Assessment of Population Variability at Subcellular Level of Some Common Sucking Tea Pests from Darjeeling Hill and its Adjoining Plain

A thesis submitted to the University of North Bengal
for the award
of
Doctor of Philosophy
in
Zoology
By

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DECLARATION

I declare that the thesis entitled "Assessment of population variability at subcellular level of some common sucking tea pests from Darjeeling hill and its adjoining plain" has been prepared by me under the guidance of Dr. Ananda Mukhopadhyay, Professor of Zoology and Dr. Min Bahadur Associate Professor of Zoology, Department of Zoology, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

We certify that Dhiraj Saha, M.Sc., has prepared the thesis entitled "Assessment of population variability at subcellular level of some common sucking tea pests from Darjeeling hill and its adjoining plain" for the award of Ph.D. degree of the University of North Bengal, under our guidance.

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ABSTRACT

Tea, *Camellia sinensis* (L.) O. Kuntze (Theaceae) is an economically important and intensively-managed monoculture plantation crop. This foliage crop provides a congenial microclimate as well as a continuous supply of food to a number of phytophagous arthropods. According to recent estimates, more than a 1000 (n= 1034) arthropods are associated with tea plantations around the globe. Insect and mite pests are the most damaging. Lepidoptera is the largest order, constituting 32%, followed by Hemiptera with 27% of the insect pest species. In India, only 300 species of arthropods are recorded and about 167 species are from North-East India. The tea plantations of three regions of Northern West Bengal i.e. Terai, the Dooars and Darjeeling hill slopes are attacked by more than two dozen (27-28 nos.) of insect and mite species. At least four of the sucking and six of the chewing arthropods are well established as a regular pest causing substantial damage to the foliage crop. The dynamic adaptations of insects have enabled them to attack every part of a tea plant with a maximum number of pests occurring on the foliage, resulting in 11–55% loss in yield if left unmanaged. Mites, thrips, jassid, aphids, tea mosquito bugs, looper caterpillars, beetles and termites are the most important among the arthropod pests. Thrips, *Scirtothrips dorsalis* Hood (Thripidae: Thysanoptera), the jassid, *Empoasca flavescens* Fabricius (Jassidae: Homoptera) and the tea mosquito bug, *Helopeltis theivora* Waterhouse (Miridae: Heteroptera) are the most important sucking insect pests that attack tea in Terai, the Dooars and lower altitudes of Darjeeling hill slopes.

Most plantations, in these regions are managed conventionally i.e. by spraying different synthetic insecticides while a few by organic farming using different herbal and microbial insecticides. In conventionally managed (synthetic insecticide-treated) tea plantations, organosynthetic insecticides of different functional groups (organochlorines, organophosphates, synthetic pyrethroids and, very recently, neonicotinoids) are routinely applied round the year to keep insect and mite pest populations under control. Despite the continuous use of insecticides, there are repeated failures in controlling the pest species in recent years in many conventional tea plantations of Terai, the Dooars and Darjeeling foothill regions. Such failures occur due to change in the susceptibility level of the pests to the applied insecticides. Changes in susceptibility levels often occur due to metabolic detoxification of insecticides through a higher level of activity of some detoxifying enzymes under the stress of different management practices.

Generally, three principal enzymes [general esterases (GEs), glutathione S-transferases (GSTs) and cytochrome P450-mediated mono-oxygenases (CYPs)] are involved in the process of metabolic detoxification of insecticides.

Polyphagous insects are associated with a wide range of host plants usually spreading over diverse families. The three sucking insect pests of tea under study i.e. *H. theivora* (tea mosquito bug), *E. flavescens* (jassid) and *S. dorsalis* (thrips) due to their association with diverse alternate host plants are more likely to encounter a wide range of biosynthetically unrelated allelochemicals (toxins) while feeding on them. An insect's nutrition, comprising food consumption and utilization in its broadest sense, links the physiological and ecological aspects of its life. Particularly, variation in host-plant allelochemicals may affect the body size of herbivorous insects which in turn, can affect

life history parameters, such as fecundity, longevity, survival and again the nutritional quality of host plants also influences the rate of larval development, adult emergence, longevity and fecundity. Irrespective of diet breadth, insect herbivores rely to some extent on enzymatic metabolism for detoxification of plant allelochemicals. An insect's ability to detoxify plant allelochemicals is a basic determinant for the selection of the range of host plants.

Therefore, enzymes responsible for the detoxification of xenobiotics in insects are most important for their survival in an unfriendly chemical environment (exposure to synthetic insecticides and host plants' allelochemicals). The biotransformation of plant allelochemicals, i.e., metabolic processing of plant chemicals, often results in the overproduction of detoxifying enzymes and degradation of xenobiotics to non toxic forms and their elimination. This mechanism is often associated with phenotypic plasticity of the pest as the production of the detoxifying enzymes is usually induced in response to the presence of plant allelochemicals in the diet of the insect. Plant allelochemicals also modify levels of detoxifying enzymes in herbivores. Induction of detoxifying enzymes by plant allelochemicals is considered as the first step toward further specialization. The capacity of insects to metabolize plant allelochemicals also cross protects them against synthetic insecticides and therefore significantly enhances the insects' tolerance to insecticides, causing difficulty in its management.

In addition to the study of biochemical variability in respect to major detoxifying enzymes, an understanding of the genetic structure of the sucking pest populations are required to develop a population specific control strategy for effective management of the pests. In small insects, like *S. dorsalis, E. flavescens,* randomly amplified polymorphic

DNA (RAPD) is an important genetic marker to study the genetic variability. The RAPD marker method has been reported to be an efficient tool to differentiate geographically and genetically isolated populations. RAPD is particularly useful to study the genetic structure of the populations, because they reveal polymorphisms in non coding regions of the genome. It has also been used to verify the existence of populations of species that have arisen either through genetic selection under different environmental conditions.

In the present study the activity of the detoxifying enzymes showed an enhanced level in populations from conventional tea plantations in all the three sucking insects than those from organic plantations. Similarly, when the pest reared on alternative hosts other than tea, the activity of all the major detoxifying enzymes showed an enhanced level of activity than the population reared on tea. The densitometric analysis of the electro-phoregram of general esterases confirmed the higher level of expression of general esterases in all the three sucking insect pest populations of tea from conventional plantations and also in pest populations reared on alternative hosts. In genetic variability study by RAPD-PCR showed different group in Unweighted Pair Group Method Analysis (UPGMA) dendrogram based on Nei's genetic distance representing three different regions of study area. The organic populations from lower altitudes of Darjeeling hill formed a different group indicating the different population than that of Terai and the Dooars for all the three species under study. So the sucking insect pest populations in this region are genetically diverse. This study of population variability at subcellular level (biochemical and genetic) would help to develop a population specific control strategy for effective management of the three sucking insect pest populations in sub Himalayan tea plantations of Northern part of West Bengal, India.

PREFACE

Polyphagous insect herbivores encounter numerous toxins (xenobiotics) as they pass through their life cycle; some toxins are produced naturally by the host plants (allelochemicals) and others by humans (insecticides) to manage these insects having pest status. The host plants have evolved defensive mechanisms for protection from herbivory, including chemical repellents and toxins (secondary metabolites). Many classes of insect repellents and toxic substances, such as iso-flavonoids, furanocoumarins, terpenoids, alkaloids, and cyanogenic glycosides are synthesized in plants. The biosynthetic pathways leading to these allelochemicals are continually evolving to generate new secondary metabolites. Similary to control the herbivorous insect pests numerous chemicals of synthetic origin are used continuously against them. In response the attacking organisms also evolve mechanisms that enable them to resist the defensive chemicals of their hosts and those toxins of synthetic origin applied for their control. A variety of defense mechanisms, including enzymatic detoxification systems, physiological tolerance, and behavioral avoidance protect insect herbivores from these xenobiotic compounds. Insect pests have evolved the mechanisms to degrade metabolically (enzymatically) or otherwise circumvent the toxic effect of many types of chemicals that we have synthesized as modern insecticides. The extent to which insects can metabolize and thereby degrade these antibiotics or toxins is of considerable importance for their survival in unfriendly chemical environment. These mechanisms continue to evolve as insects attempt to colonize new plant species or encounter newer molecules of synthetic insecticides.

Herbivorous insect groups are significantly more diverse than their non-herbivorous sister groups. The role of plant in promoting diversification in insects has occurred through coevolutionary arm races. This diversification could also have been a result of insects 'tracking' plant phylogenies, with minor chemical changes in plants allowing the evolving populations of insects to change and speciate which probably occured long after chemical changes in plants. Evolution to herbivory proceeded via mixed feeding on reproductive parts or spores, dead tissues of plants and animals, and fungi. This progression implies that omnivory precede generalized herbivory and the evolution of specialization on specific plant taxa was a later accomplishment.

Among sucking insect herbivores, the actual food used i.e. digested whole tissue particularly parenchyma (as in *Helopeltis theivora*), cell content (*Scirtothrips dorsalis*) and phloem (*Empoasca flavescens*) influences both the feeding mechanism and feeding behaviour. While the chewing insects cause extensive damage, the sucking insects cause modest to barely perceptible damage. But, sucking insects, particularly phloem and digested tissue feeding, provide an additional challenge to the plants as they deplete photosynthates, vector viruses and introduce chemical and protein effectors that alter plant defense mechanisms (signaling) and development. When these attributes are combined with broad host range, breeding strategies that promote invasiveness, highly evolved feeding strategies, the ability to adapt to a wide range plant habitats and the emergence of insecticide resistance, then it is not surprising that sucking insects cause heavy losses in agriculture and horticulture.

Resistance of insects to insecticides has been noticed greatest increase and strongest impact during the last 70 years, following the discovery and extensive use of

synthetic insecticides. The evolution of resistance to insecticides is an example of evolutionary process. An insecticide is the selection pressure, which results in a very strong but differential fitness of the individuals in a population having susceptible and resistant genotypes. The survival and subsequent reproduction of resistant individuals lead to a change in the frequency of alleles conferring resistance in the population over time. While selection pressure acts to change allele frequencies within pest populations, the phenotype upon which selection operates is a function of both genotypes and the environment. Recent studies in insect detoxifying enzymes have revealed further versatility in adaptation of insects to their environment by the phenomenon of induction. This is the process in which a chemical stimulus enhances the activity of the detoxification enzyme systems by the production of additional enzymes that metabolize toxic chemical substances. So the influence of environmental factors such as continuous usages of insecticides and the chemical constituents (allelochemicals) of host plants in phytophagous insects may have a great impact to induce the enzymatic detoxification systems of insects and thereby promoting the insecticide resistance mechanisms.

While all insects do possess detoxification ability, its magnitude is expected to vary among the species with the nature of its recent environment and feeding ecology. The level and type of detoxifying mechanisms differ greatly, which therefore, results in varying toxicity among different stages, species and populations. Variation in detoxifying enzyme activity is responsible in part for the selective toxicity of different insecticides, the development of resistance to insecticides and selective adaptation to a host plant. Overexpression of these detoxifying enzymes, capable of metabolizing insecticides can result in high level of metabolic tolerance/resistance to synthetic insecticides. Increased

expressions of genes encoding the major xenobiotic metabolizing enzymes are the most common cause of insecticide resistance in insects.

Generally three principal enzymes, general esterases (GEs), glutathione S-transferases (GSTs) and cytochrome P450 mediated monooxygenases (CYPs) are involved in the process of metabolic detoxification of insecticides. Estimation of the activities of these metabolic defence related detoxifying enzymes may give information on the levels of tolerance/resistance of the insect pest population to insecticides which appear to be a useful tool for monitoring the tolerance/resistance at population level of pests.

Early detection of the metabolic threats related to tolerance/resistance to insecticides in insect pest specimens is of crucial importance for devising pest control methods that would minimize the emergence of tolerant/resistant forms and prevent any undesirable wastage of insecticides. But, this also requires an understanding of the genetic structure of pest populations. In small insects, randomly amplified polymorphic DNA (RAPD) is an important genetic marker to study the genetic variability. The RAPD marker has been reported to be an efficient tool to differentiate geographically and genetically isolated populations. RAPD is particularly useful to study the genetic structure of populations as it reveals polymorphism in non coding regions of the genome. It has also been used to verify the existence of population of a species that has arisen through genetic selection under different environmental stresses. Continuous uses of synthetic insecticides in tea plantations of the Darjeeling foothills and its adjoining plain appear to have led to the selection of insecticide-tolerant populations and development of insecticide resistance in the concerned sucking insect pests.

So, the increasing management problem associated with the sucking pests of tea and their resistance to insecticides resulting in adaptive variability necessitates a detailed study at biochemical, molecular and subcellular levels of these pests of tea plantations from lower altitudes of Darjeeling hill and its adjoining plain. The present study is undertaken in reference to three principal detoxifying enzymes and genetic variability by RAPD-PCR to establish variability among different host plant based populations and those under different insecticide treatments (exposures i.e. field populations from conventional and organic plantations). The finding may throw light on biotype variation in the concerned pests. Further this would help to develop a population specific control strategy for effective management of the sucking insect pest populations in sub Himalayan tea plantations of northern part of West Bengal, India.

