found out that the major crops sown were castor, cotton, pigeon pea, maize. The field visits were conducted in the months of August, September and October 2016 and the initial months of the year 2017. The time in the morning was the best time, as the pest pressure was seen in early morning and sometimes during noon time. Hence visits were done in morning time 6:30 am to 8:00 am and the pests which were

observed were listed down in record books along with the crop infested. Along with this, the information was also captured for the chemical sprays done, if any. This information was taken from the farmers which remained present sometimes during these visits.

This type of information played an important role in selecting the pest for research work. The number of pests attacking various crops were noted down. The irrigation provided, the chemical sprays done, temperature and humidity conditions in field were also added to the notes. Various pests found out were belonging to different families. Some of them were collected and brought to laboratory and some were just identified and left back as it is, to the fields which it had infested. Out of all these pests, it was found out that *Spodoptera litura* was pre-dominantly seen infesting the major economically important crops like cotton, castor and maize. In some fields it had infested forming patches in the farmer's area. The level of infestation caused by this pest was a bit disturbing to the farmers of that locality. The pest had attacked severely to castor fields and mostly egg masses were identified on the underside of the leaves of castor. Larval damage was also found out near the middle of the leaves near midrib portion. This type of damage was observed in alternate rows and smaller and tender leaves. The farmers were also asked about this scenario. They too shared some photographs of their other farms, in which same kind of infestation was found out in the castor and cotton fields. It became very important to study the level of infestation. The selection of pest was done in such a manner that it would be fruitful for the scientific as well as agricultural community. Hence *Spodoptera litura* was selected as the test insect and further the studies were planned for rearing in laboratory conditions. It was not possible to bring the pest every time from the field for performing bioassay. A site survey was done in some parts of Vadodara, Gujarat and populations of *Spodoptera litura* were collected from fields nearby regions of Vadodara in Gujarat. The information on sprays occurring in these fields was recorded beforehand, taking help of the local farmers at the time of collecting the populations of the pest.

Table 2 Crops infested with various pests during field survey

Sr. No	Crop	Pest	Infestation level
1	Cotton	Locust	
		Termites	
		Aphis gossypii (Aphid)	
		Myzus persicae (Jassid)	
		Bemisia tabaci (Whitefly)	
		Maconellicoccus hirsute (Hibiscus Mealy bug)	
		Scirtothrips dorsalis (Thrips)	
		Platyedra gossypiella (Pink bollworm)	
		Helicoverpa armigera (American bollworm)	
		Spodooptera litura (Cotton leaf worm)	
		Estigmene lactinea (Hairy caterpillar)	
2	Castor	Termites	
		Nezara gramineae(Green Plant bug)	
		Achaea janata (Semi looper)	
		Spodooptera litura (Cotton leaf worm)	
3	Maize	*Chilo partellus* (Spotted stalk borer)	
		Scirpophaga incertulas (Yellow stem borer)	
		Spodooptera litura (Cotton leaf worm)	
		Myzus persicae (Jassid)	
4	Paddy	*Sesamia inferens* (Pink stem borer)	
		Spodooptera litura (Cotton leaf worm)	
		Leaf hopper	
		Beetles	
		Nezara gramineae(Green Plant bug)	
		Termites	
		Pyrilla perpusilla (Sugarcane leaf hopper)	
		Oryctes rhinoceros (Rhinoceros beetle)	
		Holotrichia insularis (White grub)	
		Myzus persicae (Jassid)	
		Scirpophaga auriflua (Sugarcane top shoot borer)	
6	Wheat	Locust	
		Termites	
		Rhopalosiphum maidis (Maize pahid)	
		Pyrilla perpusilla (Sugarcane leaf hopper)	
		Nezara gramineae(Green Plant bug)	

Low
Medium
High

Hence during the extensive survey various agricultural fields, it was found out that *Spodoptera litura* pest had caused a heavy damage and infestation in cotton and castor fields (Figure 6). This pest had caused a havoc in the castor fields as well as cotton fields, and the sprays done by the farmers in these areas, were ineffective too. The pest was re-appearing in every season, at its particular time (Figure 7).

