RESULTS & DISCUSSION

5 RESULTS AND DISCUSSIONS

5.1 Life cycle parameters and biology of *Helopeltis theivora* reared on tea

5.1.1 Developmental stages

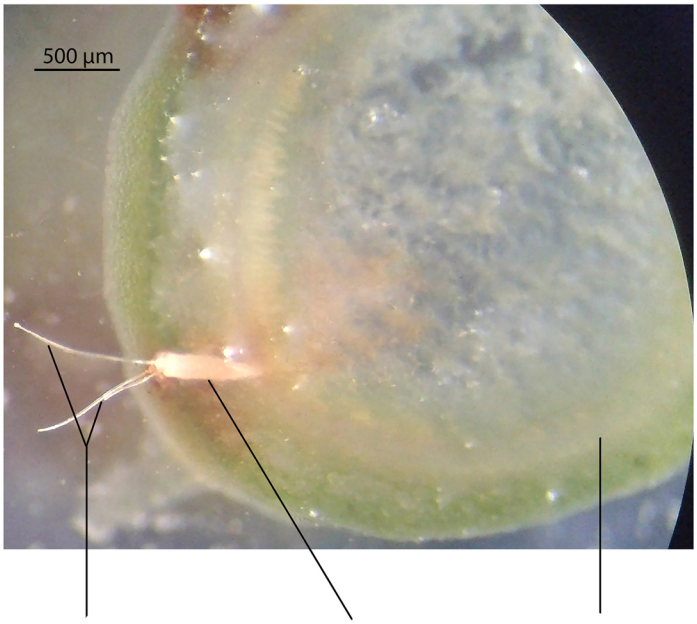
The utility of life cycle study of a pest species is immense for its proper management. Integrated pest management (IPM), the most effective and popular practice for sustainable management of pest typically involves monitoring of the population size of the insect pest. The monitoring and prediction of the population size of a pest are possible largely by studying its life cycle and biology (Apple and Smith, 1976). Studies on life cycle of *Helopeltis theivora* showed presence of egg, nymph (five instars) and adult as developmental stages, typical characteristics of hemimetabolous insect.

5.1.1.1 Eggs

The eggs were laid completely inserted into the tissues of tender stems, bases and midrib of leaves and shoots of tea twigs (Plate 5.1). Non-temperate mirid bug that breeds almost all through the year, prefer those oviposition sites that provide nutrition to the developing juveniles, till they mature (Muhamad and Way, 1995). The oviposition sites and the surrounding areas selected by the tropical insect pest *H. theivora* fulfilled nutritional and other habitat requirements of the developing individuals. Two chorionic processes per egg were seen emerging out at the site of egg deposition. The chorionic processes are also known as respiratory horns. The light coloured horns were deceptive unless observed against a dark background. The survival importance of the respiratory horn may lie in the fact that it makes the atmospheric respiration possible by projecting above the film of water flowing over the egg deposition site during heavy rains (Hinton, 1962). Such situation of heavy rains is a common feature prevailing in Terai and the Dooars regions during monsoon season. Eggs were pale in colour, slender and elongated, visible only under the microscope on the tissue sections cut through the site of the egg deposition (Plate 5.1). Endophytic (within plant tissue) oviposition is reported to provide suitable microclimate along with protection from egg parasitism (Wheeler Jr., 2001). Such adaptations may also help the pest in sustaining itself in the toxic environment of tea plantations during the time of pesticide spray besides protecting from parasite and predators.

PLATE 5.1: Transverse section of tea twig showing an egg of *Helopeltis theivora*

PLATE - 5.1



5.1.1.2 Nymphs

The life cycle of *H. theivora* reared on tea progressed through five nymphal stages as in nearly all other true bugs. Each instar lasted for 2 to 3 days. The first instar nymphs were pale yellowish in appearance. With maturation, they gained their typical greenish colour. They preferred feeding on bud and the first leaf of the tea shoots provided. All the nymphal stages were voracious sap feeder. As soon as they emerged out, they started feeding on sap from the very first tender tea twig encountered. They were wingless but were capable of cursorial (fast running) locomotion. As reported and generalised by Wheeler Jr. (2001) for heteropteran bugs, the first instar nymphs quickly dispersed in search of tender tissues for feeding. Characteristic drumstick like structure also called as scutellar horn appeared after the second instar. Nymphs were equally potent depredator of tea as adults, causing punctures and necrosis on harvestable leaves and shoots consisting of two and a bud of a tea bush, inflicting huge economic loss to the tea planters (Plate 5.2).

5.1.1.3 Adults

Newly emerged adults were pale yellowish and turned greenish black within a span of an hour. Adults were dimorphic with females being bigger than males as reported by Das (1965). Males were more slender with cylindrical abdomen. Pronotum was prominently yellow in females than in males (Plate 1.1). The curved scutellar horn with swollen apex, a characteristic of bryocorines (Wheeler Jr., 2001), the tribe to which *H. theivora* belongs to, was a prominent morphological feature in adults. They were swift but not a strong flyer. Reduced flight muscle is a common phenomenon in heteropterans (Schuh and Slater, 1995), which could be the reason for such a weak flight in *H. theivora*. They remain active all through the day with the peak of the feeding activity during dusk and dawn mostly in shaded regions of tea plantations. On an overcast condition, they could be found feeding all through the day.

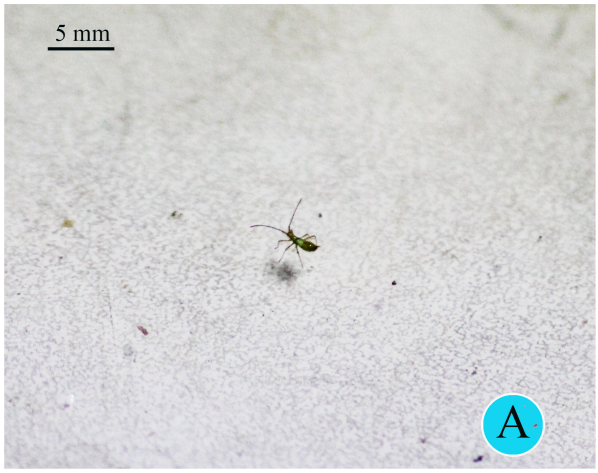
5.1.2 Life cycle parameters

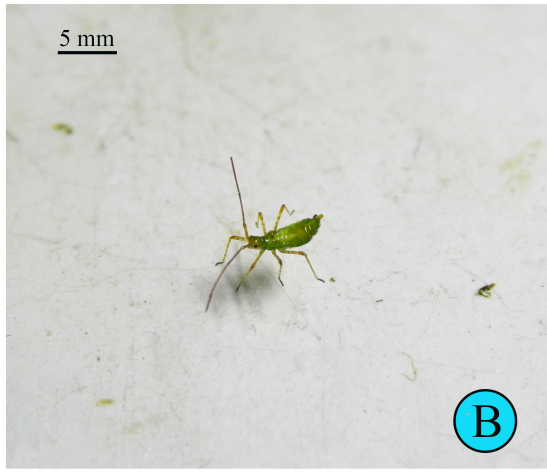
Population forecasting along with the basic biology of a pest are the important prerequisite of IPM (Sørensen et al., 2012). The information obtained by studying life cycle are useful in optimisation of population growth models for forecasting and management strategies of the pest (Córdoba-Aguilar et al., 2014). Life cycle parameters were studied for tea mosquito bug, *Helopeltis theivora* when reared on tea,

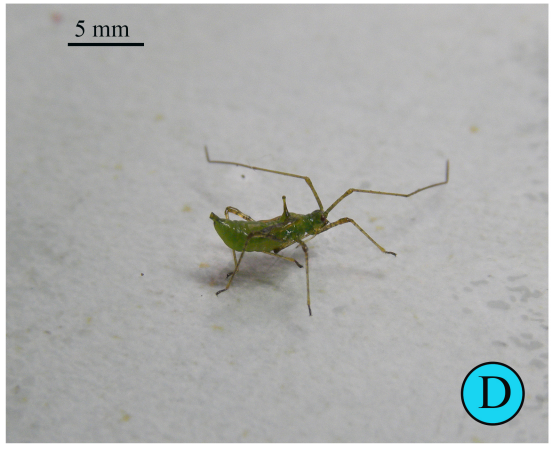
PLATE 5.2: Nymphal stadia of *Helopeltis theivora*

A) First instar B) Second instar C) Third instar D) Fourth instar E) Fifth instar

PLATE - 5.2







Camellia sinensis as host plant under laboratory condition (Table 5.1). Fecundity of *H. theivora* was recorded to be 34.38 ± 1.23 (mean ± *SE*) eggs per female from tea plantations of Terai in NE India, which is about 5.5 fold less than the reported fecundity of about 170 eggs per individual, from tea plantations of southern India (Sudhakaran, 2000). Such a variation can be due to the difference in the agro-climatic conditions of the two regions compared. As reported in other mirid bugs by Fritz (1982) and Wheeler Jr (2001), *H. theivora* also showed the iteroparous mode of reproduction, producing eggs for almost throughout its adult stage.

The developmental period of each instar ranged from 2 – 3 days with no statistically significant difference among the stadia. The total developmental period varied from 19 to 22 days during March – April which is similar to the observation of Kalita et al. (1996) on *H. theivora* from Assam region of NE India. According to Das (1984), the duration of life cycle varies with season and also the climatic condition, thus the developmental period in different seasons of the year may range from 5 to 8 weeks.

The sex ratio at birth or emergence called as secondary sex ratio (male: female) was observed to be 1: 0.5 with χ^2 value of 1.32 at $df = 1$ and $p \leq 0.05$, which is less than the table value, suggesting that the difference in observed and the expected sex ratio of 1:1 is only a matter of chance. The ratio of male to female at the time of fertilisation or oviposition, emergence or birth and the sexually matured adult stages are respectively known as primary, secondary and tertiary sex ratios (Wilson and Hardy, 2002). Sudhakaran (2000) has reported the tertiary sex ratio of 0.95:1 in sexually mature adults of *H. theivora* population collected from the tea plantation of southern India. Insects achieve the tertiary also known as operational sex ratio by the presence of various non-visual cues in their habitat (Han et al., 2012a). The female-biased tertiary sex ratio with more females for reproduction may help in the rapid proliferation of the mirid bugs (Silva et al., 2016).

The adult longevity of *H. theivora*, when reared in laboratory condition was recorded to be 13 to 15 days, while Sudhakaran (2000) have reported the longevity in the range of 28 to 48 days from the southern India. Life spans of insects are dependent on ecological conditions (Carey, 2001). The genetic makeup of a species also determines the longevity (Tower and Arbeitman, 2009). The difference observed in the longevity of the two allopatric populations of Terai in the northern part of West

Table 5.1: Life cycle parameters of *Helopeltis theivora* reared on tea in laboratory

Sl. No	Parameters	Observations*
1	Fecundity	34.38 ± 1.23 eggs per female (n = 33)
2	Total developmental period	20.66 ± 0.57 days (n = 44)
3	Secondary sex ratio (male : female)	1 : 0.5 ($\chi^2 = 1.32$, $p \leq 0.05$, df = 1)
4	Adult longevity	13.75 ± 0.56 days (n = 42)

*values are mean ± *SE*
-experiments were conducted during March-April (average temp min: 16.5^{0}C; max: 26.8^{0}C; RH-80 ±5%; average rainfall-2.5 mm).

Bengal and South India could be due to the difference in their environmental, genetic and other conditions.

5.1.3 Survivorship

To determine the suitability of tea as a complete host plant in Terai region, study on the survivability of different developmental stages of *H. theivora* was carried out. The survivorship varied among the developmental stages of the bug. The survivability was highest in egg (27.67%) and the lowest was recorded in the fifth instar (7.54%) with intermediate values in other four instars (Table 5.2). The survivability was in agreement with most of the '*r*-selected' species that includes the mirid bugs (Odum and Barrett, 2005; Córdoba-Aguilar et al., 2014; Mollá et al., 2014). In r-selected species, the survivability is highest in the initial stages of development and decreases with maturity as recorded in the mirid bug, *H. theivora*.

5.2 Pesticide tolerance level in *Helopeltis theivora* population from Terai tea plantations

The pesticide tolerance levels expressed in terms of median lethal concentration (LC_{50}) values for the populations of *H. theivora* occurring in conventionally and bio-organically managed tea plantations of Terai in the northern part of West Bengal were determined using standard bioassay technique. Five populations collected from different locations designated as Terai-I through Terai-V of *H. theivora* were assessed for the tolerance level. The coordinates and the location of the sampling sites are given in the Appendix-C. Bioassays are experiments designed to reveal the phenotypic response of pest after the exposure to one or more pesticide molecules. The bioassay involves the exposure of the pest (specimen) of interest to a series of known concentrations of a pesticide and analyses of the dose-response curve obtained along with comparing the mortality with a reference susceptible population (Anonymous, 2016). Conventionally managed tea plantations are those, wherein pests are managed using synthetic chemical pesticides and in bio-organically managed tea plantations, only bio-organically derived formulations of herbal pesticides and manures are used for pest management and enriching soils. Pesticides *viz*., monocrotophos (36% SL) and cypermethrin (10% EC), representing organophosphate and synthetic pyrethroid groups, respectively were chosen for bioassay of various

Table 5.2: Stage specific survivorship in laboratory -reared *Helopeltis theivora*

Developmental Stages/Instars	n	Survival (%)
Eggs	159	27.67
I^{st}	44	23.89
II^{nd}	38	22.64
III^{rd}	36	16.35
VI^{th}	26	11.94
V^{th}	19	07.54

populations of *H. theivora* and the results are presented in Table 5.3. The pesticides used for the bioassays were extensively used against *H. theivora* in recent past for their better efficacy (Anonymous, 2010; Saha and Mukhopadhyay, 2013; Mukhopadhyay et al., 2016) and are still the preferred one. Although the use of monocrotophos in tea plantations is not recommended by the Tea Board of India (Anonymous, 2014b) due to their greater persistence in the environment and the higher residue in the finished product, many tea planters prefer and still use the chemical for the management of pests clandestinely (per. comm.) for its efficacy and cost-effectiveness in managing pests. The Tea Board is the nodal organisation to oversee the safe usage of plant protection formulations (chemicals) for the management of pests in tea plantations of India, recommended by the Central Insecticides Board and Registration Committee (CIB), Government of India. The two pesticides used for bioassay features in the latest list of pesticides recommended by CIB for the management of various agricultural pests (http://cibrc.nic.in; accessed on 17th February 2017). Thus, they are readily available in the market and planters use them clandestinely for the management of pest occurring in tea plantations.

The population of *H. theivora* from the central Terai (Terai-I) showed the highest tolerance level expressed in terms of LC (lethal concentrations) to both the classes of pesticide tested followed by Terai-II, III, IV and V in descending order of tolerance. The result indicates that the quantum of pesticide used in the plantation of central Terai to be the highest amongst the tested plantations. As per the results of the bioassays shown in Table 5.3, it is imperative that the insecticide-exposed populations from conventionally managed tea plantations have acquired higher tolerance levels evident by the enhanced LC_{50} value.