Date: *15th March, 2013* *Dhiraj Saha*
Place: *University of North Bengal*

Contents Pages

LIST OF TABLES

LIST OF FIGURES

- Figure 4.2 Densitometric analysis of electro-phoregram of α- esterase of *H. theivora* from Conventional and organic tea plantations, showing over expression of α- esterase in *H. theivora* populations from conventional tea plantations.
- Figure 4.3 Densitometric analysis of electro-phoregram of β- esterase of *H. theivora* from Conventional and organic tea plantations, showing over expression of α- esterase in *H. theivora* populations from conventional tea plantations.
- Figure 4.4 Densitometric analysis of electro-phoregram of general esterases of *E. flavescens* from Organic and Conventional Tea Plantations. (T_C= Conventional tea plantations of Terai, D_C= Conventional tea plantations of Dooars, O_T= Organic tea plantations of Terai, O_D= Organic tea plantations of Dooars)
- Figure 4.5 Densitometric analysis of electro-phoregram of general esterases of *S. dorsalis* from Conventional and Organic Tea Plantations. (T_C= Conventional tea plantations of Terai, C= Laboratory control, D_C= Conventional tea plantations of Dooars, O_T = Organic tea plantations of Terai and O_D= Organic tea plantations of Dooars).
- Figure 4.6 An electro-phoregram of general esterases of *H. theivora* from reared on three alternative hosts i.e. *C. sinensis* (C_1, C_2), *M. micrantha* (M_1, M_2) and *P. guajava* (P_1, P_2).
- Figure 4.7 Densitometric analysis of phoregram of general esterase of male *H. theivora* reared on *Camellia sinensis* (*C*), *Mikania micrantha* (*M*) and *Psidium guajava* (*P*). Arrow (→) indicates higher expression of general esterases in *P. guajava.*
- Figure 4.8 Densitometric analysis of phoregram of general esterase of female *H. theivora* reared on *Camellia sinensis* (*C*), *Mikania micrantha* (M) and *Psidium guajava* (*P*). Arrow (→) indicates higher expression of general esterase in *P. guajava* than *C. sinensis*.

LIST OF ABBREVIATIONS

ABBREVIATIONS

AChE	Acetylcholinesterase
AD	Anno Domini
BHC	Benzene Hexa Chloride
BSA	Bovine serum albumine
CDNB	1-Chloro-2, 4-dinitrobenzene
CYP	Cytochrome P_{450}-dependent monooxygenase, also called mixed function oxidase (MFO)
DDT	Dichloro Diphenyl Trichloro Ethane
DDE	Dichloro Diphenyl Dichloro Ethylene
EC	Emulsifiable concentrate
EDTA	Ethylenediaminetetraacetic acid
FAOSTAT	Food and Agricultural Organization's Annual Statistics
Fig.	Figure
GABA	γ-Aminobutyric acid
GEs	General Esterases
GSH	Glutathione, reduced form
GST	Glutathione S-transferase
h	Hour
ha	Hectare
IPM	Integrated Pest Management
IRM	Insecticide Resistance Management
IRAC	Insecticide Resistance Action Committee
kdr	Knockdown resistance
l	Litre
LC_{50}	Lethal Concentration of a pesticide required to kill 50 per cent of the experimental population
mg	milligram
M.kgs	Million Kilograms
ml	Millilitre
min	Minutes
n	Number of replicates
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NE	North East
NN	Neonicotinoids
^{o}C	Degree Celsius
°E	Degree East
°N	Degree North
OC	Organochlorines
OD	Optical density
OP	Organophosphates
PAGE	Polyacrylamide gel electrophoresis

PBO	Piperonyl butoxide
PCR	Polymerase chain reaction
RAPD	Randomly Amplified Polymorphic DNA
RF	Resistance factor
r.h.	Relative humidity
R_m	Relative (electrophoretic) mobility
SD	Standard deviation
SE	Standard error
SP	Synthetic pyrethroids
TFPGA	Tools for Population Genetics Analysis
UPGMA	Unweighted Pair Group Method Analysis

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Terai and Mokaibari TE (Organic), Singel TE (Organic), and Castleton TE (Organic), Ambootia TE, Rohini TE of Darjeeling foothills.

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Introduction

Tea, the plantation crop with economic value: Driving the shrub to Cup

Tea, *Camellia sinensis* (L.) O. Kuntze is a native of China and the Chinese are said to have discovered its use nearly 4,700 years ago. It is believed that Shen Nung, a Chinese emperor who lived some 4,700 years ago, discovered that tea leaves falling into boiling water make a refreshing drink. Tea then became a popular drink in China for both its flavor and medicinal qualities. Eventually, the habit of drinking tea spread throughout Asia and then throughout the world. The word tea had its origin from t'e (pronounced as "tay") in "Amoy" dialect while in Cantonese it was called ch'a ("chah"). This is the name by which this wonderful beverage is known in Japan, Iran, Russia, Indonesia, Malyasia, Vietnam and in India. Tea was introduced in Japan about 800 AD and was regarded as medicine for about 500 years. Tea was introduced in Europe in early 17^{th} century with the beginning of trade between Europe and the South East Asia.

Now tea is produced in almost every region of the globe (Figure 1.1). The tea plant is predominantly grown in Asia followed by Africa and to a very small extent in Europe, South America, Australia and New Zealand. Tea is grown in thirty-six tropical and subtropical countries. Major tea producing countries are India, China, Sri Lanka, Kenya, Japan, Indonesia, Thailand, Bangladesh, Nepal, Vietnam, Turkey and Argentina (Figure 1.2).

In India, Tea is grown in wide amplitude of climatic variables; at latitudes from 9^0 to 25^0 N and longitudes 77^0 to 98^0 E. India is the world's second largest tea producer contributing approximately 23.8 per cent of the world's tea production, second to china which produces 33.7% of world's total production (Table 1.1).

Figure 1.1 Tea producing countries around the globe.

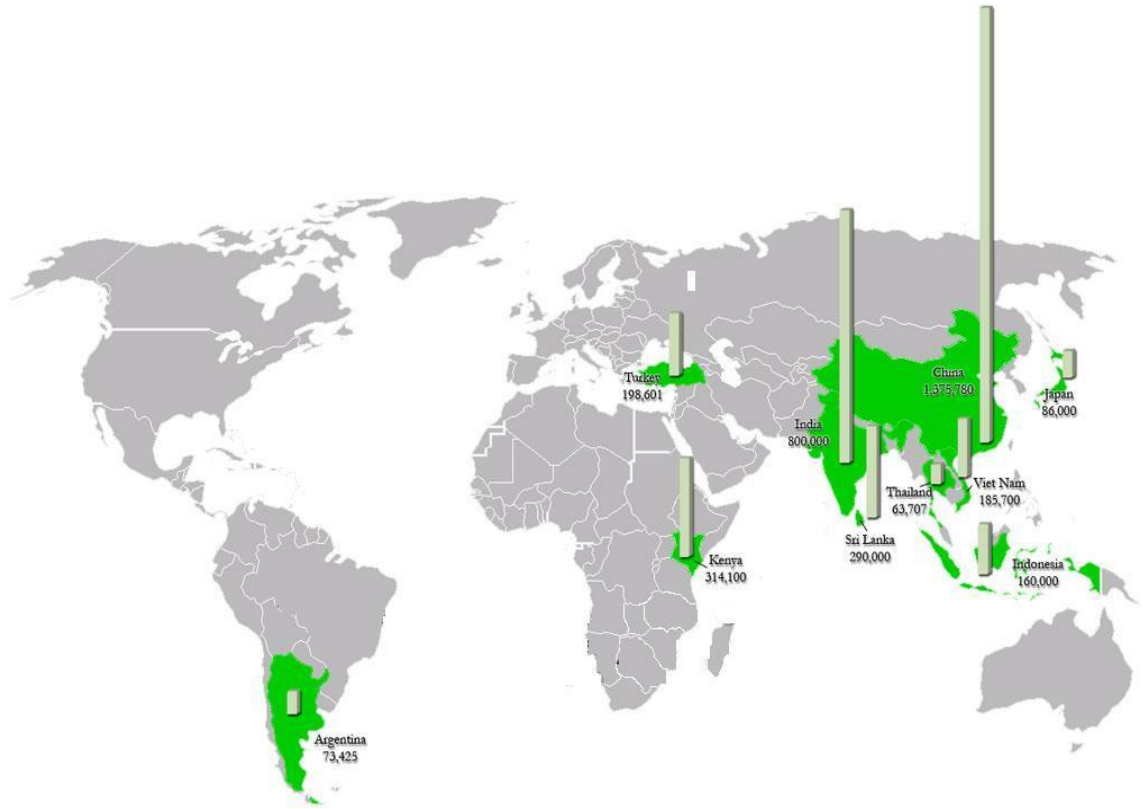

Figure 1.2 Major Tea Producing Countries around the globe. (*Data Source* FAOSTAT: *http:// faostat.fao.org*, retrieved on December 31, 2012).

Table 1.1 World tea productions in the year 2010 (*Data Source* FAOSTAT: *http:// faostat.fao.org*) (retrieved on June, 30, 2012).

Rank	Area	Production (MT)
1	China	1467467
2	India	991180
3	Kenya	399000
4	Sri Lanka	282300
5	Turkey	235000
6	Viet Nam	198466
7	Iran (Islamic Republic of)	165717
8	Indonesia	150000
9	Argentina	88574
10	Japan	85000
11	Thailand	67241
12	Malawi	66600
13	Bangladesh	60000
14	Uganda	40800
15	United Republic of Tanzania	36000
16	Myanmar	32400
17	Rwanda	24500
18	Zimbabwe	21000
19	Brazil	18400
20	Nepal	16607

In the year 2011, India produced 988.33 M.kgs of tea where as China produced 1550 M.kgs (Data source: Tea Board of India). Bulk of India's tea production is consumed locally by its large domestic population.

Table 1.2 World tea export in the year 2010 (*Data Source* FAOSTAT: *http:// faostat.fao.org*) (retrieved on 31.12.2012).

Rank	Country	Quantity (tonnes)	Value (1000 $)	Unit value ($/tonne)
1	Sri Lanka	288528	1175100	4073
2	Kenya	331594	894027	2696
3	China	305352	723933	2371
4	India	203863	583803	2864
5	EU(27)ex.int	29882	301042	10074
6	United Kingdom	27741	281126	10134
7	Germany	25301	186395	7367
8	Indonesia	92304	171628	1859
9	United Arab Emirates	23681	153786	6494
10	Viet Nam	82416	95966	1164
11	Malawi	47356	78243	1652
12	Belgium	7859	73749	9384
13	Argentina	69816	72523	1039
14	United Republic of Tanzania	30438	68140	2239
15	Russian Federation	9713	62964	6482
16	Netherlands	18158	62376	3435
17	Poland	8609	60284	7002
18	Uganda	44446	59761	1345
19	United States of America	8530	53427	6263
20	France	3633	51353	14135

In India, tea is cultivated in more than 5,78,458 hectares covering 1,57,504 numbers small and 1686 numbers big growers spreading mainly in Assam, West Bengal, Tamil Nadu and Kerala, Himachal Pradesh (Figure 1.3). Tea plantations in India are traditionally in large holdings, but in recent years small scale cultivation has gained prominence and account for 24% of the country's total production. The Country exports around 203.86 mkg and account for 10.6% world exports (Table 1.2). India is the largest consumer of black tea and per capita annual consumption is around 800g.

In West Bengal, Tea plantation is spread over three regions – the Darjeeling hill, sub Himalayan plains of Terai (western to river Teesta) and the plains of the Dooars

(eastern to river Teesta). According to the Tea Board of India, there are 308 big and 1232 small tea gardens in West Bengal, covering 1,15,095 hectares producing about 237 M kgs. However, India is the 4th largest exporter of tea with a market share of approx 11.15% in 2010.

The story of Darjeeling tea started way back in 1835 through the initiative of the British Governor General, Lord Bentinck who proposed to the council of the East India Company, setting up a committee to investigate and recommend on the suitability of tea cultivation in India. The tea committee decided to send the secretary, G.J. Gordon to China in order to acquire tea seeds and some tea workmen familiar to cultivate and manufacture tea. From this original consignment of china seed around 42,000 young plants were raised which were allocated to three main areas, 20,000 to the hill districts in the Kumaon in North India, 2000 to the hills of South India and rest 20,000 to the then North–East frontier (Weatherstone, 1992). In this first trial tea grew well in Darjeeling. As per available records Dr. Campbell, a civil surgeon and also the first Superintendent of Darjeeling, planted tea seeds in his garden at Beechwood, Darjeeling at 2134m above mean sea level as an experiment with reasonable success. Subsequently, in 1847, the government selected the area to raise tea nurseries. In 1852, with plants raised in the government nurseries, the first commercial tea gardens in Darjeeling hill area came up as Tukvar, Steinthal and Aloobarie Tea Estates (Pathak, 2004). Tea, being a labour-intensive enterprise, required sufficient numbers of workers to plant, tends, pluck and finally manufacture the produce. For this, employment was offered to people from across the border of Nepal.