Figure 6 Heavy infestation of *Spodoptera litura*

Figure 7 Damage symptoms observed in Castor leaf

This indicated the gradual development of resistance in this pest, as the insecticidal sprays used every year were not achieving the success to curb the damage caused. Moreover, the damage by this pest was also seen in the crops of tomato (Figure 8) ,maize (Figure 9) and cabbage (Figure 10).

Figure 8 Damage symptoms observed in tomato leaf

Figure 9 Damage symptoms observed in the crop of maize

Figure 10 Damage symptoms observed in the crop of cabbage

3.3 The life cycle of *Spodoptera litura*

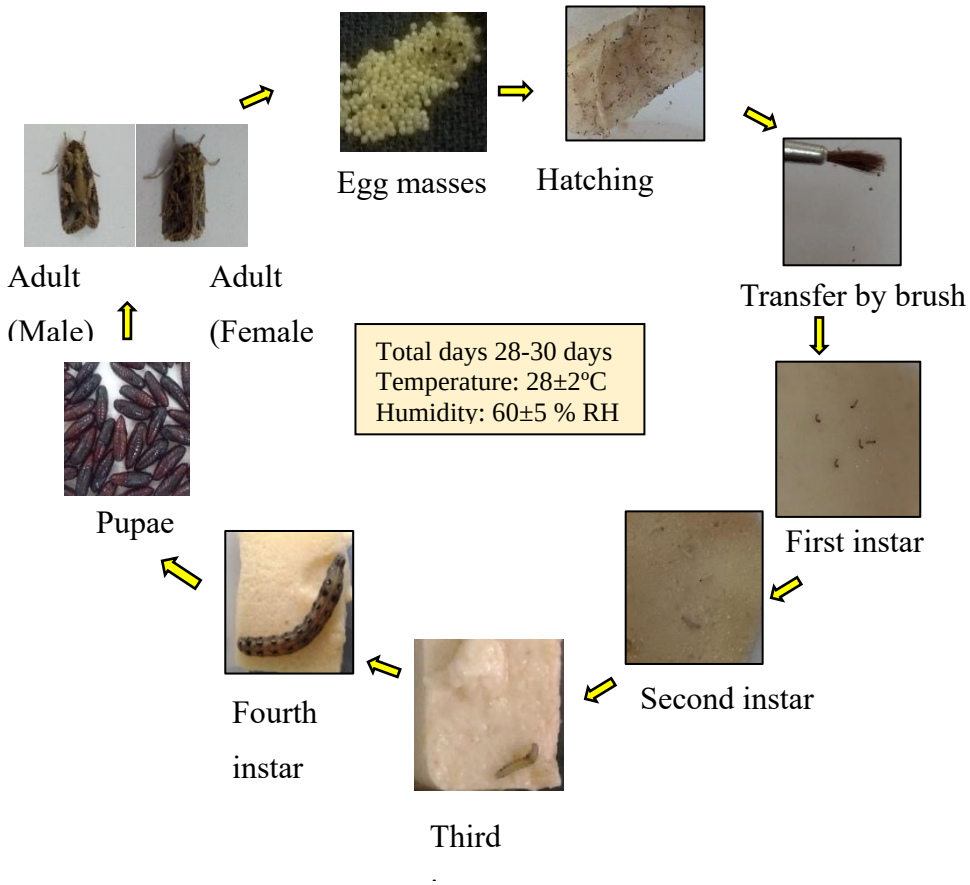

Figure 11 Life cycle of *Spodoptera litura* maintained in laboratory conditions

The whole life cycle of *Spodoptera litura* took around 26-28 days for completion of all the stages from hatching, First instar, second instar, third instar, fourth instar, fifth instar, pupal stage and adult emergence(Figure 11). The first four instars took 15 days to complete the stages, while fifth instar and pupal stage was completed in 5-6 days. After adult emergence, the females survived for longer than males (4-5 days) even after mating. All the stages were maintained in laboratory conditions (26±2º C and RH 60-65%). Thus the whole life cycle was maintained in laboratory, keeping in minds all critical points such as use of sterilized spatula for diet pieces, use of sterilized forceps for handling of the culture. All these things were taken care of, while performing the whole process of rearing.