There are reports from across the globe that indiscriminate application of synthetic pesticides results in accumulation of higher tolerance and development of resistance in pests (Cooper and Dobson, 2007; Perry et al., 2011). Bass et al. (2014) have reported that the intensive applications of chemical pesticides to control peach potato or green peach aphid, *Myzus persicae* a pest of diverse crops with cosmopolitan distribution have led to the development of widespread and varied forms of resistance. In Sri Lanka, extensive use of synthetic chemical pesticides mostly organophosphate and synthetic pyrethroid as a part of the anti-malarial campaign has secondarily led to the development of resistance in bedbug, *Climex hemipterus*, a biting nuisance (Karunaratne et al., 2007). Such reports of resistance

Table 5.3: Pesticide tolerance levels (LC_{50}) of *Helopeltis theivora* population from tea plantations of Terai region in northern West Bengal (n=150 for each population)

Sl. No	Population	Pest Management Practice	Organophosphate (Monocrotophos 36%SL)				Synthetic Pyrethroid (Cypermethrin 10% EC)			
			[§]LC_{50}	χ^2	Regression Equation	Fiducial Limits (95%)	[§]LC_{50}	χ^2	Regression Equation	Fiducial Limits (95%)
1	Terai-I	Conventional	541.6	8.48	y = 2.268x-8.01	(429.18-682.76)	30.65	10.42	y = 2.538x-6.391	(25.80-36.43)
2	Terai-II	Conventional	77.52	3.05	y = 3.717x-13.2	(67.75-88.69)	17.35	5.25	y = 4.13x-12.511	(15.89-20.17)
3	Terai-III	Conventional	40.79	2.59	y = 3.061x-9.11	(37.31-44.60)	13.39	2.32	y = 2.015x-3.316	(10.37-17.28)
4	Terai-IV	Bio-organic	15.76	1.56	y = 3.533x-9.83	(13.74-18.08)	8.35	4.01	y = 2.779x-5.901	(06.87-10.14)
5	Terai-V	Bio-organic	10.35	3.49	y = 2.331x-4.36	(8.11-13.19)	6.33	3.89	y = 2.510x-4.544	(05.31-07.55)

[§]values are expressed in ppm

development in different insect species and the varying levels of tolerance among the *H. theivora* population, as found in this study (Table 5.3) clearly indicate that the quantum of application of pesticides determines resistance level and their indiscriminate application would further aggravate the rate of resistance evolution. An excessive (no threshold) and a blanket spray of pesticide as a prophylactic measure would result in the development of heritable tolerance in insect pests (Haydock, 2005; Basnet et al., 2015). Such a practice of blanket spray needs to be abandoned completely to minimise the development of resistance in pest. Proper monitoring of tolerance/resistance level, as well as the use of the appropriate pesticide in terms of effectiveness, is required for sustainable pest management. Indiscriminate application of pesticides would just lead to wastage of resources and pesticide contamination of the environment and the most importantly the consumable/marketable tea.

5.3 Tolerance level in monocrotophos selected *Helopeltis theivora*

Selection by exposing to a high concentration (LC_{80}) of monocrotophos (36% SL) an organophosphate pesticide enhanced the tolerance level in adult *H. theivora*. As shown in Table 5.4, after the selection by the pesticide, the tolerance level expressed in terms of LC_{50} value increased about 105 fold in F_2 as compared to P generation. The repeated exposure to LC_{80} of pesticide led to the selection of pesticide-tolerant individuals from the population. The increase in tolerance level through generations was significantly different from one another at $p < 0.05$ level of significance. The tolerance levels of the generations, when selected by exposure to the pesticide was in the order of $P < F_1 < F_2$. Observed and the expected LC_{50} values were not significantly different, as the calculated values of χ^2 in none of the tested generations were greater than the table value at $df = 4$ and $p < 0.05$ level of significance (Table 5.4) Similarly, *H. theivora* population also showed an increase in its resistance ratio through generations when selected by exposure to endosulfan, a pesticide belonging to the class of cyclodienes (Roy et al., 2010a). These observations along with the discussion presented in section 5.2 further suggest that continuous and enhanced application of the same pesticide or pesticide with a similar mode of action may select individuals that are more tolerant, leading to the development of resistance in the pest population. Alternatively, use of more than one pesticide or pesticide with different modes of action may yield better results in managing such pests.

Table 5.4: Relative tolerance level (LC_{50}) across three generations of *Helopeltis theivora* when selected by exposure to LC_{80} of organophosphate pesticide (monocrotophos, 36% SL)

Sl. No.	Generation	$^{\S}LC_{50}$		χ^2	Regression equation
		Dose	Fiducial Limits (95%)		
1	P	08.2[a]	6.60-10.18	7.27	y=2.9532x-6.5587
2	F_1	15.48[b]	12.99-18.45	2.99	y=3.6623x-10.3450
3	F_2	855.38[c]	595.97-1227.74	1.07	y=1.3770x-3.1690

[§]values are expressed in ppm

[a,b,c,] mean values with different superscripts in a column were significantly different at $p < 0.05$ level of significance as per Tukey HSD and Bonferroni multiple comparison tests

Tolerance to a pesticide in arthropods is preadaptive, i.e, the mechanisms controlling the tolerance are already present in the population at very low frequencies prior to exposure to any pesticides. Such preadaptation can be the result of their exposure to other toxicants in their present environment or the evolutionary history of the arthropod (Yu, 2014). Repeated applications of pesticide lead to the artificial selection of resistant individual by exerting bottlenecking (drastic reduction) effect on the size of the parental population. Those selected individuals with higher tolerance level form the basis of artificially selected tolerant population in the later generations (Plate 5.3). Such tolerant populations are difficult to manage by resorting to the conventional practices of management.

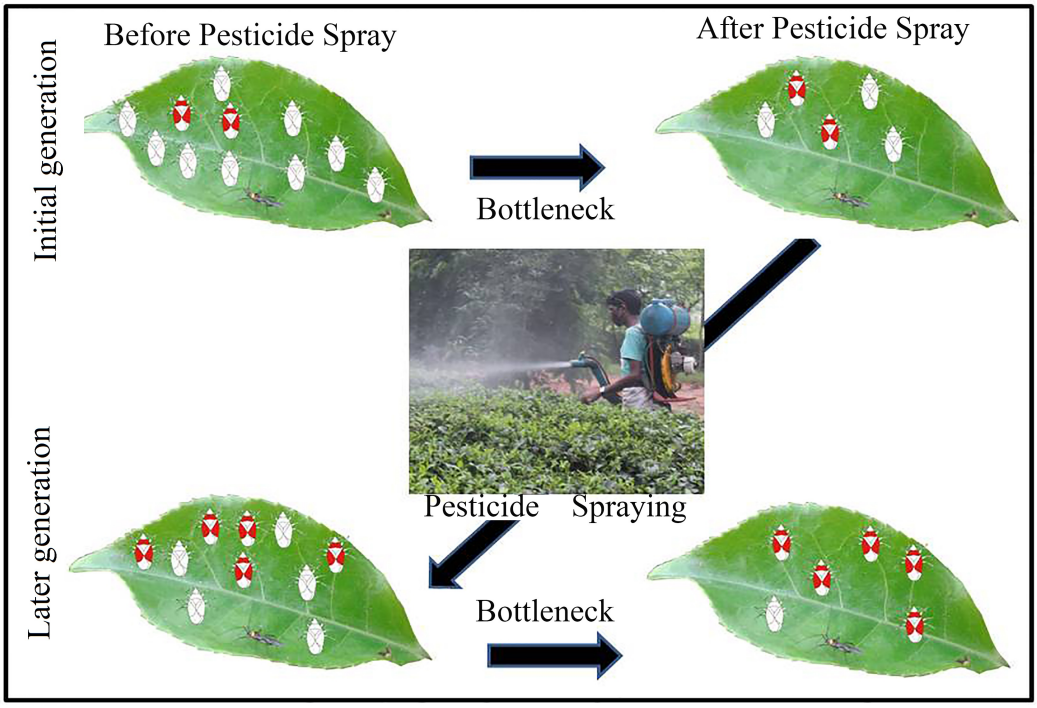
5.4 Quantitative activity of defence enzymes in *Helopeltis theivora* population from Terai tea plantations

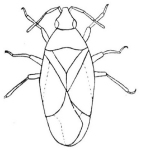
5.4.1 General esterases

The activities of general esterases (GE) in different populations of *Helopeltis theivora* from conventionally and bio-organically managed tea plantations of Terai in northern West Bengal were assessed. Independent sample *t*-test revealed that the population from conventionally managed tea plantations exhibited significantly higher activities of GE with t (59) = 6.67 and F-value of 23.25 at $p < 0.01$ than those from the bio-organically managed and the laboratory-reared reference population (Tables 5.3 and 5.5), indicating that exposure to synthetic pesticide leads to enhancement of GE activity. The observation also suggests that level of GE activity can provide clues about the pest management practices (conventional or bio-organic) prevailing in the tea plantation from which the *H. theivora* population was assessed. In many insect pests, GEs are often involved in providing higher tolerance to pesticides belonging to the groups of organophosphate, carbamate and synthetic pyrethroid to a lesser extent (Hemingway and Ranson, 2000). The higher activity of GE observed in *H. theivora* populations from tea plantations following conventional method of managing pests may be due to the greater application of organophosphate and other related pesticides. Gurusubramanian et al. (2008) have reported that organophosphate insecticides constitute about 64% of the total input of synthetic pesticide in tea plantations of NE India including Terai and the Dooars in the northern part of West Bengal.

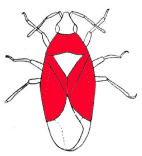
PLATE 5.3: Schematic representation of the bottleneck effect and artificial selection caused by repeated application of pesticide

PLATE - 5.3





→ Susceptible



→ Tolerant

Analysis for bivariate correlation between the resistance ratio (RR) and the GE activity ratio (AR) showed the existence of a strong positive correlation between the two covariates with a very high Pearson's correlation coefficient (r) of 0.941 at $p < 0.01$ (2-tailed) (Table 5.5). The RR is the ratio of relative tolerance (LC_{50}) of the test population (Terai-I through V) to the susceptible laboratory-reared population while the AR is the ratio of the defence enzyme (GE) activity of population (Terai-I through V) studied to the activity of the susceptible laboratory-reared population. The result also resonates that the activity of GE can act as an indicator of the relative tolerance level in the population of *H. theivora*. Higher RR and the corresponding enhanced AR in the population from conventional plantations indicates that exposure to higher doses of synthetic pesticides enhances the activity of the defence enzyme, GE.

Simple regression analysis was performed to determine the dependence of RR on the AR of GE. Linear relationship of RR and AR was found to be statistically significant with an R^2 of 0.885 and F-value of 23.06 at $p < 0.05$ (Table 5.5). These findings suggest that the resistance ratio is a function of the activity level of the defence enzyme, GE in populations of *H. theivora*. Organophosphate resistance in mosquito belonging to the genus *Culex* is also attributed to the elevated activity of GEs (Hemingway and Ranson, 2000). The elevated activity of GEs sequesters pesticide rapidly, thus confer higher tolerance to insects (Kadous et al., 1983).

Activities of GE in *H. theivora* populations collected from different conventional populations (Terai-I, II and III) were subjected to one-way analysis of variance (ANOVA), indicated that there exists a strong variation among the tested populations in terms of the activity of the defence enzyme with F-value of 20.72 at $\alpha = 0.05$ and $df = 2, 29$. The variation in GE activity among the tested population further indicated that the quantum of pesticides applied varied among the tea plantations, which possibly led to different levels of GE activity in the *H. theivora* populations imparting various levels of tolerance in them. It also showed that there existed a heterogeneity containing both resistant and susceptible individuals within and among the populations of *H. theivora*. Applications of pesticides often lead to the selection of tolerant individuals of a population and with further selection due to repeated application of a pesticide or pesticides having similar functional group, the frequency of such tolerant individual in the population becomes very high (Plate 5.3). Such populations with higher tolerance or resistance pose a major challenge for their management and a grave threat to the tea crop and the entire tea industry at large.

The higher LC_{50} values *vis-à-vis* pesticide tolerance levels in *H. theivora* populations from conventionally managed plantations can be attributed to the enhanced activity ratio (AR) of general esterases in the tested populations (Table 5.5).

5.4.2 Cytochrome P450 monooxygenases

Populations of *Helopeltis theivora* collected from different conventionally and bio-organically managed tea plantations of Terai in northern West Bengal were also assessed for the activities of cytochrome P450 monooxygenases (CYP450). Lavene's test for equality of variance showed the existence of variability in the activity of CYP450 among the conventional and bio-organically managed *H. theivora* populations with *F*-value of 20.36 at $\alpha = 0.001$ (Table 5.5). Independent sample *t*-test was performed to determine the variation in activities of CYP450 among the population of *H. theivora*. Populations from conventionally managed tea plantations were found to have a significantly higher activity of CYP450 than in the bio-organically managed population with a *t*-value of 6.79 at $p < 0.01$(2-tailed) and $df = 59$ (Table 5.5).

The relation and dependence of the resistance ratio (RR) on the CYP450 activity ratio (AR) in populations of *H. theivora* were also studied. Bivariate correlation analysis between the two variables deduced the Pearson's correlation coefficient (*r*) of magnitude 0.854 at $p < 0.05$ (1-tailed), suggesting the existance of a strong positive correlation between them. Similar to the definition given in section 5.4.1, RR is the ratio of relative tolerance (LC_{50}) of the tested population (Terai-I through V) to the susceptible laboratory-reared population, and AR is the ratio of CYP450 activity of the tested populations to the activity of the susceptible laboratory-reared reference population. The observation indicates that the activity of CYP450 can also be used as an indicator of the relative resistance level in the population of *H. theivora*. CYP450 monooxygenases are involved in the metabolism of synthetic pyrethroid, organophosphate and carbamate pesticides (Feyereisen, 1999), thus the elevated activity of CYP450 in insects confer higher tolerance and resistance to pesticides (Hemingway and Ranson, 2000; Hemingway et al., 2004).

Higher RR and the corresponding enhanced CYP450 AR in the population from conventional plantations indicated that AR is directly proportional to the extent of pesticide exposure and possibly the RR. As found in the present study, elevated level of CYP450 activity was also reported to be associated with pyrethroid resistance

in anopheline mosquitoes (Vulule et al., 1994; Brogdon et al., 1997; Hemingway and Ranson, 2000).

The dependence of RR on the AR of CYP450 was determined by simple regression analysis. The regression analysis yielded R^2 of 0.854 and F = 8.10 at $p <$ 0.06 signifying 85.4% dependence of RR on the AR of CYP450 in the populations of *H. theivora*. These results also indicated that the linear relationship of RR and AR to be statistically significant. Further, it is imperative that the RR is a function of the activity level of the defence enzyme, CYP450 in *H. theivora* populations.

Activities of CYP450 from the conventional populations (Terai-I, II, and III) when subjected to one-way ANOVA revealed the existence of a strong variation among the studied populations of *H. theivora* with F = 4.86 at α = 0.05 with df = 2, 29 (Table 5.5). The variation in the CYP450 activity among the populations of the pest may be due to varying quantum of pesticide application in the tea plantations from which the populations of *H. theivora* were collected. In other words, the quantum of pesticide application and the extent of exposure of *H. theivora* populations to pesticides determined the levels of CYP450 activity and eventually the level of tolerance or resistance.

5.4.3 Glutathione *S*-transferases

Populations of *H. theivora* acquired from different tea plantations, some managed conventionally and other bio-organically in Terai of northern West Bengal were also assessed for the activities of glutathione *S*-transferases (GST). Independent sample *t*-test revealed t (59) = 1.30 and F-value of 8.20 at $p < 0.05$ which indicated that there existed no significant difference in the activity of GST between conventionally and bio-organically managed as well as the laboratory-reared reference population of the pest (Tables 5.3 and 5.5). Statistically non-significant difference in GST activities showed that there exists no relationship between application of synthetic pesticide and the activity of GST in the pest, *H. theivora* (Table 5.5). However, the increased exposure to pesticide could enhance the activity of two other important defence enzymes, GE and CYP450 as described in the preceding sections 5.2 and 5.3. In mosquitoes and other insects, GST often acts as an important detoxifying enzyme in conjugation with CYP450 and GE (Hemingway et al., 1991). Thus, it may be possible that in the pest *H. theivora*, GST gets induced only when the defence enzymes, CYP450 and GE becomes ineffective in metabolising or detoxifying pesticides.