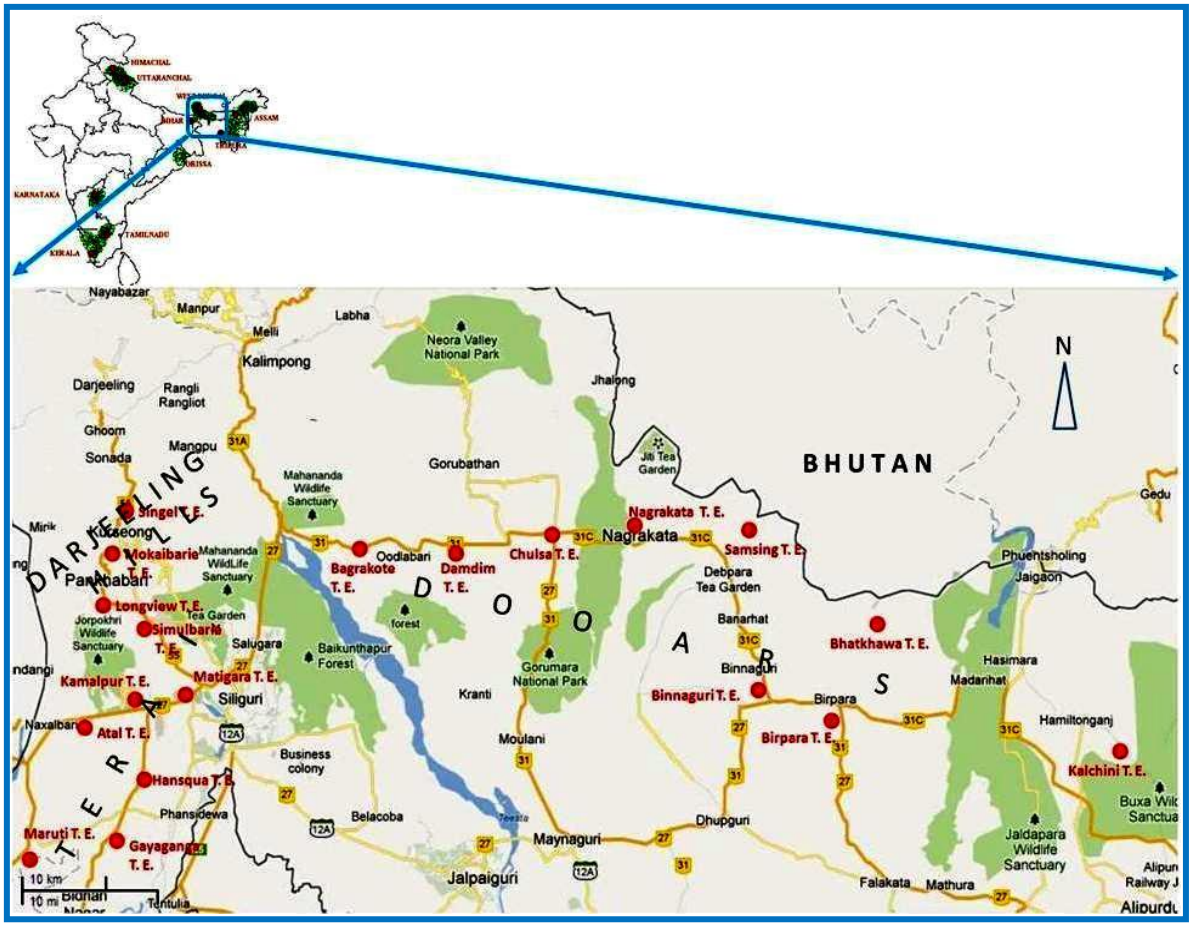

Figure 1.3 Tea producing states in India; tea growing regions in West Bengal (inset).

In 1866, Darjeeling had only 39 tea gardens producing a total crop of 21,000 kilograms of made tea. In 1870, the number of gardens increased to 56 to produce about 71,000 kgs of tea harvested from 4,400 hectares. During 1860-64, the Darjeeling Company was established with 4 gardens while the Darjeeling Consolidated Tea Co. dates back to 1896. By 1874, tea in Darjeeling was found to be a profitable venture where 113 gardens spanning approximately 6,000 hectares, were thriving.

Today there are 87 running gardens producing 'Darjeeling Tea' on a total area of 17,818 hectares. The total production ranges from 9 to 10 million kgs annually. The Terai regions have an area of 24, 359 hectares and in the Dooars region 72,918 hectares under tea plantations. Apart from tourism, Tea is the biggest industrial activity in Darjeeling, offering the largest employment in the hills. The turnover of the Darjeeling tea industry is nearly USD 7.5 million, which is acknowledged to be more than the money generated by tourism in the Darjeeling hills.

Tea plants are native to East and South Asia and probably originated around the point of confluence of the lands of northeast India, north Burma, southwest China, and Tibet.The commercially cultivated tea plants are derived from small leaved China plants, *C. sinensis*, the Assam plants, *C. assamica* (Masters) and the Cambod plants, *C. assamica lasiocalyx* (Planchon ex Watt) Wight and numerous hybrids among them. Tea plants require warm humid climate, well distributed rainfall and long sunshine hours. Shoots, comprising two or three tender leaves and a bud are harvested and processed in factories to manufacture different types of tea.

Insect Pest Occurrence in tea:

Tea plantations being perennial monoculture crop provides the congenial microclimate as well as continuous supply of food to a number of arthropods. Every part of the tea plant i.e. leaf, stem, root, flower, and seed, are subjected to the attack by at least one arthropod pest species (Figure 1.4). They may cause on an average 11%-55% yield loss, if left uncontrolled (Hazarika *et al.*, 2009). Among these arthropods, insect and mite pests are the most damaging (Muraleedharan, 2007), causing on average a 5% to 55%

yield loss (Muraleedharan, 1992; Rattan, 1992a and Sivapalan, 1999). According to recent estimates, globally, more than a thousand (1034 species) arthropods and 82 species of nematodes are associated to the tea plantations (Chen and Chen, 1989). Among the insect pests, Lepidoptera is the largest order, containing 32% of the pest species, followed by Hemiptera with 27% (Muraleedharan, 2007). In India, only 300 species of arthropods are recorded and about 167 species are from North-East India (Das, 1965).The tea plantations of three regions of Northern West Bengal i.e. Terai, the Dooars and Darjeeling hill slopes are attacked by more than two dozen (27-28 nos.) of insect and mite species. At least four of the sucking and six of the chewing arthropods are well established as a regular pest causing substantial damage to the foliage-crop. The dynamic adaptations of insects have enabled them to attack every part of the tea plant and the maximum number of pests occur on the foliage. Mites, thrips, jassid, aphids, tea mosquito bugs, looper caterpillars, beetles and termites are the most important among the arthropod pests. Thrips, jassids, tea mosquito bugs are the most important sucking insects that attack tea in North Bengal region.

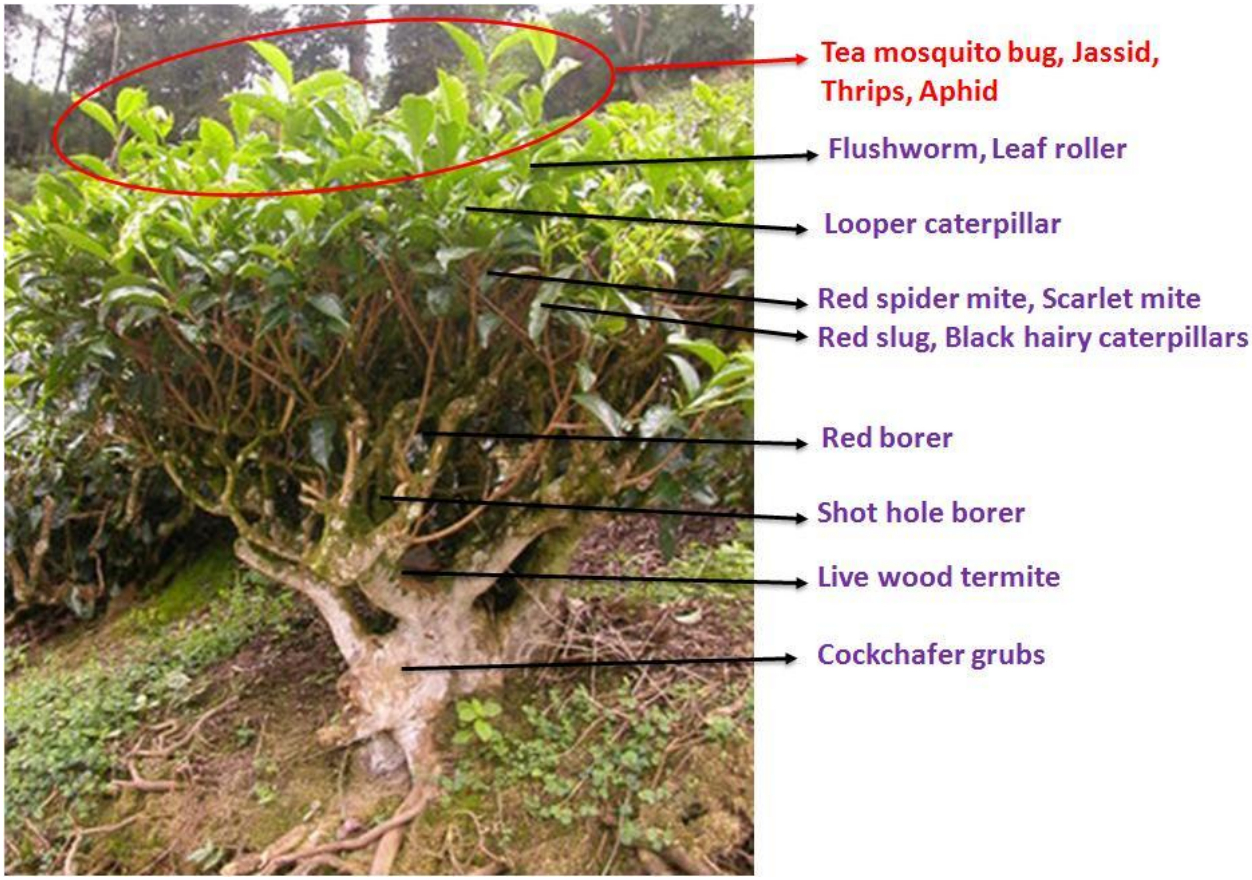

Figure 1.4 Distribution of some common tea pests on a tea bush from sub Himalayan tea plantations.

Sucking insect pests of tea from Eastern sub-Himalaya:

The most important insect pest groups that suck the sap from young tender leaves, buds and stems, the most economic part of tea plant processed in tea industry for making tea, are mainly the tea mosquito bug (*Helopeltis theivora*), tea jassid (*Empoasca flavescens*) and Assam thrips (*Scirtothrips dorsalis*) (Figure 1.5). These sucking pests cause substantial loss in yield to the tea industry.

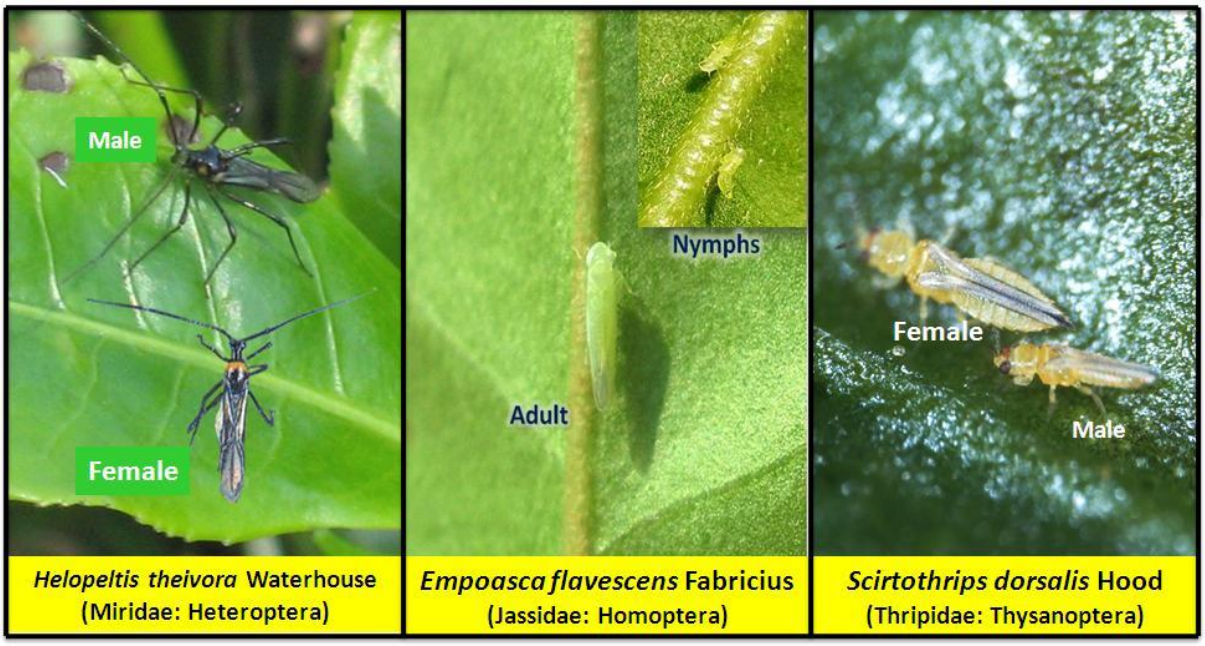

Figure 1.5 Three important sucking insect pests of tea generally attack in Himalayan tea plantations.