3.4 Collection and preservation

A mixed culture containing mostly smaller instars like second and third instars were collected in separate bowls along with healthy leaves for survival. Pupae and adults were collected in plastic jars with holes. 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 of pests was done in the early morning between 6:00 am to 8:00 am. The pest was then reared in laboratory conditions at 27±2°C and 65-70% relative humidity (RH). Incubators were used, to maintain constant conditions for the survival of test insect before conducting bioassays.

3.5 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). Larvae of *Spodoptera litura* were collected from castor and cotton fields (200-300 larvae). It was reared on artificial diet prepared in laboratory using a hot plate and the diet ingredients (Figure 12)

The diet was poured in a plastic container which had partitions in it. The larvae were carefully transferred on diet by using brush (Figure 13). The diet was changed on alternate days in the initial stages i.e. first and second instars, as it was very crucial stage. After attaining third instar, the diet was changed every two-three days depending on the condition of diet. Regular scrutiny was done for any kind of smallest infection (Fungal or bacterial) in the diet, as it had excreta of the pest. The lids of the boxes were closed and small pinholes were done on the surface of the containers to ensure ventilation. This was also done to avoid high moisture micro-environment created inside the rearing boxes. Hence rearing an infection free culture was initially a challenge as it was affected by external as well as internal factors. Thus, rearing of the pest was successfully for the further bioassays.

Figure 12 Artificial diet for rearing of *Spodoptera litura*

Figure 13 Hatching of larvae taken with care by Camlin brush

Figure 14 First instar larvae of *S.litura* transferred on artificial diet

The initial hatching in the mother culture, started in oviposition pots prepared. Hatching larvae was carefully taken with the help of Camlin hair brush (0

number and 1 number). Utmost care was taken during this process, as there were chances of damage by the brush due to smaller and delicate age of larvae. In this manner, the hatching larvae was carefully transferred on to the artificial diet. After two days, slowly the stage of larvae changed to proper first instar and was now visible clearly on the diet (Figure 14). After three to four days, the stage changed to second instar, showing a faint blackish ring like structure below the head portion. The larvae size gradually began to increase and a dark band was now clearly visible below the head portion (Figure 15). Gradually, there was instar change to post third, pre fourth and fourth instar (Figure 16) The healthy pupae obtained was a sign of healthy culture. The pupae were thoroughly checked for any deformity, cracking down, full formation etc (Figure 17). All the lab paraphernalia used for the whole process was pre-sterilized to avoid fungal and bacterial infections. The transferring of larvae becomes very crucial; hence utmost care was taken. Until pupation, the larvae were kept on artificial diet. The pupae turned to adults, male and female adults, indicating a clear difference in pattern and colour in male and female as described in the life cycle. Hence the life cycle was maintained with utmost care in laboratory conditions. The adult health was regularly checked for any kind of deformity, scales, pattern and also activeness. As discussed in the later section, other health parameters like larval size, pupal height, adult longevity were recorded regularly, to keep a keen watch on the overall health of culture. The larval activity and movements were also indicators of robustness and good health. Along with this, at the time of adult emergence, the scales developed from all sides, the activeness of adult, were all marked in record book and anything unusual was noted down. During the oviposition cages preparation, mostly the healthy males and females were taken to have a healthy and robust culture. The initial stages of the life cycle i.e hatching, first instar, second instar, and third instar were maintained in the same trays, as there was no cannibalism observed. Later during the late third instars, the larvae were separated to be kept in small boxes with partitions in it. All of it was maintained on artificial diet. The diet had a particular concentration of water, due to which it was prone to infections, if not mixed properly at a particular stage of diet preparation.