Although many researchers have reported the involvement of GST in conferring resistance (Fournier et al., 1992; Kostaropoulos et al., 2001), the exact role of this defence enzyme in pesticide tolerance and resistance is difficult to ascertain (Syvanen et al., 1996). As cited by Ranson et al. (2001), there are only two reports of a direct relationship between GST over-expression and resistance as shown in populations of diamondback moth, *Plutella xylostella* (Huang et al., 1998) and fruit fly, *Drosophila melanogaster* (Tang and Tu, 1994).

As defined in the preceding sections 5.4.2 and 5.4.3, the AR is the ratio of GST activity of the tested population (Terai-I throughV) to the activity of the susceptible laboratory-reared population. The correlation between the resistance ratio (RR) and the GST activity ratio (AR) was studied by bivariate correlation analysis, which deduced a negative, non-significant Pearson's correlation coefficient (r) of magnitude –0.1, at $p < 0.01$ (2-tailed) level of significance (Table 5.5). The observation suggests that there exists a weak negative correlation between the two variables. Such a weak correlation further suggested that GST may not be playing any significant role in imparting tolerance against pesticides in *H. theivora*.

As per the results of simple regression analysis of RR on the AR of GST, there was a linear relationship between the two variables. The relation was found to be statistically nonsignificant with an R^2 of only 0.006 and F (1, 3) value of 0.01 at $p > 0.05$ level of significance (Table 5.5). Further, it suggested that the resistance ratio was almost independent and not a function of the activity level of the defence enzyme GST in *H. theivora* population.

One-way ANOVA of the levels of the activities of GST among the conventional populations (Terai-I, II, and III) showed the lack of variation in activity among the tested populations with F-value of 2.07 at $\alpha > 0.05$ and $df = 2, 29$. It further showed the existence of homogeneity in terms of activity of GST among the tested population. Exposure to pesticides does not seem to influence the level of activity or expression of GST in *H. theivora* population.

Table 5.5: R**elative tolerance level against monocrotophos (36%SL) and the corresponding defence enzyme activity (mean ± *SE*, n=180) in *Helopeltis theivora* populations from tea plantations in Terai of northern West Bengal**

Sl. No	Population*	§RR	Defence Enzymes					
			GE		CYP450		GST	
			†Activity	¥AR	††Activity	¥AR	†††Activity	¥AR
1	Lab reared	---	0.50 ± 0.14^{d}	---	$0.08\pm0.01^{b,c}$	---	0.095 ± 0.012^{d}	---
2	Terai-I	66.04	7.43 ± 0.75^{a}	14.86	0.35 ± 0.04^{a}	4.37	$0.046\pm0.007^{a,c}$	0.48
3	Terai-II	09.45	4.17 ± 0.62^{b}	8.34	$0.25\pm0.04^{a,b}$	3.12	$0.045\pm0.005^{a,c}$	0.47
4	Terai-III	04.94	$2.27\pm0.15^{b,c}$	4.50	$0.18\pm0.01^{b,c}$	2.25	$0.061\pm0.006^{a,c}$	0.64
5	Terai-IV	01.92	1.93 ± 0.39^{c}	3.86	$0.09\pm0.01^{b,c}$	1.12	$0.026\pm0.003^{b,c}$	0.27
6	Terai-V	01.26	1.23 ± 0.20^{c}	2.26	$0.08\pm0.01^{b,c}$	1.00	$0.047\pm0.008^{a,c}$	0.49

*Terai-I, II, III are conventionally and IV and V are bio-organically managed populations

§Resistance ratio is the ratio of relative tolerance of the population (Terai-I through IV) to the susceptible laboratory-reared population

†Unit of activity is μmol α-napthol formed mg protein^{-1} min^{-1}

¥Activity ratio is the ratio of defence enzyme activity of population (Terai-I through IV) to the activity of the susceptible laboratory-reared population

†† Unit of activity is nmol cytochrome C equivalent mg protein^{-1}

†††Unit of activity is mM μg protein^{-1} min^{-1}

^{a,b,c,d} mean values with different superscripts in a column were significantly different at $p < 0.05$ level of significance as per Tukey's Honestly Significant Difference and Bonferroni multiple comparison tests

5.5 Quantitative study of defence enzymes in pesticide-selected *Helopeltis theivora*

5.5.1 General esterases

Selection by the exposure to an extreme dose (LC_{80}) of the organophosphate pesticide (monocrotophos, 36% SL) enhanced the tolerance level in adult *Helopeltis theivora*. The enhancement of the tolerance level was in concurrence with enhanced activity of GE to the tune of 5.32 and 16.4 fold in F1 and F2 generations, respectively in reference to the parental generation (P) (Table 5.6). The tolerance level expressed in terms of LC_{50} values and the activity of GE showed a strong positive correlation as suggested by a very high Pearson's correlation coefficient value (r = 0.999). Hydrolytic enzymes including GE are the most effective detoxifying agents for degradation of organophosphate compounds. They mostly function by hydrolysing the ester bonds of the organophosphate pesticides (Georghiou, 1972).

In peach aphid *Myzus persica*, a high degree of resistance after selection by application of parathion, an organophosphate pesticide for only a few generations was reported. The trait was partly inheritable and the resistant individuals were found to have gene duplications up to 64 fold (Stenersen, 2004). In insect pests, metabolic detoxification of pesticides is the principal mechanism to overcome xenobiotics including pesticide stresses. The elevated activity of general esterases (GE) has been shown to confer higher tolerance and subsequently resistance against organophosphate pesticide in many insect pests (Hemingway et al., 2004; Wu et al., 2011). Association and strong role of GE with the metabolic resistance to organophosphate in the Chinese population of cotton bollworm, *Helicoverpa armigera* was documented by Han et al (2012b). In the aphid *M. persicae*, excess production of GE as a result of gene duplication has been reported to confer resistance to parathion (Devonshire and Sawicki, 1979; Devonshire and Field, 1991).

5.5.2 Cytochrome P450 monooxygenases

The activity of cytochrome P450 (CYP450) monooxygenases were enhanced by 1.96 and 9.50 fold in pesticide-selected F1 and F2 generations, respectively in comparison to P generation of *H. theivora* (Table 5.6). It appears that the increase in the activity of CYP450 is also responsible for the rise in tolerance level of the selected generation as mentioned in preceding section 5.4.2. A high correlation coefficient value (r = 0.999) observed between CYP450 activity and the tolerance level (LC_{50}) suggested

their intimate interdependence. CYP450 also known as mixed function oxidases, are dependent on NADPH and molecular oxygen, and are the most important as well as prevalent detoxifying enzymes that provide resistance to organophosphate pesticides (Georghiou, 1972).

Insects with higher tolerance or resistance are known to exhibit greater CYP450 activity (Feyereisen, 1999). The increase in toxicity level of pesticide when applied with a synergist, piperonyl butoxide indicated involvement of CYP450 in inducing metabolic resistance to pesticides in *H. theivora* (Roy et al., 2009b).

CYP450 and GE are phase I (primary) detoxifying enzymes that biotransform xenobiotics including pesticides, by the process of oxidation, reduction and hydrolysis (Yu, 2014). Our observations in *H. theivora* system broadly concur with the hypothesis advocating detoxifying enzyme-based biotransformation of organophosphate pesticides. Enhancement of about 16.4 and 9.5 fold in the total activities of GE and CYP450, respectively in the pesticide-selected *H. theivora* was recorded. The enhancement of the activity of the defence enzymes was found to be related to the elevated level of pesticide tolerance in the pest. The activities of the defence enzymes between the less tolerant parental (P) generation and the subsequent more tolerant generations F1 and F2 were significantly different at $p \leq 0.05$, suggesting their major role in pesticide detoxification. Han et al. (2012b) have shown the enhancement of resistance and the activity of detoxifying enzymes could be correlated in the organophosphate selected Chinese strain of cotton bollworm, *Helicoverpa armigera*.

5.5.3 Glutathione *S*-transferases

Populations of *H. theivora* selected by exposure to LC_{80}, a high dose of pesticide showed enhanced resistance ratio with a reverse trend in the activity of glutathione *S*-transferases (GST). In F1 and F2, the more tolerant generations, the activity levels of GST were mere 0.52 and 0.88 fold of P, respectively (Table 5.6). It appeared that the increase in the activity of CYP450 and GE was mainly responsible for the enhancement in tolerance level and resistance ratio as mentioned in preceding sections, 5.5.1 and 5.5.2. Although many researchers have suggested the role of phase-I and II defence enzymes in pesticide sequestration and subsequently conferring the tolerance (Yu, 2014), the role of GST, the phase II defence enzyme in imparting

Table 5.6: **Relative tolerance levels and the corresponding defence enzyme activity (mean ± *SE*, n=150) in organophosphate pesticide (monocrotophos, 36%SL) selected generations of *Helopeltis theivora***

Sl. No	Generation	§Resistance Ratio	Defence Enzyme Activity					
			†GE Activity	¥Activity Ratio	††CYP450 Activity	¥Activity Ratio	†††GST Activity	¥Activity Ratio
1	P	---	0.50 ± 0.14^{a}	---	0.83 ± 0.17^{a}	---	0.095 ± 0.012^{b}	---
2	F_1	1.88	2.66 ± 0.82^{b}	5.32	1.63 ± 0.15^{b}	1.96	0.050 ± 0.005^{a}	0.52
3	F_2	104.32	8.20 ± 0.90^{c}	16.40	7.60 ± 1.37^{c}	9.50	0.084 ± 0.008^{b}	0.88

§Resistance ratio is the ratio of relative tolerance of the progeny population (F_1 and F_2) to the parental (P)

†Unit of activity is expressed in μmol α-napthol formed mg protein^{-1} min^{-1}

¥Activity ratio is the ratio of defence enzyme activity of progeny population (F_1 and F_2) to that of parental (P)

†† Unit of activity is expressed in nmol cytochrome C equivalent mg protein^{-1}

††† Unit of activity is expressed in mM per min per μg protein

^{a,b,c,d} mean values with different superscripts in a column were significantly different at $p < 0.05$ level of significance as per Tukey HSD and Bonferroni multiple comparison tests

resistance is not apparently significant in pesticide selected populations of *H. theivora*. The difference in the activities of GST as defence enzyme between the less tolerant (susceptible) P generation and the subsequent more tolerant F1 and F2 generations were not statistically significant at $p \leq 0.05$, which strongly suggests that the defence enzyme has no major involvement in conferring tolerance against organophosphate pesticide in the pest. Similarly, GST was not found to contribute to the development of higher tolerance and resistance in the cotton bollworm, *Helicoverpa armigera* in China and also in other parts of the world (Han et al., 2012b).

5.6 Qualitative study of defence enzymes in pesticide-selected *Helopeltis theivora*

5.6.1 General esterases

The native polyacrylamide gel electrophoresis (PAGE) of whole body homogenate of pesticide-selected *H. theivora* revealed four bands. Each band was considered a separate group of isozyme (s) of the general esterases (GE). The number of isozyme(s) present in each band was not known, therefore, each band was considered as a group. The bands were designated as group I (lowest mobility) through group IV (highest mobility) in the pesticide-tolerant F2 generation, while only three bands belonging to group I, II and IV were apparent in the moderately tolerant F1 and less tolerant (susceptible) P generation (Figure 5.1). Esterase belonging to group III additionally appeared in F2 generation.

Densitometric analyses of the electropherograms were carried out to estimate the contribution of various esterase isozyme groups in total esterase activity across the tested generations. As indicated by densitometric analysis, isozyme (s) belonging to group II was found to be the most active and dominant among all the esterases in the tested generations. In P and F1 generations, the group I and IV esterases contributed little to the total activity. The additional isozyme (s), belonging to group III (R_f = 0.24) found in the F2 generation had a share of about 21%, bringing down the share of the most active group II isozyme in the total esterase activity from about 85% to approximately 51% (Figure 5.2).

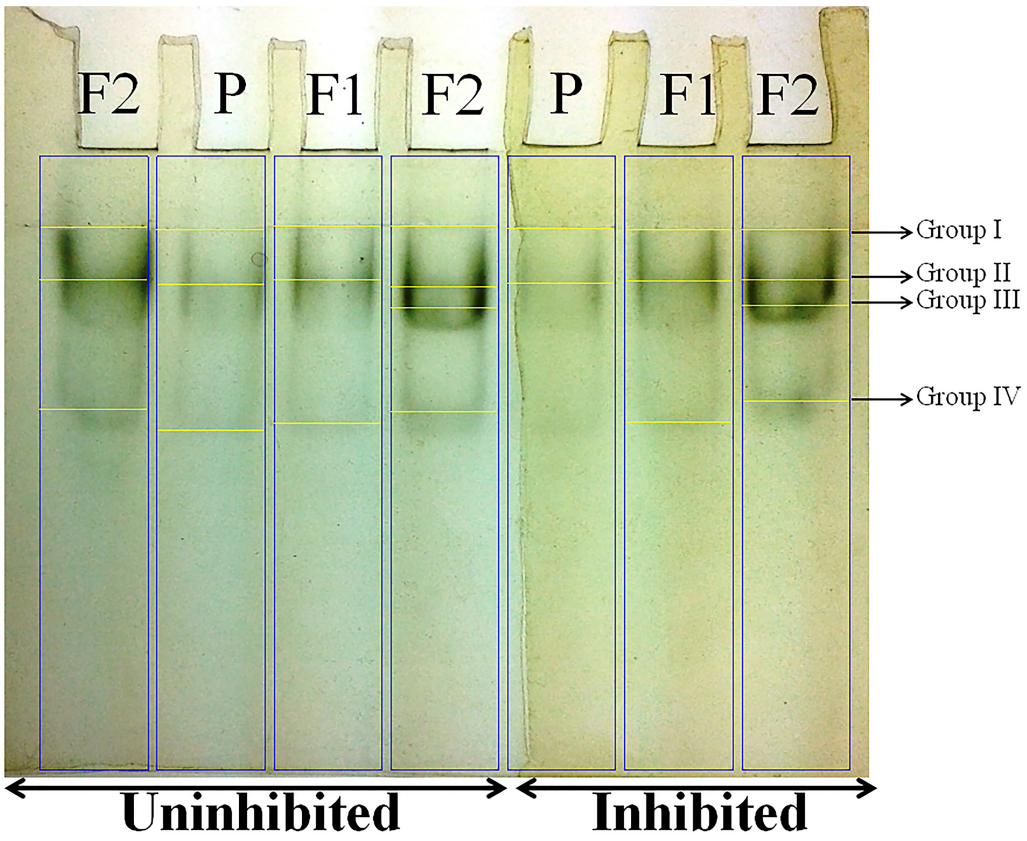


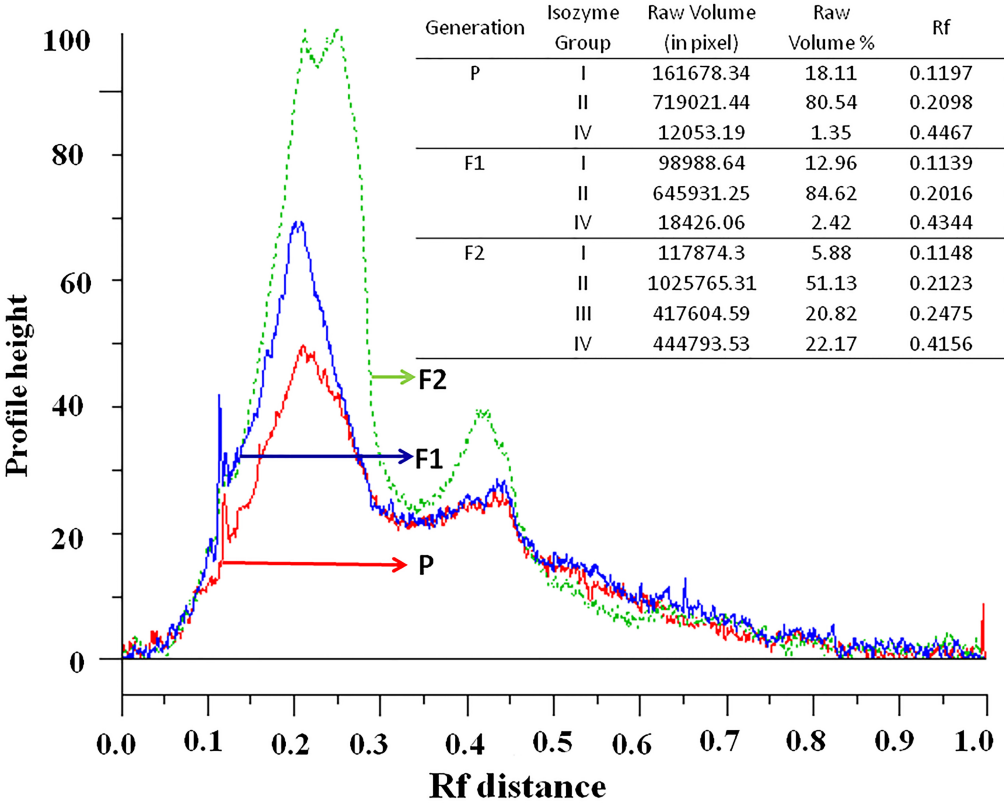
Figure 5.1: Electropherogram showing isozyme pattern of general esterase from the whole body homogenate across organophosphate selected three generations of *Helopeltis theivora*