Helopeltis theivora (Miridae: Heteroptera)

Helopeltis theivora Waterhouse (Heteroptera: Miridae), also known as tea mosquito bug, a serious pest of tea was first recorded in 1865 in Cachar causing extensive damage to tea. The tea mosquito bug was subsequently recorded in many districts of Assam and Darjeeling in 1869. Watt and Mann (1903) also reported the incidence of this pest in Assam, Terai and the Dooars. It has been the major pest of tea in the past as well as in recent times in north east India causing heavy crop loss every year. It sucks sap from tender leaves, buds and young shoots (Das, 1965). Severe outbreak of the pest and loss of crop were reported from time to time from the Dooars. In the year 1958, Das (1965) reported that the crop loss in eleven gardens of the Dooars was 750000 kgs. Prasad (1992) and Barbora and Singh (1994) reported that usually crop loss due to *H. theivora* infestation was 25%-50%. In 1992-93 one tea estate lost about 50% of the annual crop in

Central Dooars (Barbora and Singh, 1994). In recent years, *Helopeltis* has become widespread occurring both in Assam and in Darjeeling hill slope and plain. Both the nymphs and adults have sucking mouth parts and suck the cell sap from the succulent stems, buds and young leaves by inserting the stylets, while doing so, it injects toxic saliva which causes the breakdown of tissues surrounding the puncture (Das, 1965). Within 2-3 hours of sucking, a circular spot is formed around the sucking point and within 24 hours the area become translucent, light brownish which eventually becomes dark brown sunken spots and dry up (Figure 1.6). Nymphs and adults both make numerous sucking spots on the foliages which becomes curl, dried and black resulting in severe loss in crop yield. A nymph of the 1st instar produces the highest number of sucking spots (106) followed by 2nd (74), 4th (72) and 3rd Instars (28) within 24 hours (Barbora and Singh, 1994). Moreover, total number of spots produced by an adult was recorded to be 2358 within a period of 21-26 days (Barbora and Singh, 1994). A single full grown nymph (5^{th} instar) is the most voracious feeders among the all life stages, producing the most and largest feeding lesions (Bhuyan and Bhattacharyya, 2006). Further, an adult could make 150 feeding spots in a day (Hainsworth, 1952). A single female can produce lesions over an area of 412.43 square mm per day (Figure 1.6) (Kalita *et al.,* 1995). The damage to tender stems was not only due to sucking but also due to oviposition. Damage to the plant tissue takes place when adult female mosquito bug insects egg in the soft tissue of internodes, petioles and buds. The mechanism of feeding by *Helopeltis sp.* had been studied by Cohen-Stuart (1922) who showed that the proboscis penetrate the epidermal tissue and resulting the collapse of parenchyma tissue. Leach (1935) showed that the primary lesions could be recognized before the insect stops

feeding, indicating an extremely active feeding process and salivary secretions are phytotoxic. This rapidity in feeding was, of course, associated with the extensive damage inflicted by small populations of *H. theivora.* Besides causing a substantial amount of crop yield loss, the bug also causes deterioration of the quality of made tea, lowering its market value. The pest occurs throughout the year with very low infestation during December to January. About 80% area of tea plantations in north east India is affected by this pest reducing 10-50% productivity (Roy *et al.*, 2010a). The seriously affected tea plants become darker and growth is stunted. It occurs on tea almost throughout the year, but serious attack develops in May, June and July, often extending upto September-October. When the number of rainy days is large, with steady rains and dull weather conditions, its activities are increased than when the number of rainy days is small with bright sunshine in the intervals. Under the latter set of weather conditions, constant extreme variation in temperature and humidity hampers the multiplication of the pest with the decrease in its incidence. During the winter months, the Iife-cycle is completed in about 35-40 days but during March-April the life-cycle is completed in 23-28 days. The shortest life-cycle of 12-15 days was recorded during July-August (Figure 1.6). The variations in the incubation period, the stadial duration and the life-cycle in different periods of the year seem to be influenced by the environmental and weather factors. Laboratory studies show that the fecundity (egg laying capacity) is maximum at 28^{o}C with 136 eggs per female. Increasing the temperature beyond 29^{o}C reduces the fecundity. The maximum hatchability is also recorded at this temperature. The eggs laid in between 31-34^{o}C did not hatch at that temperature. When the ambient temperature dropped to 27-30^{o}C, nymphs emerged indicating the significance of the temperature

regime for *Helopeltis* build up. It is interesting to note that male female ratio was equal at 25^{o}C but at 28^{o}C, 30^{o}C and 32^{o}C the male: female sex ratios were 1:2, 1:3.4 and 1:5 respectively. The ratio is widened with the increase in temperature from 25^{o}C to 32^{o}C.

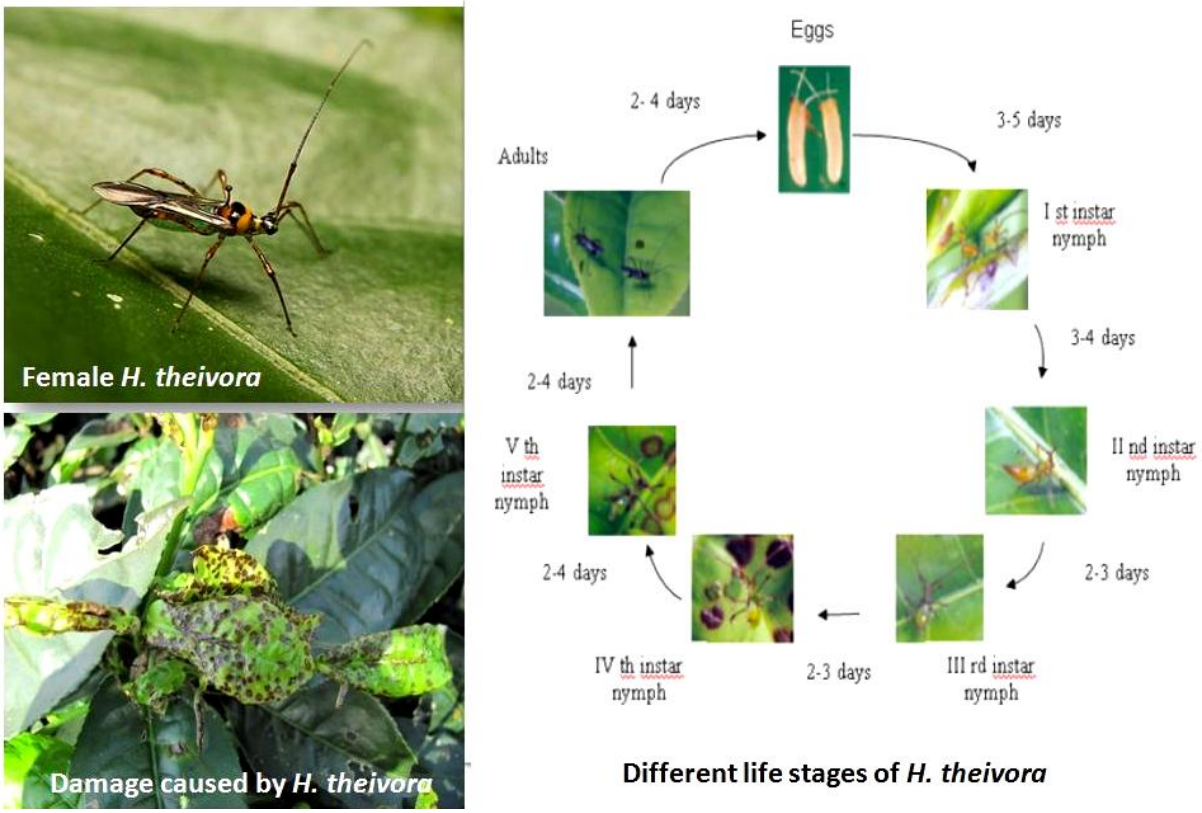

Figure 1.6 Female *H. theivora*, damage symptoms and different life stages.

Empoasca flavescens Fabricius (Jassidae/Cicadellidae: Homoptera)

Tea Jassid, *Empoasca (=Amrasca) flavescens* Fabricius (Homoptera: Jassidae or Cicadellidae) is another important sucking insect pest of tea generally occurring during first and second flush (March to June) in all tea plantations of North East India. Tea jassids are and commonly called 'Tea Green Fly', or 'Tea leaf hopper'. Fabricius (1794)

first described *Cicada flavescens* from Germany in 1794. It was later reported by other investigators as occurring generally throughout Europe and northern Africa on *Atriplex, Chenopodium, Cleinatis,* hop, potato, raspberry, sugar beet, and vines, and on many herbaceous plants and deciduous and coniferous trees. Melichar (1903) reported *E. flavescens* from Ceylon, and Distant (1910) first noticed this species in India in tea gardens. It is now commonly known as the tea green-fly and is one of the most widely distributed of the major pests of tea in north-eastern India. It has been generally accused of producing a stunted growth on the plants attacked in India. Shiraki (1920) reported *E. flavescens* as a pest of tea in Formosa and also injurious to orange trees and sugarcane, and its injury to mulberry as causing a scarcity of leaves for sericulturists. DuPasquier (1932) reported *E. flavescens* as a pest of tea, feeding on the young shoots in Indo-China, India (Assam, Darjeeling), Formosa, and Japan. It was also reported as injurious to peach and plum trees in Formosa by Kayashima (1934), and included by Kuwayama (1936) among the more injurious pests of apple in Japan. Both in India and Egypt, *E. flavescens* attacks the castor bean *(Ricinus communis)* and has been reported as particularly injurious when the plants are young, sucking the juices to such an extent that the plants fade, curl, and eventually die. Blattny (1924) listed *Empoasca flavescens* as injurious to *Mentha crispa* L. in Czechoslovakia, and reported (1933) that this species occurred in great numbers on celery in hotbeds in Bohemia and was associated with a severe outbreak of mosaic, previously vmrecorded in Czechoslovakia. Otanes and Butac (1935) mentioned *E. flavescens* among the more injurious of the sucking insects on cotton in the Philippines. It caused the leaves to curl and contributed to the failure of extensive cotton planting. It also occurred there on eggplant and potato. Investigations in Germany by

Heinze (1937) showed *E. flavescens* to be common on potato, but unable to transmit the potato virus, although its attack caused a rolling of the leaves similar in appearance to that due to the virus of leaf-roll. Esaki and Hashimoto (1938) reported *Empoasca flavescens* were abundant on rice in Oita, Kyushu, Japan. *Empoasca flavescens* is among the insects listed as attacking sugar beet in Czechoslovakia, Germany, and Sweden.

The pest remains active at various levels of intensity throughout the season. The jassids sucks the sap of growing shoot and leaves. In Darjeeling district there is a common belief that it improves the quality of made tea. *E. flavescens* also attacks tea in Bangladesh, China, Japan, Indonesia and Vietnam but are rarely seen on tea in south India. The pest remains mainly on the under surface of the leaves. Nymphs and adults of *E. flavescens* are the most important stages which damage the tea plant (Zeiss and Braber, 2001) and are mainly phloem feeder. The damage results from sap sucking. Young leaves and tender shoots are mostly affected and the nymphs are responsible for greater damage than the adults. During feeding, the rostrum is inclined downwards and the stylets are inserted into the plant tissue. They feed on the contents of the phloem vessels and reach this tissue through cortical cells. Once penetration has started, saliva is injected into the plant and the injury to the plant is probably the combined effect of feeding from the vascular tissue and the action of certain enzymes present in the saliva. The plant sap is drawn into the alimentary canal by the activity of cibarial sucking pump which is provided with strong dialator muscles. The attacked leaves become dry, uneven and usually curl downward assuming the shape of an inverted boat. The margins turn brown and subsequently dry up. This characteristic symptom is known as ‘Rim blight’or ‘Hopperburn’. The mid rib and the veins of the affected leave also show somewhat

brownish discolourations. The 'Hopperburn' and other symptoms are caused mainly by interference with the translocation of food materials and water due to the physical plugging of xylem and destruction of phloem cell (DeLong, 1971). According to Medler (1941) the plant injury is a combination of the feeding in vascular tissue and the action of a specific compound injected during feeding process. The salivary secretion causes hypertrophy of the affected phloem cells which in turn, causes an interruption of the translocation of photosynthetic materials from the leaves to a degree that causes plasmolysis of parenchymal cells resulting in 'Hopperburn'. Then the other secondary symptoms appear such as, the red and yellow colouration of leaves, stunting and curling of leaves etc. Normally the attack is confined to the undersurface of the young leaves. There is hardly any sign of puncture marks but occasionally faint brownish specks can be seen later under microscope at the site of feeding. The feeding is mainly concentrated in the middle area of the leaves but effect of the damage is reflected at the periphery where the leaves curl downward not at the site of feeding. Occasionally the leaves may curl upward and this is due to feeding on the upper surface.

E. flavescens do not like direct sunlight and therefore prefer to stay on the underside of the leaves (Simanjuntak, 2002). Too much rain or too dry weather is not favorable for the development of the species. *E. flavescens* is most damaging during the period between dry season and rainy season (Zeiss and Braber, 2001). In plains, the jassids appears during February – March and causes considerable damage to the first and second flush. The damage becomes quite visible during May –June at the time of third flush. Though, in hills, the jassids may be present for a considerable period of time. If a severe attack occurs on pruned section early in the season, the growth of the shoots is

arrested and leaves remain stunned, dry up and eventually fall off. For newly planted tea, especially when less than five months old, leafhoppers can cause the drying out of new shoots, with the plants becoming stunted and growing very slowly (Zeiss and Braber, 2001).

Scirtothrips dorsalis (Thripidae: Thysanoptera):

The most common thrips found in lower elevation of Darjeeling hill and adjoining plains is *Scirtothrips dorsalis* Hood (Thripidae: Thysanoptera). *S. dorsalis* was first noted on castor shoots and chillies in Coimbatore (India) in 1916 and was described as a new species by Hood (1919). Now-a-days, it has been found to infest about 20 hosts in India including important crops like tea, cotton, castor and mango (Kumaresan *et al.*, 1988; Ananthakrishnan, 1993). The Yellow tea thrips, *S. dorsalis* is a well-known pest of tea and chillies in India and is referred to as the "Chillies thrips" or the "Assam thrips".

S. dorsalis is a major pest of tea in almost all tea plantations in the plains of North East India and often causes severe damage to tea in Terai and the Dooars. The occurrence of *S. dorsalis* generally coincides with the new flush in tea plantations. Larvae and adults feed on buds, tender shoots, leaves and flowers of the tea plants, causing damages. During feeding on youngest open leaves they make small slits by inserting the stylets and suck the sap oozing through the wound. The sucking marks are made one after one, forming thin pale lines on the underside of the leaves parallel to the mid vein which are often described as 'silvered' or 'sand papery lines'. Damaged leaves become thicker and harder than the normal ones, duller darker green colour, and often puckered or deformed (Das, 1965). It is capable of producing several generations per year, but typically has 4-8 generations per year (Venette and Davis, 2004). The number, frequency, and duration of

generation times are dependent on temperature and moisture. Life cycles (Figure 1.7) are the slowest at the higher and lower temperature extremes (Schall, 1995), and *S. dorsalis* is capable of over wintering in the soil or protected in plant parts in the adult stage (Venette and Davis, 2004). *S. dorsalis* is capable of reproducing both sexually and parthenogenically (Pest Alert, 2005). Adults typically mate 2-3 days after their pupal molt and the females oviposit 3-5 days after emergence. Females tend to lay eggs continuously with the total number of eggs ranging from 40-68 (Venette and Davis, 2004). Severe infestations may lead to deformation and defoliation.