Figure 15 Second instar larvae of *S.litura* reared on artificial diet

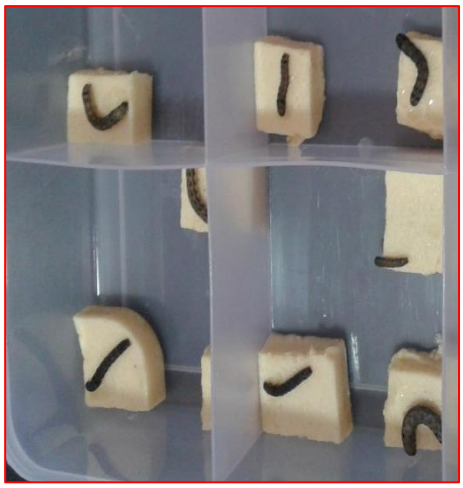

Figure 16 Pre-fourth and Fourth instar larvae of *Spodoptera litura*

Figure 17 Healthy pupae of *Spodoptera litura* reared in laboratory

Rearing in container was feasible as there was no cannibalism observed in *Spodoptera litura.* After complete formation of pupae, they were transferred to bowl. The completion of pupal stage leads 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(Male: Female). Adult diet was also provided by using honey solution. The moths were provided with water and honey solution. Another method was used for rearing i.e. rearing on natural diet. The freshly laid egg masses from fields 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. The eggs were kept in Petri dishes (11 cm dia.) They were covered with fine muslin cloth and secured with rubber bands. muslin cloth and secured with rubber bands. The larvae were kept in rearing jars (15 cm × 13 cm) covered with They were daily supplied with fresh cabbage leaves for feeding. The adults were also kept in plastic pots, available easily in local market, supplied with a piece of folded paper for oviposition (Figure 19). Cotton swabs dipped in 50 % honey solution were hanged from the top in order to provide feeding material for adults (Figure 18). The honey solution was renewed after every 48 hours. Hence rearing was done in laboratory conditions taking utmost care (Figure 19) The larvae which were reared on natural diet had many challenges like plant health, virus in plant material, fungus development, changing of diet on regular intervals. In contrast, those reared on artificial diet had different challenges like, diet developing infections, larvae preference for artificial diet etc. The rearing in lab was successfully achieved after continuous efforts and critical guidance. The adult health was regularly checked for any slightest infections. Also, the laboratory was kept clean along with the lab apparatus used for the experiments. The pots which were used for oviposition were cleaned at regular intervals, to remove the feacel matter and the wings which used to be dropped in the cages while flight in the small space of the pots. Black cloth was used to cover the whole culture to provide the dark conditions. Hence the whole procedure was developed as a standard operating procedure, by enlisting the points of check. The lab paraphernalia was always kept clean, to avoid any kind of infections. The plastic containers used to pour the diet, was cleaned immediately after use, with hot water to make it sterilized and fungus free. The diet was kept at room temperature to solidify to optimum extent, sue to which the diet pieces were not holding much water and diet was consistent.

Figure 18 Plastic box with partition for rearing process

Figure 19 Oviposition cages, larval culture of *Spodoptera litura*

Figure 20 Careful rearing of *Spodoptera litura*

3.6 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 before initiating the experiment.

3.7 Selection of insecticides

The insecticides were selected based on the market survey as well the field survey done. From 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. In this survey, the data of insecticides majorly used in various crops were listed down. As the farmer was having less knowledge about the chemistries of these insecticides, they were only asked for the names of chemicals used for the sprays. The farm owners were informed beforehand, about the research work background for getting the whole information. There were fields of Cotton, Castor, Maize, Pigeon pea, Tobacco which were surveyed. The common insecticides used for these fields were Deltamethrin, Cypermethrin, Chlorpyriphos, Coragen, Bifenthrin, Lambda Cyhalothrin, Endosulfan, Spinosad. Thus, the insecticides belonging to IRAC (Insecticide Resistance Action Committee) groups were Organophosphates, Pyrethroids, Spinosyns, Diamides and Cyclodienes.