5.6.2 Cytochrome P450

The native PAGE of whole body homogenate of *H. theivora* revealed two blue bands pertaining to the peroxidase activity of CYP450 isozymes, designated as group I (lowest mobility) and group II (highest mobility) in the F1 and F2 generations. In comparison, only one band belonging to group I with apparently minor activity (not easily detectable in the densitometric analysis) was found in pesticide susceptible P generation (Figure 5.3). Densitometric analysis revealed that the isozyme belonging to group I was the most active amongst all the monooxygenases in the tested generations but the contribution of the group towards the total activity decreased in pesticide-selected subsequent generations of F1 and F2 with an increase in the activity of the isozyme (s) belonging to group II (Figures 5.3 and 5.4).

5.6.3 General esterase *vis-à-vis* Cytochrome P450

The qualitative changes of the isozymes were evident in the overall staining intensities of the groups/band (s) of respective detoxifying enzymes in native PAGE (Figures 5.1, 5.2, 5.3 and 5.4). The overall staining intensity was in the order of P < F1 < F2 for the two detoxifying enzymes, GE and CYP450. The difference in the staining intensity of general esterases was also reported in pesticide-susceptible and resistant strains of *Helicoverpa armigera* (Srinivas et al., 2004; Han et al., 2012b), corroborating our present findings. The results of the densitometric analyses also indicated the possible involvement of various isozymes of the detoxifying enzymes in imparting a higher level of tolerance or resistance. It is apparent from our observations that the overall increase in the enzyme activity of GE and CYP450 does not necessarily mean that the activities of all the isozymes or isozyme groups get enhanced. The results also showed that the contribution of some of the isozyme of the two detoxifying enzymes got enhanced and contributed significantly to the total activity, while that of others diminished. The staining intensity of the group III of GE increased and appeared to take over the function of detoxification in pesticide selected F2 generation. The presence of group III of GE in the pesticide-selected F2 generation is in concurrence with the hypothesis of Yu (2014), which proposes the induction of new detoxifying enzymes (including isozymes) rather than activation of pre-existing one.



Generation	Isozyme Group	Raw Volume (in pixel)	Raw Volume %	Rf	Profile Height
P	I	161678.34	18.11	0.1197	26.238
	II	719021.44	80.54	0.2098	49.766
	IV	12053.19	1.35	0.4467	25.155
F1	I	98988.64	12.96	0.1139	42.046
	II	645931.25	84.62	0.2016	69.274
	IV	18426.06	2.42	0.4344	28.533
F2	I	117874.3	5.88	0.1148	37.84
	II	1025765.31	51.13	0.2123	99.803
	III	417604.59	20.82	0.2475	99.852
	IV	444793.53	22.17	0.4156	39.651

Figure 5.2: General esterase isozyme profile across organophosphate selected three generations of *Helopeltis theivora*

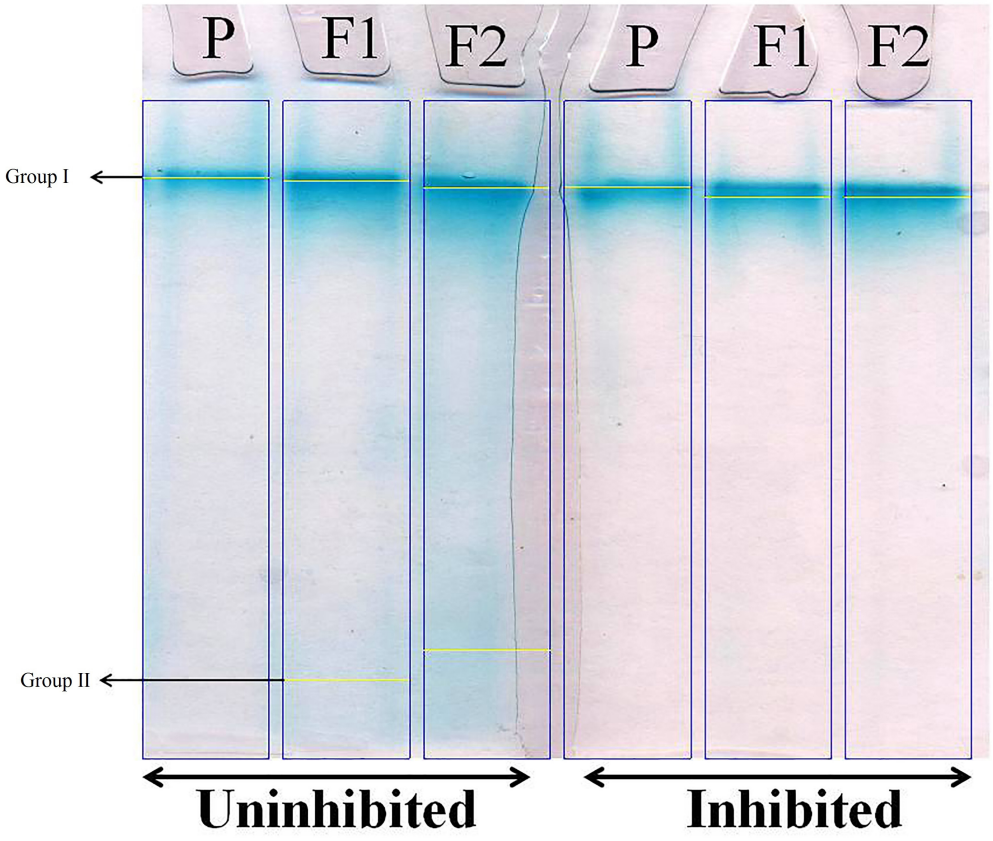


Figure 5.3: Electropherogram showing isozyme pattern of cytochrome P450 from the whole body homogenate across organophosphate selected three generations of *Helopeltis theivora*

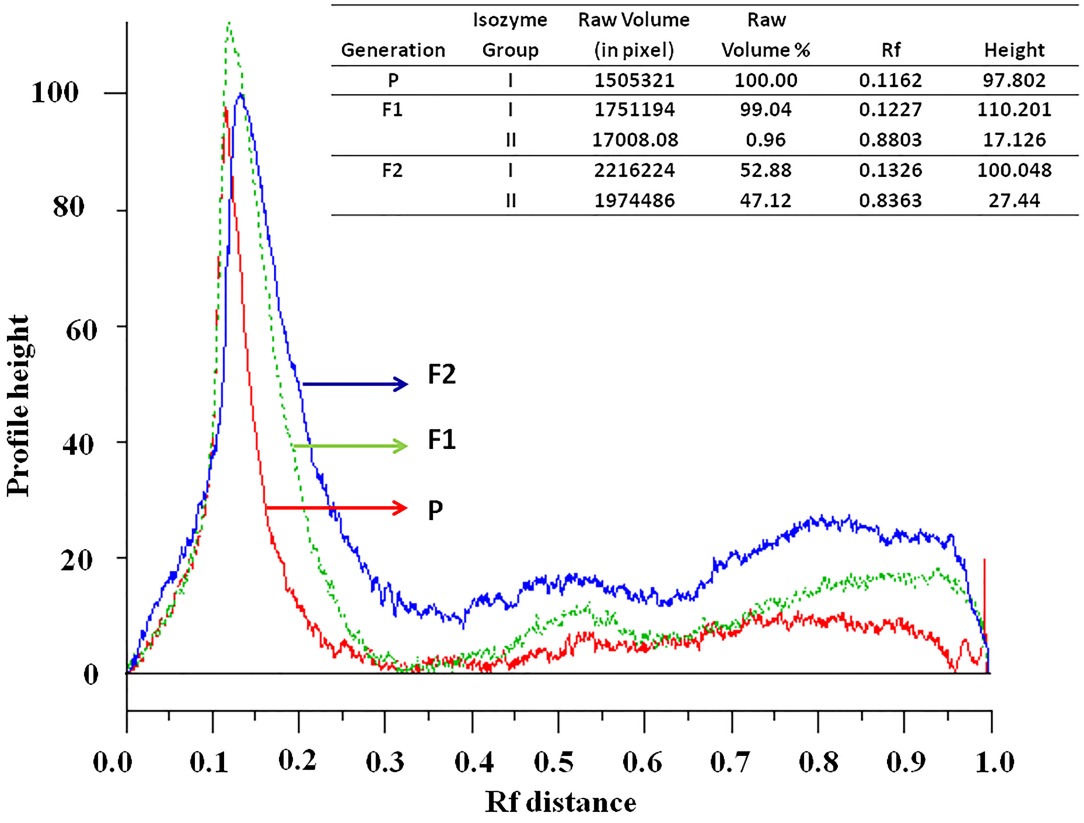
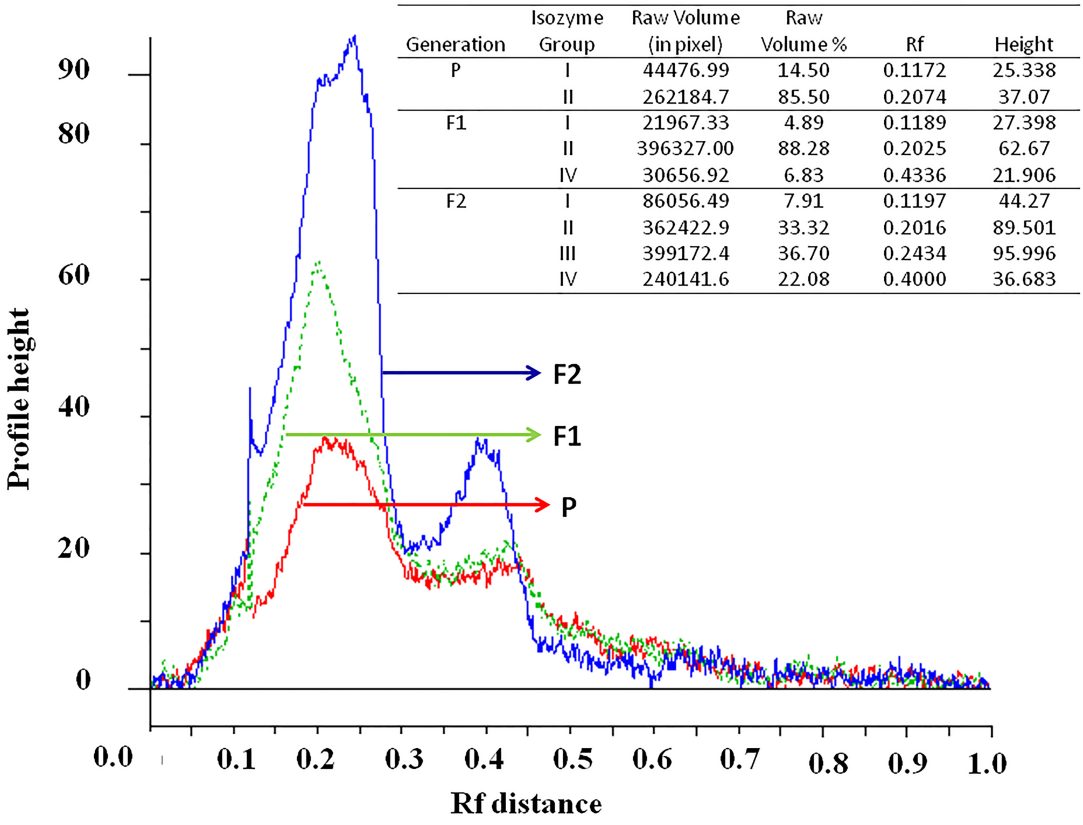


Figure 5.4: Cytochrome P450 isozyme profile across organophosphate selected three generations of *Helopeltis theivora*

Saha et al. (2012) have also reported six such esterase groups in *H. theivora* collected from chemically managed conventional tea plantations in sub-Himalayan West Bengal. The recommended field dose of most of the organophosphate pesticides for managing *H. theivora* ranges from 2,500 to 5,000 ppm (Anonymous, 2010; 2014b). Applications of pesticide at such a high dose lead to drastic reduction in the population size (bottleneck effect), an apparent initial control of the pest but results in accumulation (focusing) on allele (s) conferring resistance in the resurging population, which can be the reason for the repeated control failure of *H. theivora* reported in various tea plantations. The group I isozyme of CYP450 was marked as the most active in all the three generations. Such observations strongly suggest that the selection of *H. theivora* with pesticide or a cocktail of pesticides as is the practice in tea plantations, for generations may induce the expression of more esterase and CYP450 genes for pesticide detoxification. Wu et al. (2011) have shown that qualitative changes of esterases can also give rise to the development of resistance in insects.

5.6.4 Inhibitory effect of organophosphate pesticide on isozymes of general esterases

The esterase isozymes separated by electrophoresis on the polyacrylamide gels were inhibited by organophosphate pesticide during the process of staining, following the method described in the section 4.10. The isozyme belonging to group II with the contribution of 85.50 and 88.20% to the total activity of the esterases, respectively in P and F1 generations was found to be the most active or the least inhibited among all the esterases. While in the F2 generation, the isozyme belonging to group III with the contribution of 36.70% to the total activity was the most active amongst the isozymes after inhibition. The isozymes belonging to group III was induced additionally in the most tolerant generation F2, in response to the selection pressure exerted by exposure to pesticide. It could be possible that the tolerance conferred against the pesticide in the selected *H. theivora* was a manifestation of the elevated activity of the induced isozyme. The process of selection of *H. theivora* by exposure to pesticide is described in section 4.5. The pesticide completely inhibited the isozyme belonging to group IV in P, whereas in F1 and F2 generations, there were incomplete inhibitions leaving substantial activities, respectively of about 6.83 and 22.08% of the total activity of the esterases (Figure 5.5; Table 5.7).