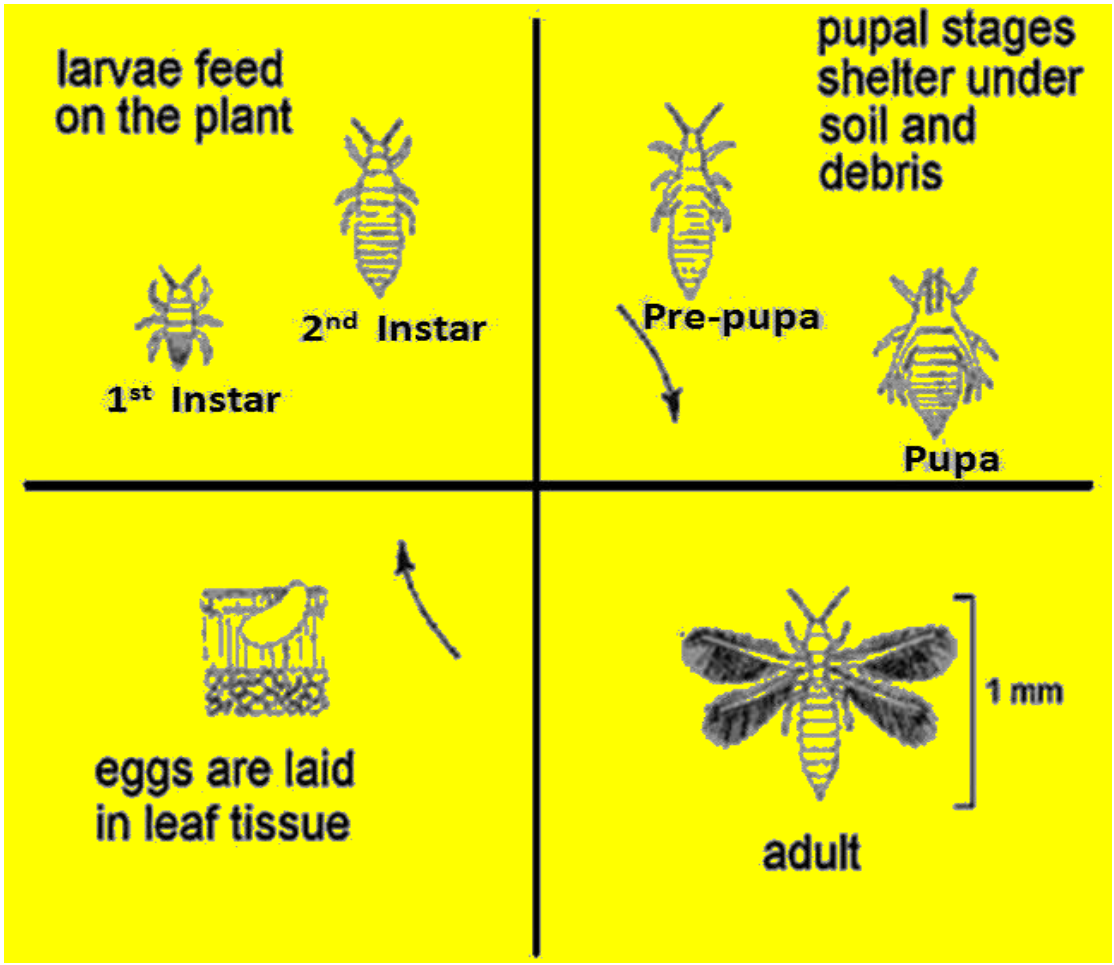

Figure 1.7 Different life stages of *Scirtothrips dorsalis*.

Management problems and variability in sucking tea pest:

Through the millions of years of life on earth, a continuous process of mutual evolution has taken place between plant and animal species that feed on them. The host plants or animals have evolved defensive mechanisms, including chemical repellents and toxins, exploiting the weaknesses in the attacking organisms. In turn the attacking organisms have evolved mechanisms that enable them to detoxify or otherwise resist the defensive chemicals of their hosts. Thus, it appears that the gene pool of most of our pest species already contains genes that enable the pests to enzymatically degrade or otherwise circumvent the toxic effects of many types of chemicals that we have developed as insecticides. These genes may have been retained at various frequencies as part of the genetic memory of the species. Resistance of insects to insecticides has a history of about 100 years, but its greatest increase and strongest impact have occurred during the last 70 years, following the discovery and extensive use of synthetic organic insecticides and acaricides.

The interdisciplinary nature of the problem is evident in the variety of living organisms that have developed resistance and the many types of chemicals that are involved (Figure 1.8). It is also apparent that insecticides and acaricides, being broad-spectrum biocides, have exceeded their intended targets and have selected for resistance not only in insects and mites but in practically every other type of organism, from bacteria to mammals. Since genetic resistance cannot be induced by any means other than lethal action, the environmental impact of such unintentional selection may be profound.

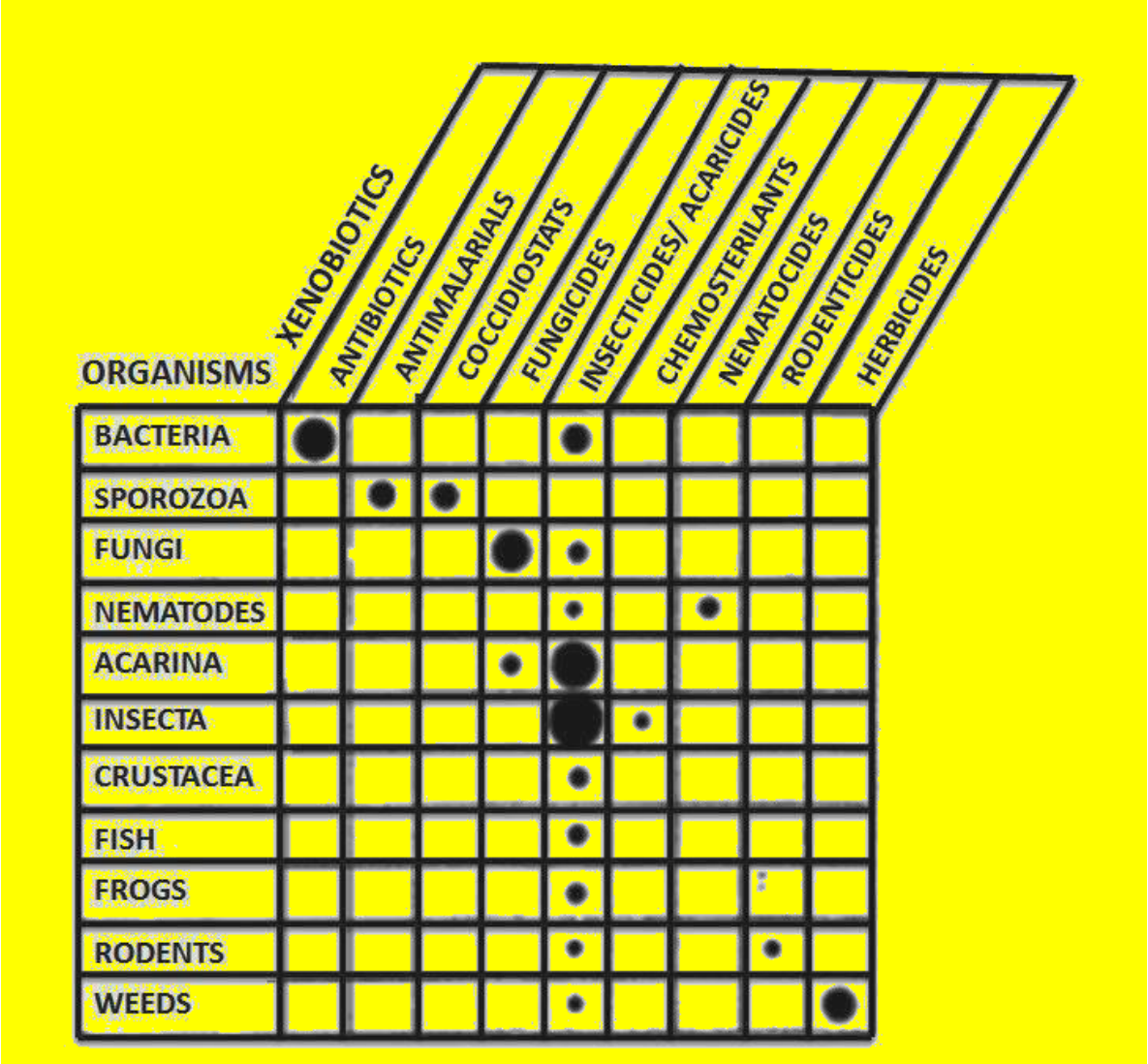

Figure 1.8 The relative frequency of resistance to xenobiotics.

In sub-Himalayan tea plantations of North East India, different management practices are followed to protect the tea crop against different insect pests. Most plantations are managed conventionally i.e. using different organo-synthetic insecticides,

whereas some organic plantations use herbal and microbial insecticides. In conventional tea plantations, organo-synthetic insecticides of different functional groups (organochlorines, organophosphates, synthetic pyrethroids and neonicotinoids) are routinely applied round the year to keep the insect (sucking and other) pest populations under control (Sannigrahi and Talukdar, 2003; Gurusubramanian *et al.* 2008a), which is a burden to planters as well as to the environment and can result in resurgence of primary pests (Sivapalan, 1999), secondary pests outbreak (Cranham, 1966), resistance development (Georghiou 1972; Kawai, 1997; Roy *et al.,* 2008a,e, 2010, 2010b) and environmental contamination including undesirable residues in made tea (Chaudhuri, 1999). Continuous uses of insecticide lead to the development of higher level of tolerance or resistance to insecticides in many insects (Scharf *et al.*, 1998a; Martin *et al.,* 2002, 2003; Yu *et al.*, 2003; Yang *et al.*, 2004; Markussaen and Kristensen 2010; Komagata *et al.*, 2010).

Review of literature suggests that from the early forties onward DDT (organochlorine) was routinely used to combat the problem of *H. theivora*, the major sucking pest (Das, 1962, 1963). Endosulfan (cyclodiene: organochlorine) was introduced in the Dooars tea plantation in the year 1968 in the form of Thiodan 35 EC (Mukerjea, 1968). At present, in the different conventional tea plantations of North East India, endosulfan, quinalphos, monocrotophos, chlorpyriphos, deltamethrin, imidacloprid and cypermethrin are extensively used to control sucking insects as reviewed by Barbora and Biswas, (1996), Sannigrahi and Talukdar (2003), Roy *et al.* (2008b) and Gurusubramanian *et al.* (2008a) (Table 1.3).

Table 1.3 Different insecticides used for controlling sucking pests in conventional tea plantations of Terai, and the Dooars.

	INSECTICIDES	TO CONTROL
1.	Endosulphan 35%EC (OC)	Thrips, Jassids, *Helopeltis*
2.	Fenpropathrin 10%EC (SP)	Thrips, Jassids
3.	Fenpropathrin 30%EC (SP)	Thrips, Jassids,
4.	Endodhan (OC)	Thrips, Jassids, *Helopeltis*
5.	Quinalphos 25%EC (OP)	Thrips, *Helopeltis*, Jassids,
6.	Monocrotophos 36% SL (OP)	Thrips, *Helopeltis* and Jassids
7.	Diclorvos 6% EC (OP)	Thrips, Jassids, *Helopeltis*
8.	Acephate 75% SP (OP)	Thrips , Jassids, *Helopeltis*
9.	Malathion 50%EC (OP)	Thrips, Jassids, *Helopeltis*
10.	Imidacloprid 17.8% SL (NN)	Thrips, *Helopeltis*, Jassids etc
11.	Cypermethrin10%EC/25%EC (SP)	Thrips, Jassids *Helopeltis* etc
12.	Deltamethrin (SP)	Thrips, Jassids and *Helopeltis*
13.	Alphamethrin-10%EC (SP)	Thrips, Jassids and *Helopeltis*
14.	Lamdacyhalothrin 2.5%EC,5%EC (SP)	Thrips, Jassids and *Helopeltis*
15.	Oxydemeton Methyl 25%EC (OP)	Thrips, Jassids and *Helopeltis*
16.	Profenphos40%EC+Cypermethrin 4%EC (OP+SP)	Thrips, Jassids and *Helopeltis*

OC: Organochloride, SP: Synthetic Pyrethroid, OP: Organophosphate, NN: Neonicotinoid.