Out of these mostly the farmers used Pyrethroids, Organophosphates, Diamides and Spinosyns were used extensively in some areas. Another survey was done to know the locations from where these pesticides were available in the market i.e pesticides shops from where the farmer used to buy these chemicals. This was done to know the exact information about the insecticides and to know the approximate amount the farmer is spending in lure of getting a healthy crop. Fortunately, or unfortunately, the results of this survey were eye openers for me. The farmers were spending a whole lot of money just to maintain the crop. In

response to this what they are getting in long run was only “Resistance development” to the same insecticides sprayed in the field. The survey done for the local pesticide market shops (Table 3) created a great interest and was a very useful piece of information, for selection of insectides for the bioassays. The commercial formulations of all the insectides were very easily available in the market.

Table 3 Survey of Pesticide shops in Vadodara, Gujarat

Sr.no.	Name of the shop/agency	Area	Postal address
1	Dharti Pesticide	Navapura	A-9. Sardar Patel Vegetable Market, Opp Kevda baug, Bagikhana Road, Navapura, Vadodara-390001. Phone number: 0265-2974713
2	Kanani Pesticides & Biochemical Pvt. Ltd.	Navapura	H-22, City Enclave Society, Near Polo Ground, Navapura, Vadodara, Gujarat 390001 Phone: 094288 20196
3	Gayatri Pest control	Kothi	Surat Blood Bank Building, Kothi, B/S Kuber Bhavan, Kothi, Vadodara, Gujarat 390001 Phone: 02652424381
4	Sun Agro Sales	Chhani	Panchayat Complex, Bus Stand Road, 15, Sokhda Rd, Chhani, Vadodara, Gujarat 391740 Phone: 09426508431
5	Shreeji Pecticides	Raopoura	Laxminarayan Bhavan, Behind Khanderao Market, Raopura, Raopura, Vadodara, Gujarat 390001 Phone: 02652433779

Sr.no.	Name of the shop/agency	Area	Postal address
6	Bharat Traders	Kevda Baug	9, Jam Chamber, Kevda Baug, Opposite Sardar Patel Market, Kevda Baug, Vadodara, Gujarat 390001 Phone: 089808 04125
7	Akshar Krushi Seva	Kevda Baug	Shop No A/4 Sardar Patel Vegetable Market, Main Road, Kevdabaug, Vadodara - 390001, Nr Jayratna Building (Map)
8	Krishna Trading corporation	Por	12, Shreenath Plaza,No 8,National Highway ,Por,, Behind Shah Petrol Pump, Vadodara-390001
9	Krushiko Sales and Service	Jubilee Baug	Tarkeswar Mandir, Jubilee Baug, Vadodara-390001
10	Intensive pest control PVT. LTD.	Alkapuri	Intensive pest control PVT. LTD. 510, 511, 512, 5th floor, premier chamber, near LIC Office building, alkapuri (O): 0265-2432239,2343856
11	A Saj Agricare PVT. Ltd.	Kirtistumbh	A Saj Agricare PVT. Ltd 3 lad apartments, opp. Pologround, V.K. Marg, vadodara- 390001. (O): 0265-2433355,2433292 (M): 9898139649
12	Goverdhan Traders	Sardar patel market	Mukesh Fertilizers 10, Jam chembers, opp. Sardar patel market, nr. Kevda baug. Vadodara-390001. (O): 0265-24260