Generation	Isozyme Group	Raw Volume (in pixel)	Raw Volume %	Rf	Height
P	I	44476.99	14.50	0.1172	25.338
	II	262184.7	85.50	0.2074	37.07
F1	I	21967.33	4.89	0.1189	27.398
	II	396327.00	88.28	0.2025	62.67
	IV	30656.92	6.83	0.4336	21.906
F2	I	86056.49	7.91	0.1197	44.27
	II	362422.9	33.32	0.2016	89.501
	III	399172.4	36.70	0.2434	95.996
	IV	240141.6	22.08	0.4000	36.683

Figure 5.5: General esterase isozyme profile of *Helopeltis theivora* across three generations when inhibited by organophosphate

Table 5.7: Comparison of the isozyme profile of general esterases in organophosphate pesticide selected *Helopeltis theivora*

Generation	Isozyme Group	UNINHIBITED				INHIBITED			
		Raw Volume (in pixel)	Raw Volume (%)	R_f	Profile Height	Raw Volume (in pixel)	Raw Volume (%)	R_f	Profile Height
P	I	161678.34	18.11	0.11	26.238	44476.99	14.5	0.11	25.33
	II	719021.44	80.54	0.20	49.766	262184.7	85.5	0.20	37.07
	IV	012053.19	01.35	0.44	25.155	----------BLOCKED COMPLETELY----------			
F1	I	098988.64	12.96	0.11	42.046	21967.33	04.89	0.11	27.39
	II	645931.25	84.62	0.20	69.274	396327.0	88.28	0.20	62.67
	IV	018426.06	02.42	0.43	28.533	30656.92	06.83	0.43	21.90
F2	I	117874.30	05.88	0.11	37.84	86056.49	07.91	0.11	44.27
	II	1025765.3	51.13	0.21	99.803	362422.9	33.32	0.20	89.50
	III	417604.59	20.17	0.24	99.852	399172.4	36.70	0.24	95.99
	IV	444793.53	22.17	0.41	39.651	240141.6	22.08	0.41	36.68

5.6.5 General esterase isozymes in presence and absence of pesticide inhibition

As indicated by the densitometric analysis of the electropherogram of the general esterases, there was a marked difference in the activities of isozymes of general esterases in presence and absence of inhibitor. In general, as per the raw volume (in pixel) of the electrophoregram, there was a drastic reduction in the activity of the defence enzyme when inhibited by the pesticide.

In absence of inhibition, the contribution to the total activity of the defence enzyme, the isozyme belonging to group IV in P generation was 1.35%. The activity of the isozyme got obliterated completely when inhibited by pesticide during staining. Similarly, the activity of the isozymes belonging to the group I got reduced in parental generation. The contribution to the total esterase activity of isozyme (s) belonging to group I decreased from 18.11% in uninhibited condition to 14.5% when inhibited. Interestingly, the activity of group II isozyme increased, thus the contribution to the total activity rose from 80.5% to 85.5% after inhibition in the same generation (Figure 5.6).

In F1 generation, the share in the total activity of the group II and IV isozyme increased respectively from 84.62% and 2.42% in absence of inhibition to 88.28% and 6.83% when inhibited by the pesticide with the concomitant decrease in the activity of group I from 12.96% to 4.89%.

In the most tolerant F2 generation, the share of group II isozyme to the total esterase activity decreased from 51.13% to 33.32% when inhibited. The decrease was compensated by an increase in the activities of groups I and III. The activity of group I increased from 5.88% in absence of inhibtion to 7.91% when inhibited and that of group III increased from 20.82% to 36.08%, respectively in absence and presence of inhibition. There was no such difference in the contribution of group IV isozyme towards the total activity of the esterases (Figures 5.1, 5.2, 5.5, and 5.6). These observations suggest that the isozymes induced by the exposure to pesticides are the least inhibited and plays a pivotal role in conferring higher tolerance level and resistance, when challenged by pesticides.

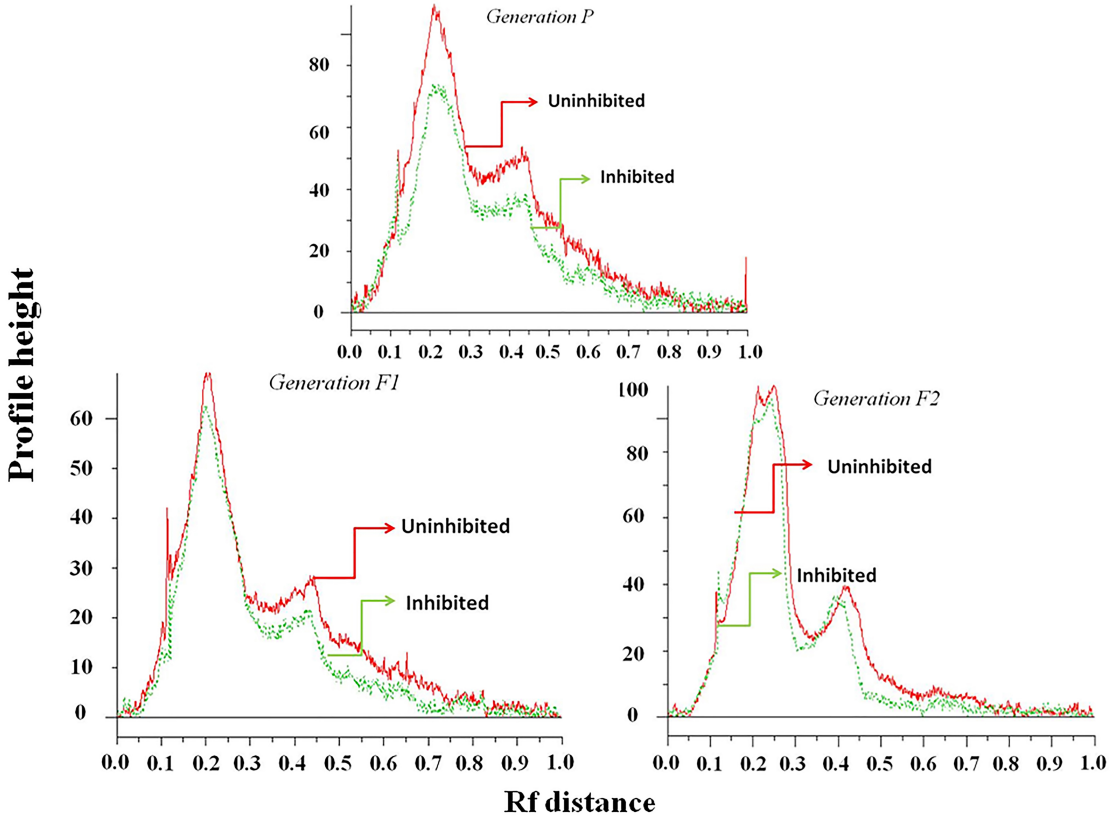


Figure 5.6: Comparison of general esterase isozyme profile across organophosphate-selected generations of *Helopeltis theivora*

5.6.6 Inhibitory effect of organophosphate pesticide on isozymes of cytochrome P450

Densitometric analysis of the electropherogram of the isozymes of CYP450 revealed that, when inhibited by the organophosphate pesticide during the process of staining, the heights and the volumes of isozyme profile were found to be significantly reduced in comparison to pesticide uninhibited electropherogram profile. In both F1 and F2 generations, the isozyme (s) belonging to group II along with other less prominent minor bands were totally blocked and were not detectable in the electropherogram (Figures 5.3, 5.4, 5.7 and Table 5.8).

5.6.7 Cytochrome P450 isozymes in presence and absence of pesticide inhibition

When inhibited by the organophosphate pesticide, the isozymes belonging to group II were totally blocked in all the tested generations, consequently, the group I isozyme(s) contributed 100% to the total activity of CYP450 monooxygenases. The overall raw volume corresponding to the activity of the isozyme(s) also got reduced considerably in all the generations (Figures 5.3, 5.4, 5.7 and 5.8).

Inhibition by organophosphate pesticide did not block the isozymes of the defence enzyme completely. Additional isozymes induced in pesticide-selected generations were the least inhibited. Those non-inhibited isozyme(s) could be the one conferring higher tolerance. The GE isozyme belonging to group III expressed in the F2 because of pesticide selection was found to be the most prominent with 36.70% of total enzyme activity after inhibition. CYP450 isozyme groups were not completely blocked by the pesticide inhibition. Although the total activity was reduced, the activity of the CYP450 isozyme belonging to group I was significantly visible (Figures 5.3, 5.7 and 5.8) indicating its involvement in detoxification of the organophosphate pesticide. The induction of CYP450 and GE in pesticide-exposed insects (Han et al., 2012b) and their roles in pesticide metabolism and detoxification are well documented (Zhu and Luttrell, 2012; Yu, 2014).

From these observations, it appears that different alleles of detoxifying enzymes responsible for imposing higher tolerance or resistance are operative in *H. theivora* population. Further, these observations indicate that continuous application of the same pesticide or pesticides with a similar mode of action at very high dose may have implication in selecting more tolerant individuals.

Table 5.8: Comparison of the isozyme profile of cytochrome P450 in organophosphate pesticide selected *Helopeltis theivora*

Generation	Isozyme Group	UNINHIBITED				INHIBITED			
		Raw Volume (in pixel)	Raw Volume (%)	R_f	Profile Height	Raw Volume (in pixel)	Raw Volume (%)	R_f	Profile Height
P	I	1505321	100	0.11	97.80	1088739	100	0.13	99.61
F1	I	1751194	99.04	0.12	110.20	1236218	100	0.13	109.64
	II	17008.08	0.96	0.88	17.12	----------BLOCKED COMPLETELY----------			
F2	I	2216224	52.88	0.13	100.04	1745694	100	0.14	119.43
	II	1974486	47.12	0.83	27.44	----------BLOCKED COMPLETELY----------			

It is also possible that the application of pesticide can develop a cross-resistance to another pesticide with a similar mode of action. There are many instances of development of cross-resistance in insects in response to application of pesticides (Yu, 2014). Cross-resistance refers to a condition in which a population of insect resistant to one pesticide develops resistance to another pesticide by default to which it has not been exposed. Cross-resistance can develop from enhancement of nonspecific defence enzymes such as CYP450 and GEs. Thus, in populations of *H. theivora* also, enhancement of defence enzymes by exposure to monocrotophos as shown in this study can result in the development of resistance against other organophosphate compounds such as quinalphos, as they do share a common basic structural skeleton and the mode of action. The mode of action of the two pesticides is by blocking acetylcholinesterases (AChE) to disrupt the axonal transmission of nerve impulses.

An endemic population of a pest usually comprises a variety of biotypes with subtle differences from one another in terms of survival. Directional selection results in survival of the fittest having gene (s) for the greater tolerance or resistance. These tolerant forms have much reduced mortality in the toxic environment created by the application of the pesticide (s). Subsequent generations of such selected pests, therefore, have an increased frequency of the alleles responsible for the resistance, a case of bottleneck effect (ffrench-Constant et al., 2004). As suggested by Basnet et al. (2015), periodic change of pesticide with different functional group or mode of action along with the augmentative release of natural enemies such as lynx spider (Basnet and Mukhopadhyay, 2014) is expected to perform better in managing such tolerant *H. theivora* populations. Further, the pest population may be better tackled with effective pesticides in such situations with proper surveillance of tolerance level (Basnet and Mukhopadhyay, 2014).

5.7 *In vitro* reaction pattern of synergists with detoxifying enzymes of *Helopeltis theivora*

A synergist is a chemical added to pesticide formulations containing active and other ingredients, which increases the efficacy of the pesticide. They usually are structural analogues of chemical pesticides and effective inhibitor of defence/detoxifying enzymes.

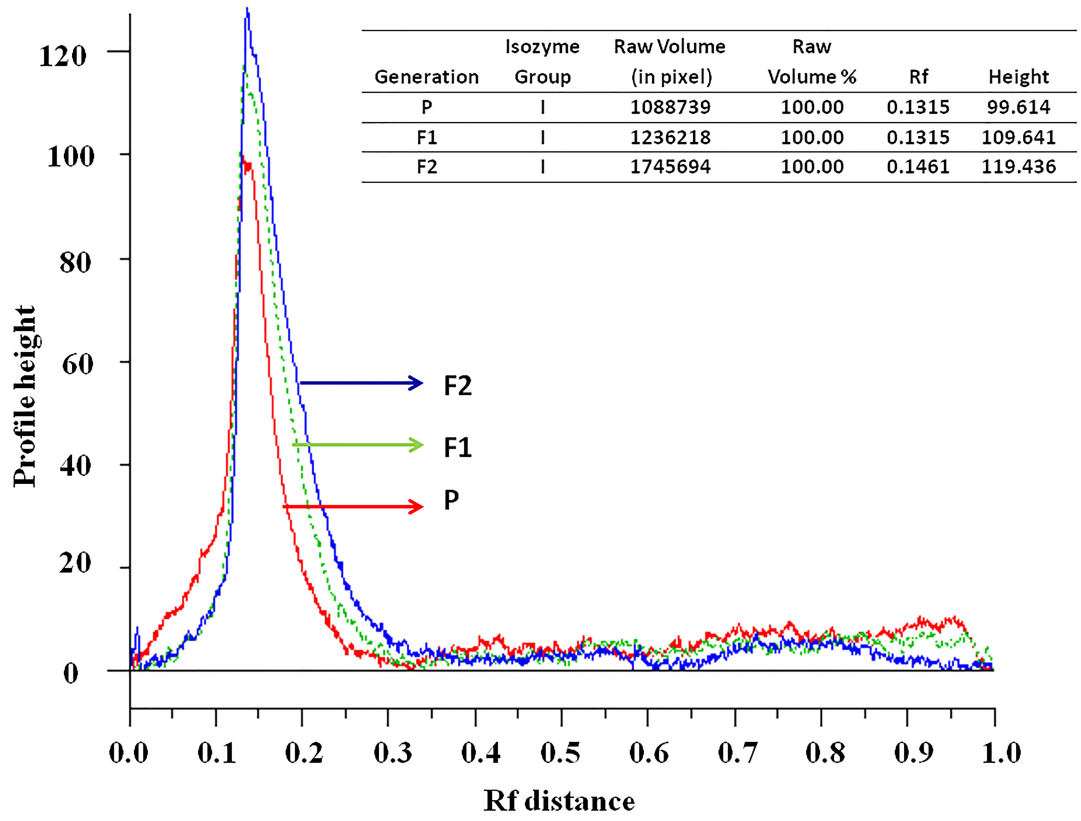


Figure 5.7: Cytochrome P450 isozyme profile of *Helopeltis theivora* across three generations when inhibited by organophosphate

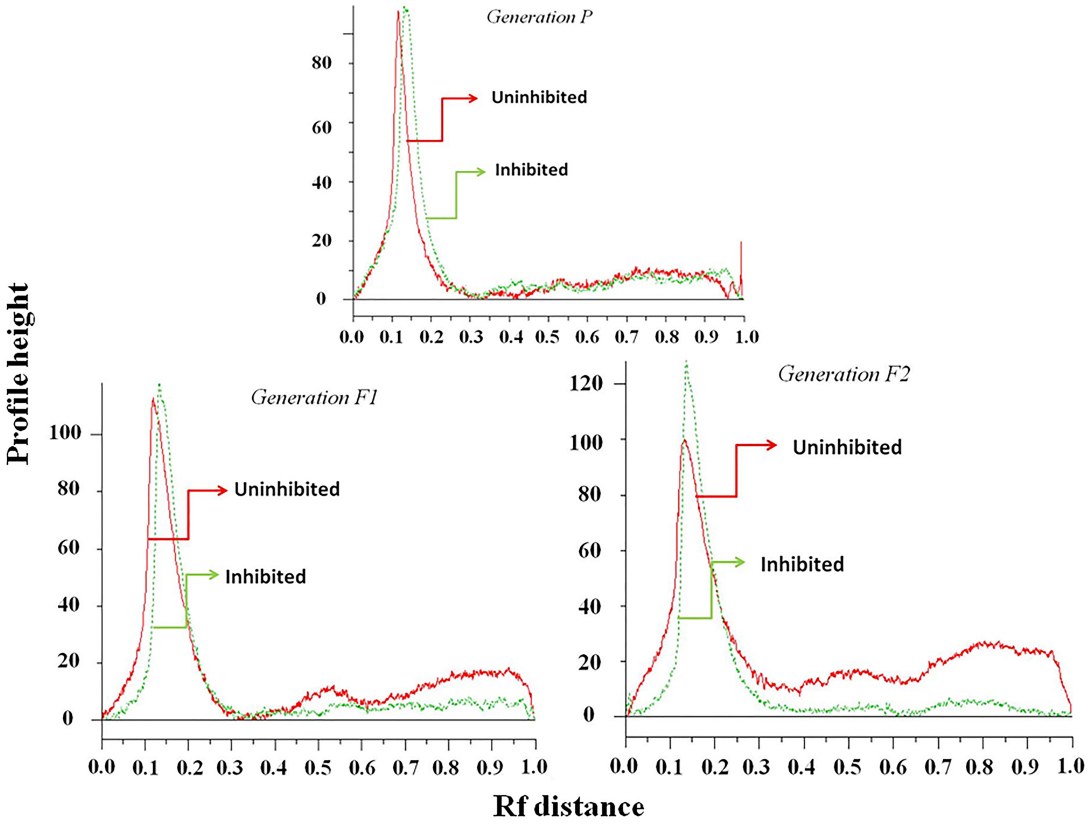


Figure 5.8: Comparison of cytochrome P450 isozyme profile across organophosphate-selected geneations of *Helopeltis theivora*

For their metabolic enzyme inhibiting ability, synergists have been used extensively both for enhancing the effectiveness of pesticides and as an analytical tool for diagnosing resistance mechanism as well (Bernard and Philogène, 1993; Young et al., 2006). Synergists have evolved as an invaluable tool for studying metabolic resistance mechanisms and pesticide detoxification in insects (López-Soler et al., 2011). They are the most promising tools for overcoming metabolic resistance as they can directly inhibit the resistance mechanism itself (Raffa and Priester, 1985). The two common pesticide synergists S, S, S - tributylphosphorotrithioate and piperonyl butoxide have been tested in present study by applying as the inhibitor of GE and CYP450, respectively.