An average of 7.5 l/ha of insecticides is used per year in the Dooars tea plantations (Roy *et al.*, 2008b) wherein, endosulfan is the most common insecticide accounted for 73.5% and found to be ineffective in controlling the pest in different parts of the Dooars

tea plantations (Roy *et al.*, 2010a). Decrease in the susceptibility to different classes of insecticides may be one of the causes for resurgence of pests and persistence on tea crop (Bora *et al.*, 2007; Gurusubramanian and Bora, 2008; Gurusubramanian *et al.*, 2008b; Rahman *et al.*, 2005; Sarker and Mukhopadhyay, 2003, 2006a, b; Sarmah *et al.*, 2006; Roy *et al.*, 2008a,b,c). A major problem in the control of *H. theivora* is its potential to develop resistance rapidly to regularly used insecticides (Gurusubramanian and Bora, 2008). In *Helopeltis spp.* attacking cocoa, high insecticide tolerance have been reported (Dzolkhifli *et al.*, 1986; Liew *et al.*, 1992; Ho, 1994). Their high reproductive potential and numerous annual generations, combined with continued and repeated use of an array of active substances of insecticides on tea cultivations for many years, have limited the control of *H. theivora* populations by killing the natural enemies and were led to resurgences in the bug populations (Roy *et al.* 2009b, c, d). Added to this, in recent days, the situation has further worsened as it has developed resistance to many commonly used insecticides and leading to controls failure (Roy *et al.*, 2008b, 2011; Mukhopadhyay and Roy, 2009). Such failures are already known in case of organochlorides (OC), organophosphorus (OP) and synthetic pyrethroid (SP) insecticides and more recently for the newer compound such as neonicotinoids (NN) (Gurusubramanian and Bora 2007; Gurusubramanian *et al.* 2008b; Roy *et al.* 2008a,b,c,d, 2011). The resistance developed in *H. theivora* populations in North East India ranged from 1.47 - 62.99 folds for males and 1.25 - 62.82 folds for females to different classes of insecticides (Gurusubramanian and Bora, 2008). Variations in the relative toxicity to commonly used insecticides have been observed in *H. theivora* populations from Jorhat, Assam (Gurusubramanian and Bora, 2007), Darjeeling (Bora *et al.*, 2007), and the sub-Himalayan Dooars region of north east

India (Roy *et al.*, 2008a). Variation in relative toxicity was also observed between male and female populations of Jorhat and Darjeeling and among the populations of *H. theivora* collected from different sub districts of Dooars to the tested insecticides (Rahman *et al.*, 2005; Bora *et al.*, 2007; Gurusubramanian and Bora, 2008; Roy *et al.*, 2008a, c, 2010). A comparison of expected effective dose of thirteen insecticides against tea mosquito bug based on their LC_{50} values with recommended dose revealed a pronounced shift in the level of susceptibility of *H. theivora* to all the chosen insecticides except acephate (Gurusubramanian *et al.*, 2008b). Gurusubramanian and Bora (2008) observed that Nagarkata population of the Dooars region showed a high level of resistance than Jorhat population of Assam in both males (7.998 folds in Jorhat and 8.522 folds in Nagrakata) and females (4.739 folds in Jorhat and 8.326 folds in Nagrakata) to endosulfan during 2006 -2007.

Like *Helopeltis,* the management of other sucking insects such as thrips, insecticides have also been used targeting *S. dorsalis* in conventional tea plantations. In India, *S. dorsalis* populations have shown resistance to a range of organochlorine (DDT, BHC and endosulfan), organophosphate (acephate, dimethoate, phosalone, methyl-O-demeton and triazophos) and carbamate insecticide (carbaryl) in chili ecosystem (Reddy *et al.*, 1992). Vanisree *et al.* (2011) has shown that *S. dorsalis* has developed a high degree of resistance to various insecticides *viz*., monocrotophos, acephate, dimethoate, phosalone, carbaryl and triazophos. Recently, Seal *et al.* (2006, 2007) tested several insecticides on *S. dorsalis* in chili ecosystem in USA and found limited success with chlorfenpyr, spinosad and imidacloprid. The performance of novaluron, abamectin, spiromesifen, cyfluthrin, methiocarb and azadirachtin failed to provide effective control

of this pest (Dogramaci *et al.,* 2011). To date there is no report of development of insecticide resistance of *S. dorsalis* in tea ecosystem. Though there is a report of control failure of *S. dorsalis* in tea ecosystem (Gurusubramanian *et al.,* 2008a), a high basal level of insecticide resistance-related enzymes have been reported in tea ecosystem from north east India (Saha *et al.,* 2010, 2012b; Saha and Mukhopadhyay, 2013). Metabolic resistance to synthetic insecticides has also been reported in western flower thrips, *Frankniella occidentalis* (Jensen, 2000; Maymo´ *et al.,* 2002, 2006; Espinosa *et al.,* 2005).

In another emerging sucking insect pest of tea, *E.* flavescens, there is no report of insecticide resistance in tea ecosystem from north east India. But there are reports of repeated control failure of *E. flavescens* in sub-Himalayan tea plantations of nort east India (Gurusubramanian *et al.,* 2008a). Chemical insecticides including fenvalerate, cyfluthin, cypermethrin and imidacloprid have been used to control the leafhoppers as frequently as seven times annually or even more frequently in China (Tao *et al.,* 1996; Shi *et al.*, 2001). In China, high level of resistance to many insecticides has been reported in allied species, *E. vitis* (Nian-Wu *et al.*, 2004). Resistance to thiamethoxam was highest and to cyperemethrin was lowest in *E. vitis.* In Fujian province of China, Jia-Xiang *et al.* (2009) found a regional diversity of resistance to eight insecticides in *E. vitis* in tea ecosystem and reported higher resistance level to bifenthrin, acetamiprid, imidacloprid, cartap and chlorfenapyr.

Detoxification of insecticides is an important mechanism for insect pests to tolerate regularly applied insecticides (Brown and Brogdon, 1987; Soderlund and Bloomquist, 1990; Yu, 1996 Yu *et al.*, 2003; Scarf *et al.*, 1998a, 1999; Nehare *et al.*,

2010; Wu *et al.*, 2011). Despite the continuous use of insecticides there are repeated failures in controlling the insect pest species in recent years (Gurusubramanian *et al.*, 2008a; Roy *et al.*, 2008a) in different conventional tea plantations of Terai, the Dooars and Darjeeling foothill regions. Such failures occur due to change in the susceptibility level of the pest species to the applied insecticides. Susceptibility levels change mainly due to metabolic detoxification of the insecticides through higher activity levels of some insecticide-detoxifying enzymes under the stress of different management practices (Sarker and Mukhopadhyay, 2003, 2006; Buès *et al.*, 2005; Limoee *et al.*, 2007; Cao *et al.*, 2008a,b; Wu *et al.*, 2011).

Generally three principal enzymes, general esterases (GEs), glutathione S-transferases (GSTs) and cytochrome P450 mediated monooxygenases (CYPs) are involved in the process of metabolic detoxification of insecticides. Estimation of the activities of these metabolic defense related detoxifying enzymes may give information on the level of tolerance/resistance of the insect pest population to insecticides and are useful tool in monitoring the tolerance/ resistance to insecticides at population level in the pest.

Early detection of the metabolic threats related to tolerance/resistance to insecticides in pest specimens is of crucial importance for devising pest control methods that would minimize the appearance of tolerance/resistance forms and prevent any undesirable wastage of insecticide. Further, this also requires an understanding of the genetic structure of pest populations.

In small insects, Randomly Amplified Polymorphic DNA (RAPD) is an important genetic marker to study the genetic variability. The RAPD marker has been reported to be

an efficient tool to differentiate geographically and genetically isolated populations. RAPD is particularly useful to study the genetic structure of the populations because the reveal polymorphisms in non coding regions of the genome (Vucetich *et al.,* 2001). It has also been used to verify the existence of population of species that have arisen either through genetic selection under different environmental conditions (Fuchs *et al.*, 1998). RAPD markers have been used to estimate genetic variability (Skinner and Camacho, 1995; Stewart and Excoffier, 1996) and to determine genetic structure of populations of various organisms (DeSousa *et al.,* 1999). The RAPD technique has been used to identify genetic polymorphisms in a great many biological species (DeOliveira *et al.*, 2007). Continuous uses of synthetic insecticides in tea of the Darjeeling foothill and its plain appear to have led to the selection of insecticide-resistant populations and development of insecticide resistance in the concerned sucking pests.

So, the increasing management problem associated with the sucking pests of tea and their resistance to insecticides resulting adaptive variability necessitates a detailed study on the biochemical of these pests from lower altitudes of Darjeeling hill and adjoining plain with reference to three principal xenobiotic detoxifying enzymes and genetic variability by RAPD-PCR to establish variability among different population which may throw light on biotype variation too. This would help to develop a population specific control strategy for effective management of the sucking insect pest populations in sub Himalayan tea plantations of northern part of West Bengal, India.

Review of Literature

Insect face numerous toxins (xenobiotics) as they go through their life cycle. Some toxins are produced naturally by the host plants (allelochemicals) and others by humans (insecticides). The host plants have evolved defensive mechanisms, including chemical repellents and toxins (secondary metabolites) (Wink, 2003). Many classes of insect repellents and toxic substances, including iso-flavonoids, furanocoumarins, terpenoids, alkaloids, and cyanogenic glycosides are synthesized in plants (Wink and Waterman, 1999). The biosynthetic pathways leading to these allelochemicals are continually evolving to generate new secondary metabolites) (Wink, 2003). In turn the attacking organisms evolved mechanisms that enable them to resist the defensive chemicals of their hosts (Musser *et al.*, 2002). A variety of defense mechanisms, including enzymatic detoxification systems, physiological tolerance, and behavioral avoidance, protect insect herbivores from these hazardous compounds. Insect pests have evolved the mechanisms to degrade metabolically (enzymatically) or otherwise circumvent the toxic effect of many types of chemicals that we have synthesized as modern insecticides. The extent to which insects can metabolize and thereby degrade these toxic or otherwise detrimental chemicals is of considerable importance for their survival in a chemically unfriendly environment (Johnson, 1999). These mechanisms continue to evolve as insects attempt to colonize new plant species or encounter newer molecules of synthetic insecticides.

Resistance of insects to insecticides has been noticed greatest increase and strongest impact during the last 70 years, following the discovery and extensive use of synthetic organic insecticides (Mullin and Scott, 1992). The evolution of resistance to insecticides is an example of evolutionary process. The insecticide is the selection

pressure, which creates a very strong fitness differential between susceptible and resistant genotypes (Dominiguez-Gill and McPheron, 1999). The survival and subsequent reproduction of resistant individuals lead to a change in the frequency of alleles conferring resistance over time. While selection pressure acts to change allele frequencies within pest populations, the phenotype upon which selection operates is a function of both genotypes and the environment (Dominiguez-Gill and McPheron, 1999). Recent studies in insect detoxifying enzymes have revealed further versatility in adaptation of insects to their environment by the phenomenon of induction. This is the process in which a chemical stimulus enhances the activity of the detoxification enzyme systems by the production of additional enzymes that metabolize toxic chemical substances (Terriere, 1984). So the influence of environmental factors such as continuous usages of insecticides and the chemical constituents (allelochemicals) of host plants in phytophagous insects can have a great impact to induce the enzymatic detoxification systems of insects and thereby affecting the insecticide resistance mechanisms.

While all insects possess detoxifying ability, the magnitude can be expected to vary among the species with the nature of its recent environment and feeding ecology (Fragoso *et al.,* 2002; John and Graeme, 2008; Despres *et al.,* 2007; Mullin, 1985, 1988; Hung *et al.,* 1990). The level and type of detoxifying mechanism differ greatly, which therefore, results in varying toxicity among different stages, species and populations. Variation in detoxifying enzymes activity is responsible, in part, at least for the selective toxicity of different insecticides, the development of resistance to insecticides and selection of host plants (Li *et al.,* 2007; Maymo *et al.,* 2002, 2006; Claudianos *et al.,* 2005). Over expression of these detoxifying enzymes, capable of metabolizing

insecticides can result in high level of metabolic tolerance/ resistance to synthetic insecticides (Wu *et al.,* 2011). Increased expressions of genes encoding the major xenobiotic metabolizing enzymes are the most common cause of insecticide resistance in insects (Komagata *et al.,* 2010).

THREE IMPORTANT SUCKING PESTS OF TEA:

Tea is a gift of nature and is most widely used non – alcoholic beverage all over the world. Tea industry is one of the most important agro-industrial crops in India and sustains the economy of a large number of people in North East India. Tea originated from the borders of Assam and Mayanmer in Southeast Asia centered around 29°N and 98°E. The indigenous tea, in India was used as a medicinal plant from early times but its commercial cultivation did not begin until the 19th century. The first viable plantations in Assam date back to around 1835 and the first crop were sold in 1838. During the latter part of the 19th century, the entomologists working at Indian Museum were involved in the identification and publishing of information on insects associated with tea. The dynamic adaptations of insects have enabled them to attack every part of a tea plant with maximum number of pest occuring on the foliage, resulting in 11%–55% loss in yield if left uncontrolled (Hazarika *et al.,* 2009). As the tea crop is derived from the young foliage and because maximum production is achieved by enhancing the leaf cover by pruning, it is not surprising that the major pests of the crop are those associated with the foliage. The sucking insect pests damage these young foliages, the most economic part of the plant to be processed in the tea industry.

The study of insects associated with tea i.e. tea entomology (Temology) is relatively recent. Early accounts of tea pests in India and Sri Lanka include "*Notes on tea in Darjeeling*" published in 1888, "*Insect Pests of the tea plant*" by E. E. Green covering pests found in Sri Lanka, in 1890, and "*An account of the insects and mites which attack the tea plant in India*" by C.E Cotes in 1894.Watt and Mann produced a good account of Indian tea insects in their "*The Pests and Blights of the Tea Plant*" published in 1898 with a second expanded edition in 1903. They bring together scattered information from different sources such as "*The Tropical Agriculturist*" which began publication in 1881 and is still being published. The "*Tea Cyclopaedia*" also published in 1881, and "*Indian Museum Notes*" published annually in Calcutta between 1889 and 1903 which replaced two previous issues of "*Notes on Economic Entomology*".

The most important pest groups in sub Himalayan tea plantations are sucking insect pests. Tea mosquito bug, thrips, jassids are common in almost all the sub-Himalayan tea plantations in north east India.

A. *Helopeltis theivora* Waterhouse (Tea Mosquito Bug):

One of the earliest disorders of tea recorded in India was the leaf spotting and die-back caused by *Helopeltis theivora*, the most important sucking pest till today, commonly known as tea mosquito bug. This was first noticed in Cachar, Assam in 1865 and was at first thought to be a fungal disease. It was not until around 1873 that it was shown to be caused by the feeding of *H. theivora* described by Waterhouse in1886. Species of *Helopeltis* have been recorded on tea from most of the tea growing countries in Asia and Africa apart from Japan and Korea.