Sr.no.	Name of the shop/agency	Area	Postal address
13	Ideal Pest Control Services	Gorwa	Ideal pest services in gorwa, 3-2, bakul park, b/h sahyog society, G.R. Road, Gorwa, Vadodara-390016 Ph: 2391480, 2395391
14	Gujarat Agro Agency	Khanderao market	Khanderao Market, Ahead of Gurudwara, Near market Char Rasts, Vadodara-390001
15	Pest World	Baroda Dairy	Pest World, 12- ground floor, Annapurna bhavan, silver coin, near aagan tower, Makarpura three way, Manjalpur. (M): 9825017622
16	Azad Agencies	Dandia Bazar	Opp. Siddhivinayak Temple, Dandia Bazaar, Vadodara- 390001. (O): 0265-2433622
17	Gujarat pest control	Lehripura	Gujarat pest control in fatepura, opp. Nyay mandir, khajuri market, lehripura road, Fatepura- 390006 (O)- 0265 2429007
18	Narmada Pest Control Services	Kareli Buag	Narmada Pest Control Services, Ground floor, prasant apartment, opp. Mental hospital, karelibaug, vadodara-390018
19	Patel pesticides	Tarsali	Sayona Chambers, R.M Road, Near Tarsali Bus stand, Tarsali, Vadodara, 390001
20	P.R. Corporation Private limited	Alkapuri	301. Capri House, Behind Express Hotel,Alkapuri, Vadodara-390007
21	Ashok Pesticides	Kareli Baug	23/24, Near Char Bhuja Complex Dhawal Park, Behind Char Bhuja , Kareli Baug

This type of survey helped me for the selection of insecticides for the research work, as it gave me insight of the actual scenario of its applications in farmer's fields.

3.7 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 (Figure 21)

Healthy and infestation free cotton leaves were collected from field and they were washed in laboratory using distilled water. Leaf discs of five centimetres 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. These test units were then carefully opened, and the larvae were checked for mortality, one by one by using Camlin brush and forceps. Hence utmost care was taken to keep the mother culture as well the tested culture, infection free. The monsoon season was full of challenges, as there were maximum chances of getting infection through fungus and virus due to humid conditions. The lab paraphernalia was kept very clean and tidy every time the culturing work was performed. Proper sterilization of pupae and forceps used to transfer the larvae were done on a regular basis. This served as a base of infection free culture. After performing the leaf dip bioassay, the observations were completed within

2 hours on the day of observation. On completion of observations, every time the leaf discs were discarded in a proper manner so that the mother culture would not get exposed to any kind of infections. The chemicals were properly disposed as per the disposal guidelines of the department.

Figure 21 Different concentrations of insecticide solutions

Figure 22 Healthy cotton leaf for leaf-dip bioassay

Figure 23 Leaf discs cut out of healthy cotton leaf

Anything unusual was captured in datasheet. At 96 hours, the observation was taken using camel hair brush, which was pre-sterilized. The leaf- dip bioassay was performed with the help of healthy cotton leaves brought from the unsprayed cotton fields (Figure 22) Leaf discs were cut using disc cutter, which was available in local market and these discs were further used for performing the bioassay (Figure 23)

The leaf discs which were cut were kept in moist tissue paper to avoid desiccation of leaves. These leaves were then dipped in various concentrations of insecticides for 10 seconds and then dried for half an hour.

Uniform shaped leaf discs were cut with the help of leaf-cutter. Leaf cutter was procured from a local vendor. Leaf discs were dipped in the insecticide solution for 10 seconds for all the concentrations of treatments. Separate forceps were used for separate treatments. Untreated check was also kept to compare the results of treated ones. After dipping, these discs were dried in room temperature on aluminium foil. After ensuring the drying of leaf discs, they were placed in petri plates (Borosilicate Glass, 40 X 12 mm) and larvae were released. Third instar larvae were released with extra care on these dried leaves and 30 larvae were released per treatment to the treated leaf discs as well to the untreated control (10 larvae per replication and 3 replicates per treatment) and These discs were placed in small petri plates. All the petri plates were previously marked according to the diluted concentrations of the treatments. In each petri plate the discs were placed on a moist filter paper, to avoid desiccation of the leaf disc. The larvae were released with the help of Camlin hair brush. After release of larvae the petri plates were sealed with Parafilm (125’ Length X 4’ width).