5.7.1 Inhibition of general esterase by synergist, S, S, S - tributylphosphorotrithioate

Percent inhibition of GE activity of the whole body homogenate of *H. theivora* was subjected to one-way ANOVA, which deduced *F*-value of 3195.75 at $p \leq 0.001$ and *df* = 8, 297 (Table 5.9), showing a strong variation in the *in vitro* inhibition of the defence enzyme. The variation suggested that the inhibition was dependent on the effective concentrations of the inhibitor S, S, S – tributylphosphorotrithioate (DEF) in the reaction mixture.

Bivariate nonparametric correlation analysis between the concentration of DEF and the percent inhibition of GE activity deduced a statistically significant Pearson's correlation coefficient '*r*'= 0.979 at $p < 0.001$ (2-tailed), suggesting the prevalence of a positive correlation between the two covariates (Table 5.10). The observation further asserts that the inhibition of the activity of GE by DEF is a dose-dependent interaction.

Simple regression analysis showed that the linear relationship between the percent inhibition of GE activity and concentration of the synergist used as inhibitor to be statistically significant with an $R^2 = 0.958$ at $p < 0.001$ (Table 5.11). The observation too asserts the dependence of inhibition of GE activity on the concentration of DEF used. The finding further shows that the *in vitro* GE activity inhibition by DEF is a linear function of the concentration of the inhibitor for *H. theivora* population.

Table 5.9: One-way analysis of variance of percent inhibition of general esterases activity and cytochrome P450 by S, S, S – tributylphosphorotrithioate and piperonyl butoxide, respectively in *Helopeltis theivora*; n=305

Source	*df*	Inhibition of General Esterases				Inhibition of Cytochrome P450			
		[¥]SS	[§]MS	*F*	Sig.	[¥]SS	[§]MS	*F*	Sig.
Between Groups	8	252434.50	31554.31	3195.754	0.000	2.07	0.26	11.58	0.000
Within Groups	297	002932.52	9.87			6.64	0.02		
Total	305	255367.03				8.71			

[¥]Sum of squares

[§]Mean of square

Table 5.10: Correlation between the concentration of inhibitor and the percent inhibition of the defence enzymes in *Helopeltis theivora*

	Covariates	Percent inhibition of General Esterase		Percent inhibition of Cytochrome P450	
		[¥]Concentration (mM)	[§]Inhibition	[†]Concentration (mM)	[††]Inhibition
Pearson Correlation	[¥†]Concentration	1	0.979	1	0.987
Sig. (2-tailed)			0.000		0.000
n		306	306	306	306
Pearson Correlation	[§††]Inhibition	0.979	1	0.987	
Sig. (2-tailed)		0.000		0.000	
n		306	306	306	306

[¥]Concentration of S, S, S – tributylphosphorotrithioate

[§]General Esterase Inhibition

[†]Concentration of piperonyl butoxide

[††]Cytochrome P450 Inhibition in mM

Table 5.11: Simple regression analysis of inhibitor concentration of percent inhibitions of defence enzymes in *Helopeltis theivora*

Model Summery	Defence Enzyme versus Inhibitor	
	General Esterases versus [#]DEF	Cytochrome P450 versus [§]PBO
r	0.979	0.987
R^2	0.958	0.975
Adjusted R^2	0.958	0.975
SE of the estimate	5.930	4.763
F	6939.18	11676.69
Sig.	0.000	0.000

[#] S, S, S-tributylphosphorotrithioate

[§] Piperonyl butoxide

The observation needs careful interpretation as the dose-response curve (Figure 5.9) obtained is a non-linear hyperbola indicating the increase in the concentration of the inhibitor failed to inhibit the defence enzyme proportionately beyond a certain point. The graph attained an asymptote beyond the point, suggesting that 100% inhibition is not achievable with the increase in the concentration of the inhibitor. The lack of cent percent inhibition indicates the presence of more than one isozymes of GE in the whole body homogenate of the pest. Qualitative studies on GE, as described in section 5.6.1 also have shown the presence of multiple isozymes (groups) of the defence enzyme. Inhibition of all the isozymes by DEF may not be trivial due to their substrate specificity and variability in active sites, leaving a substantial amount of GE activity intact. The half the maximum inhibitory concentration, commonly called as IC_{50} of DEF as computed through probit analysis was found to be 0.52 mM with a 95% confidence interval of 0.47 – 0.58 mM (Figure 5.10). The concentration of the DEF needed to inhibit half (50%) of the maximum activity of GE *in vitro* assay at a specific substrate concentration is its IC_{50}.

DEF is an organophosphate compound (Lewis, 2004) widely acclaimed as an effective synergist of pesticide (Alves et al., 2008). Most pesticides are esters of substituted phosphoric, carbamic or cyclopropane carboxylic acid and are prone to degradation by the action of hydrolytic enzymes such as GE (Devonshire, 1991; Devonshire and Field, 1991). Resistance to pesticides in insects is due to the enhanced activities of defence enzyme including GE (Perera et al., 2008). DEF functions by inhibiting hydrolytic defence enzymes especially GE involved in the metabolism of pesticides with ester linkages (Soderland and Bloomquist, 1990; López-Soler et al., 2011), making pesticide effective and persistent. The ability of synergists like DEF to biochemically inhibit defence enzymes can be harnessed for increasing the effectiveness of pesticides *vis-à-vis* for the management of resistant or more tolerant pests. For using DEF as pesticide synergist in the field, care must be taken as the chemical also act as a plant defoliant when applied at a dose beyond certain concentration in cotton plantations (Potter et al., 2002) and may show such defoliant activity in tea plantations as well. Moreover, increasing the concentration of DEF did not attend 100% inhibition as stated above, which suggests that application of the higher dose of the synergist to improve the efficacy of pesticide would lead only to waste of resources and chemical contamination of the environment.

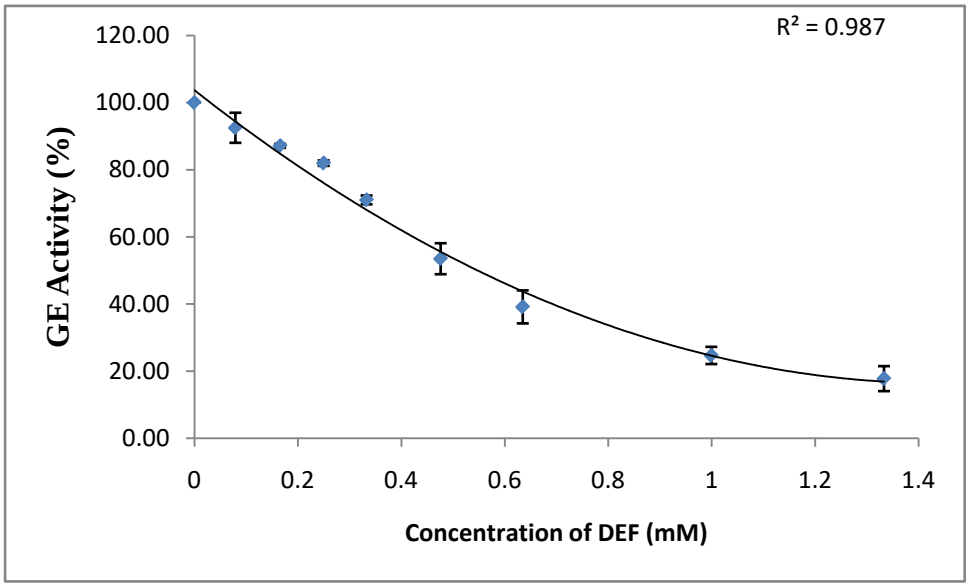


Figure 5.9: Inhibition of general esterase by S, S, S - tributylphosphorotrithioate

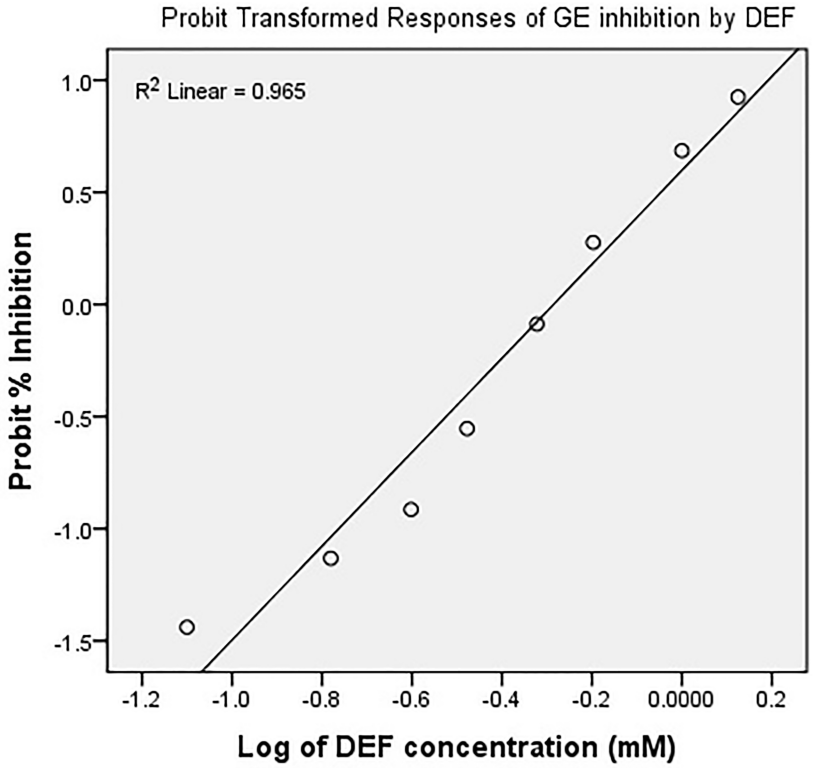


Figure 5.10: Probit transformed curve showing the probability of general esterase activity inhibition in response to S, S, S - tributylphosphorotrithioate in *Helopeltis theivora*

The aim of this study was to ascertain the inhibitory effect of DEF on the esterases of *H. theivora* for possible application of the chemical as a synergist of suitable pesticide for the management of insect pest with higher pesticide tolerance or resistance in tea plantations.

5.7.2 Inhibition of cytochrome P450 by synergist, piperonyl butoxide

The percent inhibitions of CYP450 activity by various concentrations of pyperonyl butoxide (PBO) were subjected to ANOVA. The analysis deduced *F*-value of 11.589 at $\alpha = 0.05$ and $df = 8, 297$ (Table 5.9) suggested strong variations in inhibition both within and between the tested populations. The observed variation in inhibition of CYP450 activity in whole body homogenate of *H. theivora* by PBO suggested that the *in vitro* inhibition was dependent on the concentrations of the synergist used in the present study as an inhibitor for the analysis.

The correlation between the concentration of PBO and the percent inhibition of CYP450 activity was determined by bivariate correlation analysis. A very high Pearson's correlation coefficient (r) of magnitude 0.987 at $p < 0.001$ (2-tailed) was found, suggesting the prevalence of a strong positive correlation between the two variates (Table 5.10). The observation also indicated that the inhibition of the activity of CYP450 by PBO is a concentration-dependent interaction.

Regression analysis revealed that there exists a statistically significant linear relationship between the percent inhibition of CYP450 activity and concentration of the inhibitor. The coefficient of determination (R^2) of 0.975 at $p < 0.001$ (Table 5.11) also implies a strong dependence of the inhibition on the concentration of the inhibitor. The finding further shows that *in vitro* inhibition of CYP450 activity by PBO is a linear function of the concentration of the synergist acting as an inhibitor for *H. theivora* population. The non-linear hyperbolic dose-response curve obtained (Figure 5.11) suggested that the increase in the concentration of the synergist failed to inhibit the defence enzyme proportionately beyond a certain level. Therefore, care must be taken to deduce the importance of the apparently linear dependence of inhibition on the concentration of the synergist used as an inhibitor for the assay. The asymptote in graph attained after a point suggested that the complete inhibition is not possible by increasing the concentration of the inhibitor. Such a lack of cent percent inhibition clearly indicated the presence of more than one isoforms of CYP450 in the whole body homogenate of the pest. Qualitative studies of CYP450, as described in

section 5.6.2 also have shown the presence of multiple isozymes (groups) of the defence enzyme. As there exist a substrate specificity and variability in active sites among different isozymes, inhibition of all of them by PBO may not be possible. Therefore, a substantial amount of CYP450 activity may remain intact even after treatment with inhibitor. The IC_{50} of PBO as deduced by probit analysis was found to be 0.88 mM with a 95% confidence interval of 0.79 – 0.98 mM (Figure 5.12). IC_{50} is the concentration of the inhibitor needed to inhibit half (50%) of the maximum activity of an enzyme *in vitro* assay at a specific substrate concentration (Yung-Chi and Prusoff, 1973). The IC_{50} value acts as a reference for choosing the appropriate dose of synergist needed for management of the pest by planters.