Systematic Position:

Order: Heteroptera
Intraorder: Cimicomorpha
Superfamily: Miroidea
Family: Miridae
Subfamily: Bryocorinae
Tribe: Dicyhini
Subtribe: Monaloniina

All over the World 35 different species of mirids are associated with tea, out of which 18 are different species of the genus *Helopeltis* (Table 2.1). *H. theivora* is a polyphagous pest with a wide range of alternative host plants (Table 2.2).

Table 2.1 Different species of *Helopeltis* associated with tea all over the world.

SN	Pest Species	Status	Distribution
1.	*Helopeltis anacardii* Miller	Leaf feeder and Shoot feeder	Malawi (Sweeney, 1965)
2.	*Helopeltis antonii* Signoret	Leaf feeder and Shoot feeder	India (Gopalan and Perumal, 1973), Indonesia Java (Koningsberger, 1908), Indonesia Sumatra (Leefmans, 1916), Sri Lanka (Hutson, 1920), Vietnam (Du Pasquier,1932)
3.	*Helopeltis bergrothi* Reuter	Leaf feeder and Shoot feeder	Kenya (Le Pelley, 1959), Malawi (Petch and Light, 1928), Tanzania (Harris, 1933a), Uganda (Gowdey, 1917)
4.	*Helopeltis bradyi* Waterhouse Synonyms *Helopeltis antonii bradyi* Waterhouse, *Helopeltis romundei* Waterhouse	Leaf feeder and Shoot feeder	Indonesia Java (Leefmans, 1916), Indonesia Sumatra (Leefmans, 1916), Malaysia W.Malaysia (Anon, 1941)
5.	*Helopeltis cinchonae* Mann	Leaf feeder and Shoot feeder	Indonesia Java (Leefmans, 1916), Indonesia Sumatra (Leefmans, 1916), Taiwan (Sonan, 1924) Malaysia W.Malaysia (Corbett, 1932),

6.	*Helopeltis clavifer* (Walker)	Leaf feeder and Shoot feeder	Papua New Guinea (Smith *et al.*, 1985)
7.	*Helopeltis cuneatus* Distant	Leaf feeder and Shoot feeder	Indonesia Java (Leefmans, 1916), Indonesia Sumatra (Leefmans, 1916)
8.	*Helopeltis fasciaticollis* Poppius	Leaf feeder and Shoot feeder	Taiwan (Sonan, 1924), China (Xie, 1993)
9.	*Helopeltis maynei* Ghesquiere	Leaf feeder and Shoot feeder	Uganda (Hargreaves, 1936)
10.	*Helopeltis orophila* Ghesquiere	Leaf feeder and Shoot feeder	Zaire (Ghesquiere, 1939), Uganda (Foster-Barham, 1957)
11.	*Helopeltis orophila lutea* Ghesquiere	Leaf feeder and Shoot feeder	Zaire (Ghesquiere, 1939)
12.	*Helopeltis orophila rubida* Ghesquiere	Leaf feeder and Shoot feeder	Zaire (Ghesquiere, 1939)
13.	*Helopeltis schoutedeni* Reuter	Leaf feeder and Shoot feeder	Uganda (Ingram *et al.*, 1966), Kenya (Benjamin, 1968a), Tanzania (Benjamin, 1968a), Mali (Fofana, 1978), Malawi (Peregrine, 1991)
14.	*Helopeltis sp.*	Leaf feeder and Shoot feeder	Taiwan (Sonan, 1923), Vietnam (Du Pasquier, 1924) Malaysia W.Malaysia (Corbett,1930),
15.	*Helopeltis sumatranus* Roepke	Leaf feeder and Shoot feeder	Indonesia Sumatra (Menzel, 1929)
16.	*Helopeltis theivora theobromae* Miller	Leaf feeder and Shoot feeder	Malaysia W.Malaysia (Lever, 1949)
17.	*Helopeltis theivora* Waterhouse *Synonyms* *Helopeltis febriculosa* Bergroth	Leaf feeder and Shoot feeder	Indonesia Java (Koningsberger, 1908), India (Andrews, 1913), Indonesia Sumatra (Leefmans 1916), Laos (Du Pasquier, 1932), Vietnam (Du Pasquier, 1932) Bangladesh (Mir, 1990),

Table 2.2 Alternative hosts of *Helopeltis theivora*

Name of the Alternative hosts	Family
1. Acalypha sp	Euphorbiaceae
2. Ehretia acuminata	Boraginaceae
3. Enthocephalus cadamba	Rubiaceae
4. Eugenia jambolans	Myrtaceae
5. Eurya acuminata D.O.	Theaceae
6. Ficus hispida	Moraceae
7. Gardenja jesminoid	Rubiaceae
8. Jasminum sandens Vahl	Oleaceae
9. Maesa ramentacae A.D.C.	Maesaceae
10. Melastoma malabathricum L.	Melastomataceae
11. Mikania micrantha	Asteraceae
12.Morus alba	Moraceae
13.Oxalis acetosella	Oxalidaceae
14. Phlogocanthus pubinervious	Acanthaceae
15. Polygonum chinens	Polygonaceae
16. Premna latifolia	Verbenaceae
17.Psidium guajava	Myrtaceae
18.Similax herbaceae L.	Liliaceae
19. Bidens pilosa	Compositae

B. *Empoasca flavescens* Fabricius (Tea jassid/Greenfly)

Systematic Position:

Order: Homoptera
Intraorder: Auchenorrhyncha
Superfamily: Cicadoidea
Family: Cicadellidae (=Jassidae)
Subfamily: Typhlocybinae
Tribe: Empoascini (=Helionini)

Different species of jassids associated with tea all over the tea growing countries are summarized in Table 2.3. There are, 21 different species are recorded in tea ecosystem out of which six species are of *Empoasca*.

Table 2.3 Different species of jassids associated with tea all over the world.

SN.	Pest Species	Status	Distribution
1.	*Bothrogonia formosana* (Matsumura)	leaf feeder	Taiwan (Shiraki, 1920)
2.	*Bothrogonia ferruginea* (Fabricius)	No record	China (Cheo, 1936)
3.	*Bothrogonia ferruginea apicalis* Walker	No record	China (Cheo, 1936)
4.	*Bothrogonia japonica Ishihara*	Leaf feeder	Japan (Ishihara, 1962), Korea (Kwon, 1983)
5.	*Bothrogonia sp.*	No record	Papua New Guinea (Szent-Ivany, 1958)
6.	*Chanohirata theae* (Matsumura)	No record	Taiwan (Schumacher, 1915)
7.	*Cicadella sp.*	No record	China (Cheo, 1936)
8.	*Cicadella viridis* (Linnaeus)	No record	Papua New Guinea (Szent-Ivany, 1958)
9.	*Empoasca formosana* Paoli	leaf feeder,	Taiwan (Chen and Tseng, 1988)

		shoot feeder	
10.	*Empoasca onukii* Matsuda	Leaf feeder	Japan (Mochizuki *et al.,* 1994)
11.	*Empoasca paraobliqua* Ghauri	No record	Argentina (Ghauri, 1964)
12.	*Empoasca pirisuga* (Matsumura)	No record	China (Xie, 1992)
13.	*Empoasca thea* Distant	No record	India (Fletcher, 1920)
14.	*Empoasca flavescens* Fabricius	Leaf feeder	China (Cheo, 1936), India (Andrews and Tunstall, 1915), Indonesia Java (Menzel, 1929), Japan (Du Pasquier, 1932), Sri Lanka (Evans, 1952), Taiwan (Shiraki, 1920), Vietnam (Du Pasquier, 1932)
15.	*Penthimia bella* Stal	No record	Kenya (Benjamin, 1968a)
16.	*Penthimia nitida* Lethierry	No record	Japan (Minamikawa, 1957)
17.	*Penthimia sp.*	No record	Japan (Sonan, 1933)
18.	*Penthimia testacea Ge*	No record	China (Ge, 1991)
19.	*Pseudonirvana unicolor* Kuoh & Kuoh	Leaf feeder	China (Kuoh and Kuoh, 1983)
20.	*Sophonia orientalis* (Matsumura)	Leaf feeder	China (Kuoh and Kuoh, 1983), Hawaii (Zee *et al.,* 2003)
21.	*Vulturnus ornatus Distant*	No record	Sri Lanka (Hutchinson *et al.,* 1963)

C. *Scirtothrips dorsalis* Hood (Assam/Yellow Tea Thrips)

Systematic Position:

Order: Thysanoptera
Suborder: Terebrantia
Family: Thripidae

Different species of thrips associated with tea in India and all over the world has been summarized in Table 2.4.

Table 2.4 Different species of thrips of *family Phlaeothridae and Thripidae* associated with tea all over the world.

Family		*Species*	*Status*	*Distribution*
Phlaeothripidae	*1.*	*Aleurodothrips sp.*	No record	Malaysia W. Malaysia (Yunus and Ho, 1980)
	2.	*Apelaunothrips consimilis* Ananthakrishnan	Leaf feeder	India (Varatharajan *et al.*, 2007)
	3.	*Arrhenothrips longisetis* Sen	Leaf feeder	India (Varatharajan *et al.*, 2007)
	4.	*Haplothrips gowdeyi* (Franklin)	Leaf feeder	India (Varatharajan *et al.*, 2007)
	5.	*Haplothrips inquinatus* Karny		Indonesia (Karny, 1921)
	6.	*Haplothrips tenuipennis* Bagnall	Predator	India (Andrews, 1925)
	7.	*Hoplandrothrips sp. near tristissimus* (Priesner)	Leaf feeder	Malawi (Benjamin, 1968a)
	8.	*Neoheegeria sp.*	Leaf feeder	India (Rau, 1936)

Thripidae	1.	*Anaphothrips sp.*		Indonesia, Malaysia W.Malaysia (Karny, 1926, Yunus and Ho, 1980)
	2.	*Aptinothrips rufus* Haliday	flower feeder	India (Bagnall, 1918a)
	3.	*Ceratothrips lefroyi* (Bagnall)	leaf feeder	Taiwan (Chen, 1979)
	4.	*Chaetanaphothrips sp.*		Malaysia, W. Malaysia (Yunus and Ho, 1980)
	5.	*Chaetanaphothrips theiperdus* (Karny)	leaf feeder	Indonesia (Karny, 1921), Indonesia Java (Menzel 1929)
	6.	*Danothrips theifolii* (Karny)	leaf feeder	Indonesia (Karny, 1921), Indonesia Java (Menzel, 1929)
	7.	*Dendrothrips minowai* Priesner	leaf feeder	Japan (Okada and Kudo, 1982)
	8.	*Heliothrips haemorrhoidalis* (Bouche)	leaf feeder	China (Cheo, 1936), Georgia (Tulashvili, 1930), Hawaii (Zee *et al.,* 2003), India (Rau 1935), Indonesia Java (Koningsberger, 1908), Kenya (Benjamin, 1968b), Malawi (Smee, 1937), Sri Lanka (King, 1941), Tanzania (Harris, 1933b), Uganda (Ingram *et al.*, 1966)

9.	*Lefroyothrips lefroyi* (Bagnall)	flower feeder	India (Bagnall, 1918b)
10.	*Lefroyothrips obscurus* (Ananthakrishnan & Jagadish)		India (Ananthakrishnan and Jagadish, 1966)
11.	*Megalurothrips distalis* (Karny)	flower feeder, leaf feeder	India (Ananthakrishnan and Jagadish, 1966), Japan (Okada and Kudo, 1982)
12.	*Microcephalothrips abdominalis* (D.L.Crawford)	leaf feeder	Japan (Okada and Kudo 1982)
13.	*Mycetothrips setiventris* Bagnall	flower feeder	India (Bagnall, 1918b), Malawi (Benjamin, 1968a)
14.	*Mycterothrips setiventris* *Synonyms* *Taeniothrips setiventris*	leaf feeder	India (Pathak and Mukhopadhyay, 2005)
15.	*Scirtothrips aurantii* Faure		Malawi (Rattan, 1992b), Zimbabwe (Jack,1940)
16.	*Scirtothrips bispinosus* (Bagnall)	leaf feeder	India (Bagnall, 1924)
17.	*Scirtothrips dorsalis* Hood	leaf feeder	China (Xie, 1993), India (Sannigrahi and Mukhopadhyay, 1992), Japan (Takahashi, 1935), Taiwan (Chen, 1979)
18.	*Scirtothrips kenyensis* Mound	leaf feeder	Kenya (Mound, 1968)
19.	*Scirtothrips sp.*	leaf feeder	India (Rau, 1936)
20.	*Scolothrips sp.*	Predator	Korea (Lee *et al.*, 1995)
21.	*Scolothrips takahashii* Priesner		Japan (Omata, 1997)

22.	*Taeniothrips sp.*	leaf feeder	Japan (Sonan, 1933), Malaysia W. Malaysia (Yunus and Ho, 1980)
23.	*Thrips coloratus* Schmutz		Japan (Okada and Kudo, 1982)
24.	*Thrips flavus* Schrank		Japan (Okada and Kudo, 1982), Taiwan (Chen, 1979)
25.	*Thrips florum* Schmutz	Flower feeder	India (Rau, 1936)
26.	*Thrips formosanus* (Priesner)		India (Varatharajan *et al.*, 2007)
27.	*Thrips hawaiiensis* (Morgan)	leaf feeder	Indonesia (Karny, 1921), Indonesia Java (Menzel, 1929), Japan (Okada and Kudo, 1982), Taiwan (Chen, 1979)
28.	*Thrips sp.*		Georgia (Demokidov, 1916), India (Rau, 1936), Mauritius (Ramlogun, 1971)
29.	*Thrips tabaci* Lindeman		Japan (Okada and Kudo, 1982)

Insecticide Resistance Mechanisms in Sucking Insects of Tea:

Herbivorous insect groups are significantly more diverse than their non-herbivorous sister groups (Mitter *et al.*, 1991). The role of plant in promoting diversification in insects has occurred through coevolutionary arm races (Ehrlich and Raven, 1964). This diversification could also have been a result of insects 'tracking' plant phylogenies, with minor chemical changes in plants allowing the evolving populations of insects to change and speciate which probably occured long after chemical changes in plants (Bernays, 1998). Evolution to herbivory proceeded via mixed feeding on reproductive parts or spores, dead tissues of plants and animals, and fungi. This progression implies that omnivory precede generalized herbivory and the evolution of specialization on specific plant taxa was a later accomplishment (Dethier, 1954).