As discussed in preceding sections, CYP450 monooxygenases are involved in the metabolism of synthetic pyrethroids, organophosphates and carbamate pesticides (Feyereisen, 1999). Roy et al. (2009b) have shown the synergistic action of PBO on the toxicity of various pesticides against *H. theivora*. PBO delays detoxification of pesticides in insects by inhibiting detoxifying enzymes especially CYP450 monooxygenases (Knowles, 1991; Hodgson and Levi, 1998). The addition of PBO as a synergist to pesticide reduces the dose of the active ingredient required to generate the desired pesticide effect. The half-life of PBO ranges from 4.3 days in the soil to 3.4 hours in the air. Thus, PBO in the environment is not persistent and gets degraded rapidly thus, it does not act as a serious pollutant (Arnold, 1998). Therefore, PBO appears to be a safe and appropriate synergistic chemical that is reliable for the management of the resistant pest. Although PBO is widely used as household and storage pest control formulation, its potential as a synergist to maintain the efficacy of pesticide is not tapped to the fullest in horticulture and agricultural systems (Young et al., 2005) including tea plantations. Hence, the inhibition of CYP450 by using the precise dose of the inhibitor as a synergist of appropriate pesticide can help planters in managing the resistant pest sustainably.

5.8 Defence enzyme based techniques to determine pesticide tolerance levels in *Helopeltis theivora*

The level of pesticide tolerance is a vital component, a plant protectionist should consider while developing and formulating pest management strategies for the optimal use of pesticides. The desired goal of the minimal use of pesticide requires

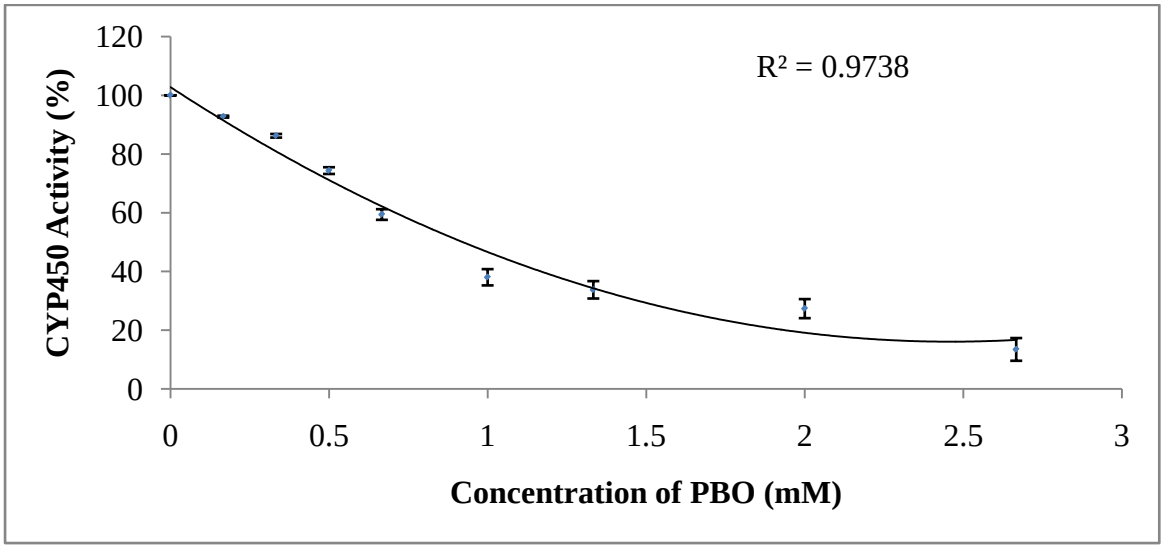


Figure 5.11: Dose response curve showing inhibition of cytochrome P450 by piperonyl butoxide
-error bar represents *SE*

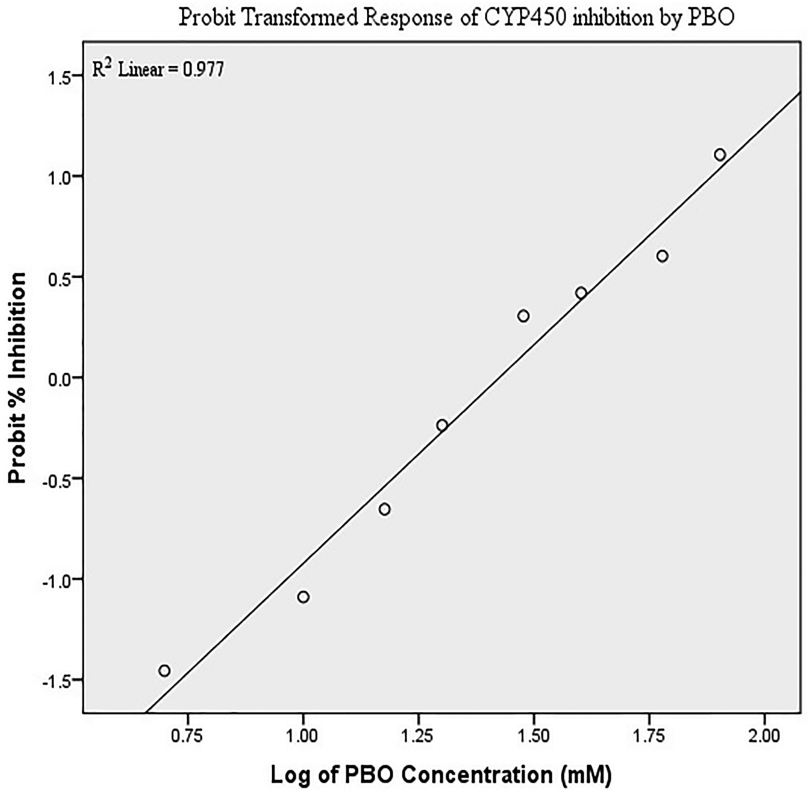


Figure 5.12: Probit transformed curve showing the probability of cytochrome P450 activity inhibition in response to piperonyl butoxide in *Helopeltis theivora*

continuous monitoring of the emergence and development of tolerance and resistance to pesticides in a given pest. Biochemical assays, especially based on the activity of defence enzymes appear as an important technique to determine the levels of pesticide tolerance in a pest (Anonymous, 2016).

5.8.1 Microplate-based Assay

The observed and the expected LC_{50} values, respectively were computed based on the tolerance level determined by bioassay performed as per the technique described in materials and methods section and by fitting the data of enzyme activities to a reference equation (Table 4.1). The two reference equations used as standard were prepared based on the tolerance level of *H. theivora* population against pesticide of class organophosphate and synthetic parathyroid and the corresponding defence enzyme activity in the population. Chi-square (χ^2) test was performed to assess the goodness of fit between the observed and the expected values of LC_{50}. In none of the tested populations, the calculated χ^2 was more than the table value of 7.81 at $\alpha = 0.05$ and $df = 3$ (Table 5.12 and 5.13). The observation convincingly suggests that the expected and the observed LC_{50} values were not significantly different from one another. The standard equation developed, therefore, appears to be robust enough for determining the relative LC_{50} values of unknown populations of *H. theivora* for the pesticides belonging to the group organophosphate and synthetic pyrethroids. However, care must be taken to interpret the LC_{50} obtained based on the standard equation, as the tolerance level in insect as reviewed in section 2.2 is determined by an array of factors. The tolerance level obtained should be taken as relative instead as an absolute value. Detection of the activity levels of detoxifying enzyme appears to act as an alternative to the conventional bioassays for indexing pesticide tolerance level in several insect pests (Maa and Liao, 2000; Lopez-Soler et al., 2008; Perera et al., 2008).

Our observations also bear out the idea of applying metabolic enzyme-based detection and indexing of the level of tolerance in *H. theivora* population. Such biochemical-based technique can, therefore, help to determine the level of tolerance of a pest population with a much lower number of insects compared to the conventional labour intensive, time-consuming bioassays.

Table 5.12: The observed and the expected tolerance levels (LC_{50}) of *Helopeltis theivora* populations based on GE activity

Population	GE Activity	Organophosphate			Synthetic Pyrethroid		
		Observed LC_{50}	Expected LC_{50}	χ^2	Observed LC_{50}	Expected LC_{50}	χ^2
1	4.17	77.52	80.72	6.9	17.35	18.23	0.83
2	2.27	40.79	32.88		13.39	10.78	
3	1.93	15.76	24.36		8.35	9.45	
4	1.23	10.35	6.81		6.33	6.71	

Table 5.13: The observed and the expected tolerance levels (LC_{50}) of *Helopeltis theivora* populations based on CYP450 activity

Population	CYP450 Activity	Organophosphate			Synthetic Pyrethroid		
		Observed LC_{50}	Expected LC_{50}	χ^2	Observed LC_{50}	Expected LC_{50}	χ^2
1	0.25	77.52	74.50	1.86	17.35	20.43	1.13
2	0.18	40.79	47.58		13.39	14.34	
3	0.09	15.76	12.96		8.35	6.51	
4	0.08	10.35	9.12		6.33	5.64	

Such a rapid, easy to monitor technique can be adopted at the field laboratory level as an on-spot tool for quick determination and continuous surveillance of tolerance status of a pest population during the control programme. Information on resistance status would guide planters to choose proper pesticide and design pest management strategy accordingly, saving both the cost of application of pesticides as well as reducing environmental contamination.

5.9 Life cycle parameters of spider *Oxyopes javanus*

5.9.1 Courtship and Mating Behaviour

The male *O. javanus* performed an intricate courtship dance by shaking or moving body up and down also by raising the prosoma and drumming pedipalps to lure the female (Plate 5.4). The courtship dance always preceded the successful mating. The responsive female showed interest by moving her pedipalps up and down alternately, simply once or twice. During the process, the male and female came very close and made a very short physical contact with their first pair of legs. The male then approached the female very cautiously from the side and mounted her from the front or directly from behind. The female twisted her abdomen in such a way that the male could insert one of the palps in the ventrally located genitalia of the female for depositing the sperm packets within seconds with utmost elegance. No long-term physical contacts lasting more than few seconds were observed between them. The process of courtship followed by mating that lasted for 10 – 20 minutes. Successful mating was marked by the change in attitude of the female towards the male. Subsequent to mating, behaviour of both the genders changed dramatically. The female now approached the male as a predator and tried to get hold for eating him up. The majority of such attempts were not successful, as after mating males were in a hurry to flee the scene to find a safe hideout. Those males successful in avoiding sexual predation were observed to be able to mate with a new female every alternate day.

Finding a potential mate is the first step towards successful reproduction, which may not be trivial for all spiders for their solitary nature of life (Foelix, 2011). Proper self-introduction by male spiders to females is necessary as females do often treat males as a prey.

PLATE 5.4: *Oxyopes javanus* performing courtship dance

PLATE - 5.4

Courtship signalling identifies males as conspecific mating partner, arouses receptivity and reproductive behaviour along with suppressing the hunting behaviour of predatory females. Courtship involves sequential movements of appendages and body, creating visual and vibratory signals. The correct display prevents sexual cannibalism before mating (Herberstein, 2011). As the courtship behaviour is species specific, it maintains reproductive isolation preventing cross-fertilisation (Barth, 1993). During short physical contact (copulation), specialised tarsal segment of the pedipalp of the male is inserted into the female's genital opening and the sperm packets are deposited in her seminal receptacles (Foelix, 2011).

5.9.2 Ovipositon, fecundity and incubation period

The female *O. javanus* spun a protective cocoon of silk with fine mesh also called as egg sac around newly laid eggs. In tea plantations, egg masses were mostly observed to be deposited on mature leaves below the tea canopy. They lay eggs on the dorsal surface of mature leaves. In laboratory conditions, egg masses were mostly (about 70%) deposited on the muslin cloth used for covering the mouth of the rearing jar. In some cases ventral side of the leaves of the tea shoots kept inside the jar were also selected for the deposition of the egg sac. Occasionally, egg sacs were also found to be deposited on the sidewalls of the rearing jar (Plate 5.5).

After deposition of eggs, females were found to be vigilantly guarding the eggs against any threat (Plates 5.5) and became very aggressive throughout the incubation period. In the laboratory, the incubation period varied from 13 to 30 days during summer season spanning from May – July. The guardian female seldom fed with minimal physical activities during the egg incubation period.

Fecundity ranged between 72 to 148 eggs per egg sac. Each female spider laid 1 – 3 batches of eggs in her lifetime. The hatchability and the emergence was recorded ranging from 58 – 140 spiderlings per egg sac or cocoon (Table 5.14). Although a firm and tough cocoon provides protection and maintains microclimatic condition, especially humidity within for the developing spiderlings, (Hieber, 1985) while hatching, it also poses a problem for the neonates in their exit from the egg-case during hatching.

PLATE 5.5: Female *Oxyopes javanus* guarding egg mass/cocoon
In tea plantations

PLATE - 5.5

Nevertheless, the spiderlings manage to create a small opening by enzymatic digestion of the cocoon for the exit (Herberstein, 2011). Maternal care in female *O. javanus* was demonstrated as she actively guarded the cocoon until the end of incubation period. The spider males were found to be polygamous and parental care was largely lacking in them. Normally the newly hatched spiderlings cling by abdominal hairs onto the body of mother (Rovner et al., 1973), however, spiderlings of *O. javanus* did not show such behaviour.

5.9.3 Developmental stages

5.9.3.1 Spiderlings

Very tiny spiderlings hatched out inside the cocoon. The first stage was an immobile pale white larva with a pale greenish tinge in the abdomen. Larvae observed to be covered by an embryonic membrane and were non-feeding, indicating the retrieval of nourishment from the yolk possibly present within their abdomen. After two moults within a week, the larva changed into a mobile spiderling, which is also known as the nymphal stage (Plate 5.6). In the present study, spiderlings were recorded to undergo 9 – 10 moults to attain adulthood depending upon the gender, with male requiring lesser number of moults. A very high mortality (70 ± 2%) of the post-embryonic stages before reaching adulthood was recorded in the laboratory conditions (25 ± 2^{o}C, 85 – 90% RH and 12 L: 12 D photoperiod). After the last moult, the functional sexual organ developed and further growth in size was not observed. At this stage, the tarsal segment of pedipalp of male was found to be covered by hair-like structures or bristles which grew bulb-like housing several sex-related structures.

5.9.3.2 Morphology of adult *Oxyopes javanus*

Body and all the legs were found to be light brownish. The abdomen was green in male while chalky white in female. The cephalic region was marked by two longitudinal black lines on the either side. There were eight simple eyes in the cephalic region positioned to cover the vision angle of almost 360°. Males were smaller than the females and had a pair of pear shaped terminal tarsal segments of pedipalp or male palp (Plate 5.7). Adults used their strong legs clothed with fine hairs and conspicuous long spines for fast running and capturing prey.

PLATE 5.6: Spiderlings of *Oxyopes javanus*

A) Newly hatched

B) Under binocular microscope (Magnification≈40X)

PLATE 5.7: Adult *Oxyopes javanus*

A) Female

B) Male

PLATE - 5.6

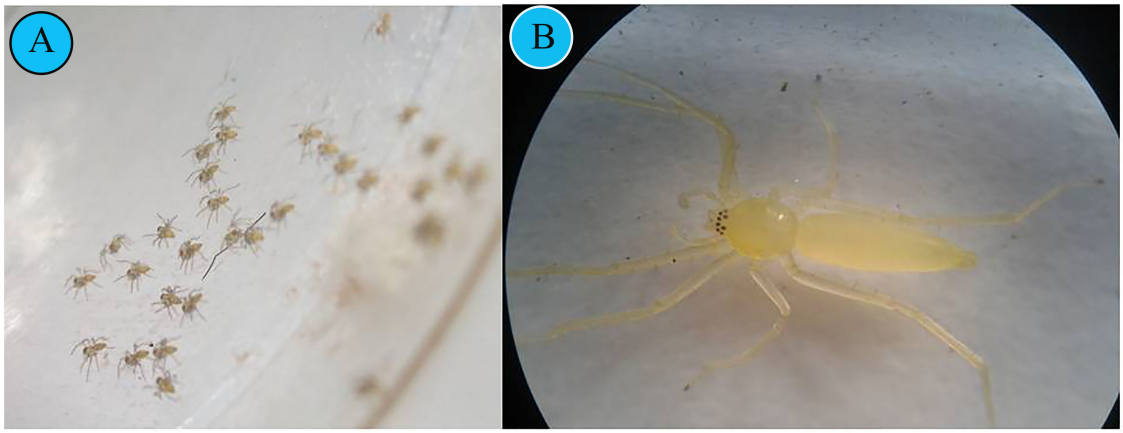


PLATE - 5.7

They used their strong hind legs for jumping and at times found clearing up to a distance of 15 cm in a leap. For this ability, they are also known as jumping or lynx spiders. The average longevity of adult female and male *O. javanus* was recorded as 93.3 ± 5.11 and 51.2 ± 6.42 days (mean ± *SE*), respectively (Table 5.8a).

The ontogeny of a spider is divisible into three stages: an embryonic, a larval and a nympho-imaginal period. The embryonic period starts from the time of egg fertilisation until the features of spider's body without morphological characters of the adult are established (within cocoon). The larval stage is unable to feed and depends upon their yolk supply for nutrition and energy. All the organ systems develop during the nympho-imaginal period. Imagoes (adult) differ from juveniles by attaining sexual maturity (Foelix, 2011). A developing *O. javanus* passed through all these stages, completing life cycle within a short span of about 3 months. Both genders of spider usually took a similar developmental period to reach maturity, but the life span of females is often somewhat longer than males (Levy, 2009). As found in other spiders, females of *O. javanus* were found to be bigger with greater longevity than males.

5.10 Predation efficiency and biological control potential of *Oxyopes javanus* against *Helopeltis theivora*

5.10.1 Functional response

With the increase in the number of adult prey (*H. theivora*) per experimental arena, the predation rate increased in both the sexes of *O. javanus*. When the sufficient number of prey were provided, females of *O. javanus* (n = 5) showed mean per capita predation of 11.67 (SD = 1.53) much higher in comparison to a male (n = 5), predating only on 3.67 (SD = 1.52) adult *H. theivora*. Independent sample t-test showed that the mean per capita predation was significantly higher in females than in males of *O. javanus* with t (8) = 6.41 at p = 0.001. Thus, it could be inferred that the female spiders were associated with significantly greater predation potential than males. Higher predation rate of females may be necessary to meet the requirement of their enhanced investment on reproduction than males (Givens, 1978; Walker and Rypstra, 2002). It may also be needed to maintain larger body size of female *O. javanus* demanding a high development and maintenance cost.

Table 5.14: Life cycle parameters of *Oxyopes javanus* reared in laboratory

Sl. No	Parameters	Observations
1	Fecundity	72 to 148 eggs per egg sac (n=13)
2	Egg sac per female	1 to 3 in life time (n=13)
3	Hatchability	48 to 140 per egg sac (n=13)
4	Mortality of spiderling	70±2% (n=144)
5	Adult longevity	female - 93.3 ± 5.11
	(in days; n=10; mean ± *SE*)	male - 51.2 ± 6.42

-experiments were conducted during May-June with RH 85 – 90% and 12L: 12D photoperiod

The proportion of prey consumption was highest, which reached up to 100% at lower prey density in both the sexes, implying that the predator, *O. javanus* has the potential to eliminate smaller populations of the prey, *H. theivora*. In the present study, both sexes of *O. javanus* showed 'type II' form of functional response (Holling, 1959). In type II response, prey consumption increases at a decreasing rate, usually because of reduction in capture rate attributed to the handling time (Holling, 1959; Rypstra, 1995; Marc et al., 1999) and with the increase in prey density beyond certain threshold, the predation rate becomes stable or reaches an asymptote (Holling, 1965). The predation rate in male *O. javanus* became almost constant beyond the prey density of 9 per experimental arena. On the other hand, predation rate of female *O. javanus* continued to increase until 12 insects (*H. theivora*) as prey were there per arena. After such a high density of prey, the predation rate attained a stable phase (Figure 5.13).Type II responses are common in spiders (Rypstra, 1995; Marc et al., 1999). Searching efficiency for female spider was comparatively higher than males. The difference in predation effectiveness across the two genders was of the tune of about 59 fold, where female spider predator being more efficient (Table 5.15 and 5.16).

5.10.2 Aggregative response

The effect of increasing density of spider on predation rate is defined here as 'aggregative response'. Aggregative response study showed that at lower density, *O. javanus* fed cooperatively on *H. theivora*. However, with an increase in density, the average per capita predation rate of *O. javanus* decreased in both the sexes, which is a common phenomenon for most prey-predator interaction. With the increase in the number of *O. javanus* predating on the same number of prey, their propensity expressed in prey consumed per individual spider (♀&♂) got reduced (Table 5.10 and Figure 5.14). This implies the existence of some interference and competition among the spider predators more so among females than in males as indicated by the gradient of the slope of the trend lines in Figure 5.14. Thus, it is imperative to maintain a suitable density of the spider *O. javanus* in tea plantations for their deployment as a biological control agent of the pest.

Table 5.15: Functional responses of *Oxyopes javanus* to adult *Helopeltis theivora* as prey

Parameters	Spider predator	
	Male	Female
a	0.84	4.60
T_H	0.14	0.01
a/ T_H	6.08	356.58

a = predation efficiency
T_H = handling time (days)
a/T_H = effectiveness of predation

Table 5.16: Regression of *Oxyopes javanus* density on predation efficiency

Statistics	Spider Predator	
	Female	Male
R^2	0.95	0.74
SE	0.77	0.26
p-Value	0.001	0.02
Regression Equation*	y = 15.816 - 1.5362x	y = 3.7285 - 0.2084x

*Regression of spider number versus predation efficiency

5.10.3 Prey consumption time

The prey consumption time i.e., the time required for consumption of one live adult *H. theivora* was found to be 47.73 ± 1.69 minutes and 63.13 ± 6.01 minutes (mean ± *SE*) for male and female *O. javanus*, respectively (Figure 5.15). The population density of *O. javanus* in the tea plantations of Terai was found to vary from 1 – 2 spiders per bush during May-October. The density of the spider was largely dependent on the availability of prey population. The population started building up during April-May with the onset of the population of *H. theivora* and gradually declined with the setting in of monsoon as the population of *H. theivora* also dwindled in tea plantations.

For deploying predators as biocontrol agents, the predator-prey relationship has to be well understood. The lynx (hunting) spider, *O. javanus* is predominantly found in the canopy (top tier or table) of tea bushes sharing the same habitat with tea mosquito bug, *H. theivora*. As mentioned in the introductory chapter, the bug feeds on tender leaves, inserts eggs in leaf tissue and the internodes for incubation and completes its life cycle in tea bush canopy. Most hunting spiders prey upon insects that they encounter during their 'active period' (Riechert and Lawrence, 1997; Marc et al., 1999). From the activities and the foraging behaviour of *O. javanus*, it appears that their 'active period' is during dusk and dawn which coincides with the feeding period of *H. theivora* too enabling *O. javanus* as an effective predator of the bug. Competition, intra-guild predation i.e., predation upon members of same trophic level and cannibalism are the prominent limiting factors for the aggregative response of spider (Riechert and Lockley, 1984; Riechert and Lawrence, 1997; Marc et al., 1999). Some spiders reduce their own density through intra-cohort cannibalism, (Riechert and Lockley, 1984; Wise and Chen, 1999). Such self-limiting tendencies may result in depressed aggregative response and lead to eruption of pest population (Fagan and Hurd, 1991). In *O. javanus*, no such behaviours were recorded in the present study.

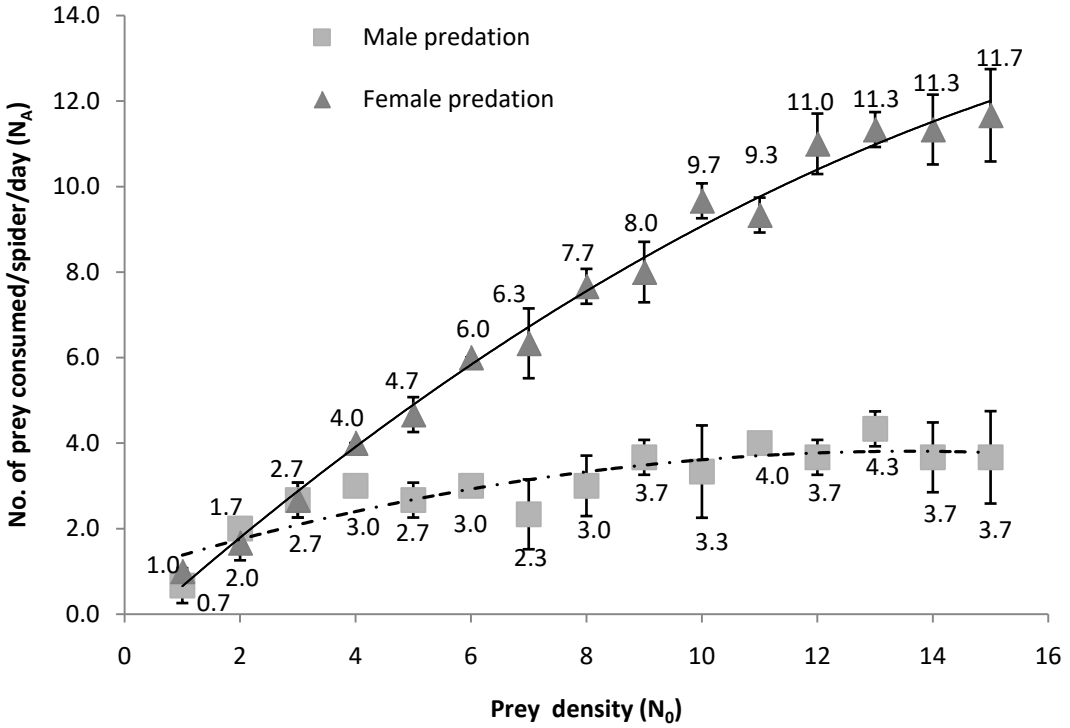


Figure 5.13: Predatory efficacy of *Oxyopes javanus* (♂&♀) with variable density of adult *Helopeltis theivora*
-error bar indicates *SE*

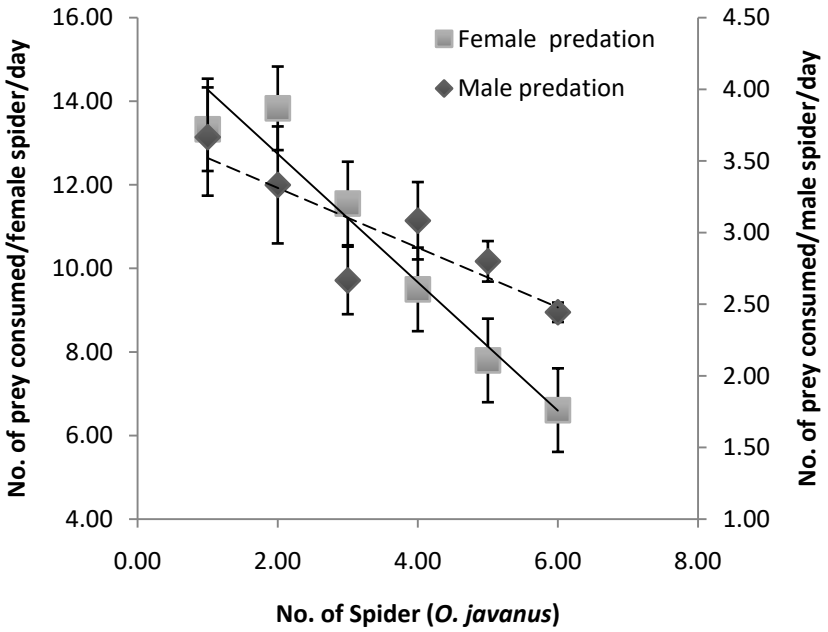


Figure 5.14: Predation efficacy of *Oxyopes javanus* (♀&♂) with increase in its density
-error bar indicates *SE*

Specialist predators may drive the pest (prey) to local extinction and then die off themselves, setting the niche free for secondary pest outbreak. Generalist predators subsist on alternative prey when the pest density is low (Symondson et al., 2002; Stiling and Cornelissen, 2005). The population of *H. theivora* also fluctuated with the change in climatic condition in the tea plantation areas of Terai and the Dooars of North Bengal and their density touched the lowest mark during winter (December through February) (Mukhopadhyay and Roy, 2009). In such period of insufficient prey abundance, *O. javanus* being a generalist predator can thrive well on other alterative preys. Thus, it is highly promising to deem *O. javanus* as a potential biocontrol agent for managing population of *H. theivora* in tea plantations under IPM of NE India including North Bengal region.

About 107 species of spiders belonging to 53 genera distributed over 20 families are recorded from forest and agricultural areas of the Dooars in West Bengal and Assam in NE India (Raychaudhuri, 2009). Conserving, augmenting and finally integrating such a diverse spider fauna (Raychaudhuri et al., 2016), especially *O. javanus* along with other practices of pest management, may help in keeping a check on the population of not only *H. theivora* but also of other soft-bodied tea pests. This would reduce pesticide load in the tea plantations and the cost of tea production. Crop with negligible or less pesticide exposure supports greater density and diversity of spider than those with repeated spraying of pesticides (Yardim and Edwards, 1998; Marc et al., 1999; Holland et al., 2000). Spider diversity and density is higher in organic than in conventional fields (Marc et al., 1999; Das et al., 2010a). Avoiding spray of pesticides during the active period of spider can help to conserve them (Riechert and Lockley, 1984). The risk associated with spiders being used as biocontrol agent is minimal (Stiling and Cornelissen, 2005). Therefore, conservation and augmentation of spiders and other natural enemies should be encouraged especially in the bio-organically managed tea plantations of sub-Himalayan foothills and plains of Darjeeling and NE India as a whole for sustainable production of tea.

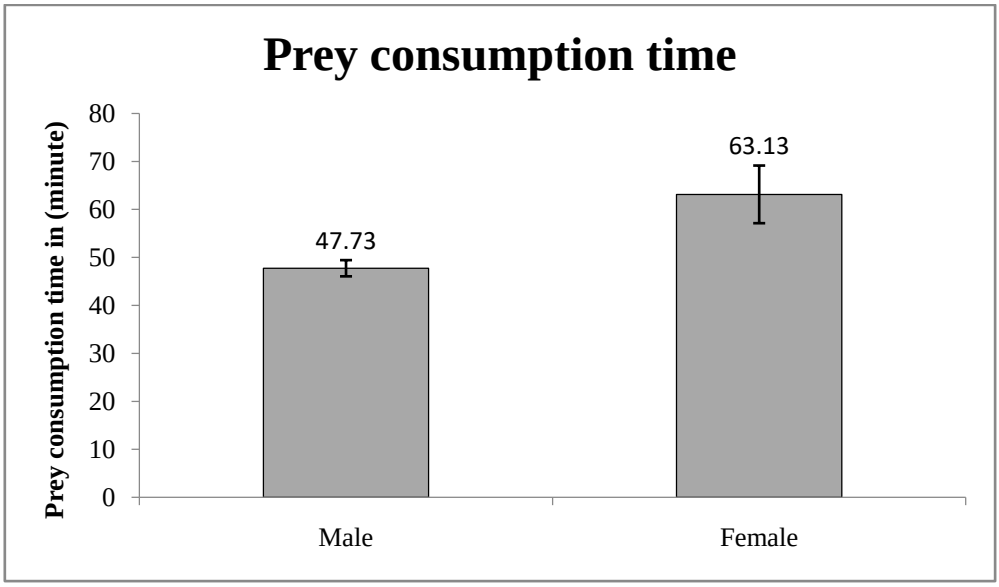


Figure 5.15: Prey consumption time of *Oxyopes javanus*

-error bar indicates *SE*