Among sucking insect herbivores, the actual food used i.e. digested whole tissue particularly parenchyma (as in *Helopeltis sp.*), cell content (Thrips) and phloem (*Empoasca sp.*) influences both the feeding mechanism and feeding behaviour (Bernays, 1998). While the chewing insects cause extensive damage, the sucking insects cause modest to barely perceptible damage. But, sucking insects, particularly phloem and digested tissue feeding, provide an additional challenge to the plants as they deplete photosynthates, vector viruses and introduce chemical and protein effectors that alter plant defense mechanisms (signaling) and development (Kaloshian and Walling, 2005). When these attributes are combined with broad host range, breeding strategies that promote invasiveness, highly evolved feeding strategies, the ability to adapt to a wide

range plant habitats and the emergence of insecticide resistance, then it is not surprising that sucking insects cause heavy losses in agriculture and horticulture (Goggin, 2007).

Development of resistance is an example of 'microevolution'. Variation within a population may include individuals with genetic traits that make them better adapted to survive exposure to an insecticide. If these individuals survive the insecticide exposure, then the resistance traits can be passed on to the next generation, thereby enriching the gene pool with those genes. The subject of this part of the review is the resulting mechanisms of the genetic alterations that give rise to resistance. The mechanisms of resistance can be divided into four levels:

1. **Behaviour** – avoidance of contact with the insecticide.
2. **Barrier tissues** – reduced penetration of the insecticide.
3. **Detoxification** – metabolism of the insecticide.
4. **Alteration** – at the target site for the insecticide

The first level, at which resistance can develop, is when the insect encounters the insecticide. An altered behaviour can help the insect to avoid coming into contact with the insecticide. Once the insect has come into contact with the insecticide, a delayed penetration through the integument reduces the effect of the insecticide at the target site. Inside the insect, the insecticide may be metabolized and thereby inactivated. At this third level of resistance mechanisms, three systems of detoxification enzymes operate: esterases, glutathione *S*-transferases, and cytochrome P450-dependent monooxygenases. Increased activity of one of these enzyme systems in inactivating insecticides will result in resistance. Alterations at the target site for the insecticide are the last level of resistance mechanisms. The different classes of insecticides bind to specific target sites. Reduced

binding at the target site or increased number of target site molecules may confer resistance.

A. *Behavioural resistance*

Behavioural resistance mechanisms are the least studied resistance mechanisms in insects, although behavioural resistance is not significant. Sparks *et al.* (1989) reviewed the role of behaviour in resistance. They defined behavioural resistance as 'evolved behaviours that reduce an insect's exposure to toxic compounds or that allow an insect to survive in what would otherwise be a toxic and fatal environment'. Behavioural resistance has been shown in more than 30 species of insects (Sparks *et al.*, 1989). Avoidance is the first step in the evolution of behavioural resistance (Brattsten, 1988). This kind of resistance has been noticed in *H. theivora* (Roy and Mukhopadhyay, 2011). *H. theivora,* show a different egg laying strategy to avoid insecticide exposure. *E. flavescens* do not like direct sunlight and therefore prefer to stay on the underside of the leaves. This behaviour of *E. flavescens* cross protects them from the direct insecticide exposure in conventional tea plantations during spraying (Simanjuntak, 2002). No studies on resistance due to behavioural mechanisms in *S. dorsalis* have yet been reported.

B. *Reduced penetration*

Reduced penetration of insecticides through barrier tissues of insects is one way in which an insect can modify the effective dose of insecticide at the target site. The mechanism may not prevent the insecticide from eventually entering the insect, but it can reduce the rate at which the insecticide reaches the target site. Reduced penetration has been shown to function as a resistance mechanism to many different insecticides, and, by the nature of

this mechanism, cross-resistance is often found (Price, 1991). The rate of penetration of insecticides through the cuticle or other barriers depends on the physicochemical properties of the insecticide and the barrier. For example, reduced penetration can contribute to DDT resistance in the tobacco budworm, *Heliothis virescens* F. (Lepidoptera: Noctuidae). Vinson and Law (1971) showed that DDT resistant larvae had an altered composition of the cuticle; the protein and lipid content were greater in the cuticle of resistant larvae and, furthermore, the cuticle of the resistant larvae probably had a higher degree of sclerotization. In *M. domestica***,** two resistant strains, with reduced penetration as one of the resistance mechanisms, also showed increased cuticular lipid content; more total lipids, monoglycerides, fatty acids, sterols and phospholipids were present in the resistant strains compared to a susceptible strain (Patil and Guthrie, 1979). Reduced penetration has been documented as a resistance mechanism only at the level of the insect cuticle, but any biological membrane may serve as a barrier and thereby give resistance (Scott, 1990). As a single resistance mechanism it usually only confers low levels (<3-fold) of resistance (Scott, 1990). Reduced penetration has been shown to function as a resistance mechanism to many different insecticides, including insecticides of the three major classes, OPs, carbamates, and pyrethroids. Reduced penetration of OPs through the cuticular barrier has reported for e.g. diazinon in *M. domestica* (Forgash *et al.,* 1962), azinphos-methyl in the pear psylla, *Psylla pyricola* Foester (Hemiptera: Psyllidae) (van de Baan and Croft, 1991), and profenofos in *H. virescens* (Kanga and Plapp, 1995).

However, by slowing the penetration rate of insecticides, this mechanism reduces the risk that the insects' detoxification systems become overloaded and the dose of

insecticide reaches a fatal level at the target site. In female *H. theivora* higher level of body lipid effectively reduces the penetration of insecticide to the target site (Roy *et al.*, 2008c). No studies on resistance due to reduced penetration in *E. flavescens* and *S. dorsalis* have been reported. Mutero *et al.* (1994) and Martinez-Torres *et al.* (1999) suggest that when different resistance mechanisms are combined in the same individuals, a synergistic effect, resulting in a high level of resistance, may arise. Therefore, even a small degree of reduced penetration may contribute significantly to the overall resistance of the insect.

C. *Metabolic Detoxification*

Metabolic detoxification of insecticides is an important toxicokinetic mechanism for insects to reduce the toxic effects of insecticides. Organophosphates, organochlorines, carbamates and pyrethroids are generally hydrophobic compounds and detoxification enzymes transform the insecticides to more hydrophilic and less biologically active compounds that can be eliminated by excretion. Increased detoxification of insecticides has often been reported in many resistant populations (Soderlund and Bloomquist, 1990). Three enzyme systems are generally recognized as the major detoxification systems involved in insecticide resistance in insects. These are esterases, cytochrome P450-dependent monooxygenases, and glutathione *S*-transferases (Soderland and Bloomquist, 1990). Therefore, detoxification of insecticides is an important mechanism for insect pests to tolerate regularly applied insecticides (Brown and Brogdon, 1987; Scarf *et al.*, 1998a, b, 1999, 2001; Yu *et al.*, 2003; Nehare *et al.*, 2010; Wu *et al.*, 2011).

D. *Alteration at the target site for the insecticide*

The insecticides of the four major classes, organophosphates, carbamates, organochlorine and pyrethroids, have receptor in the nervous system as the site of action. OPs and carbamates have a single enzyme as target, acetylcholinesterase, and the pyrethroids and DDT have the voltage-gated sodium channel of the nerve membrane as target. Other target sites for neurotoxic insecticides are the y-aminobutyric acid (GABA)-receptor (Casida, 1993; Sattelle, 1990; Narahashi, 1996) and the nicotinic acetylcholine receptor (Sattelle, 1985; Narahashi, 1996). The GABA-receptor is the target for cyclodienes ***(****e.g.* dieldrin and endosulfan), y-HCH (lindane) and fipronil, and the nicotinic acetylcholine receptor is the target for nicotinyl insecticides **(**imidacloprid and nicotine). Alteration at the target-site, to less a sensitive target for neurotoxic insecticides, is an important toxicodynamic resistance mechanism in insects. Altered GABA-receptor, as a resistance mechanism has been reviewed (Feyereisen, 1995; Soderluand and Bloomquist, 1990).

The target for OPs and carbamates is the enzyme acetylcholine esterase (AChE) (EC 3.1.1.7). The function of AChE is to break down acetylcholine in the synaptic cleft (Zubay, 1983). Acetylcholine is an excitatory neurotransmitter that transmits a signal from a presynaptic to the postsynaptic neurone. Arrival of an action potential to the presynaptic membrane of a cholinergic synapse triggers the release of acetylcholine into the synaptic cleft. Acetylcholine diffuses to the postsynaptic membrane and binds to a specific receptor. The insect acetylcholine receptors have been reviewed by Sattelle (1985) and Gundelfinger (1992). Upon binding to the receptor, acetylcholine ultimately causes the development of an action potential in the postsynaptic neurone (Zubay, 1983). For proper function at the cholinergic synapse, acetylcholine must be rapidly broken

down in the synaptic cleft to restore the resting potential of the postsynaptic membrane. This is achieved by AChE that hydrolyses acetylcholine to acetate and choline (Zubay, 1983).

In insects, AChE is mainly located in the central nervous system. Localisation, properties and structure of insect AChE have been reviewed by Eldefrawi (1985) and Toutant (1989). OPs and carbamates are potent inhibitors of AChE. These compounds act by forming a stable covalent intermediate with AChE thus preventing the enzyme from hydrolysing acetylcholine. An accumulation of acetylcholine leaves the cation channel of the receptor permanently open, which eventually is lethal. OPs and carbamates are quasi-irreversible inhibitors of AChE; OPs phosphorylates and carbamates carbamylates the active-site serine in AChE (Main, 1979; Eldefrawi, 1985). The time of reactivation of phosphorylated or carbamylated AChE is generally long. However, half-lives of reactivation vary considerably, from minutes to several days, depending on the compound interacting with AChE (Aldridge and Reiner, 1972; Main, 1979). Carbamylated AChE generally reactivates faster than phosphorylated AChE. Reduced sensitivity of AChE to inhibition by OPs and carbamates is an important resistance mechanism in insects and is often referred to as altered or insensitive AChE. This mechanism has been found in several insect populations resistant to these compounds and several extensive reviews have covered this topic of altered AChE as a resistance mechanism (Hama, 1983; Oppenoorth, 1985; Fournier and Mutero, 1994).The presence of insensitive AChE conferring resistance was first suggested by Smissaert (1964), from a study on OP-resistant mites, *Tetranychus urticae* Koch (Acari: Tetranycidae). Inhibition of AChE activity *in vitro* by diazoxon and paraoxon was assayed in resistant and

susceptible mites, and it was found that AChE activity of resistant mites had reduced sensitivity to inhibition by the two OPs. Fournier and Mutero (1994) list 25 examples of insensitive AChE in species of Hemiptera, Coleoptera, Diptera, and Acarina. Insensitive AChE has been reported in the greenbug, *Schizaphis graminurn* (Siegfried and Ono, 1993a,b), the peach-potato aphid, *Myzus persicae* and the tobacco aphid, *M. nicotianae* Blackman (Hemiptera: Aphididae) (Moores *et al.*, 1994), the pear psylla, *Cacopsylla pyri* L. (Hemiptera: Psyllidae) (Berrada *et al.*, 1994).

The level of AChE activity may differ in susceptible and resistant populations. Several examples of increased AChE activity in OP-resistant insects have already been described, *e.g.* in the California red scale, *Aonidiellu auruntii* Mask. (Hemiptera: Coccoidae), increased AChE activity is associated with chlorpyrifos resistance (Levitin and Cohen, 1998); in the greenbug, *S. gruminum,* three resistant populations all showed increased AChE activity (Zhu and Gao, 1999); in the lesser grain borer, *Rhyzopertha dominica,* all of the 15 resistant populations tested, had higher AChE activity than the susceptible population (Guedes *et al.*, 1997); in the tobacco budworm, *Heliothis virescens,* nine of 10 resistant strains showed significantly increased AChE activity (Harold and Ottea, 1997); in the fruit fly, *Drosophila melanogaster,* selection for resistance to parathion and fenthion was accompanied by a correlated increase in AChE activity (El-Abidin Salam and Pinsker, 1981), and in several resistant strains of the housefly, *M. domestica,* increased V, (maximum initial rate of an enzyme reaction) of AChE has been shown **(**Tripathi and O'Brien, 1973; Voss, 1980; Yeoh *et al.*, 1981; Oi *et al.*, 1990).

Sarkar and Mukhopadhyay (2006b) reported that higher level of AChE activity in *H. theivora* sampled from conventional tea plantations than from organic tea plantations

indicating the presence of resistance to insecticides in conventional tea ecosystems. There is no report on *S. dorsalis* and *E. flavescens* in conventional tea ecosystems. But such kind of resistance has been reported in *Frankniella occidentalis* (Jensen, 2000) and *Bemisia tabaci* (Vassiliou *et al.*, 2011).

MAJOR METABOLIC DETOXIFYING ENZYMES IN INSECTS

Carboxylesterases, glutathione S-transferases and cytochrome P450 mediated monooxygenases are the three principal enzymes that facilitate insects to metabolize different kind of toxins. These large enzyme families contain multiple forms with overlapping substrate specificities. Knowledge of insecticide detoxification helps in understanding the mechanism of insecticide resistance, hence development of a sound resistance management strategy. Detoxification can be divided into Phase I (Primary) and Phase II (Secondary) processes (Figure 2.1).

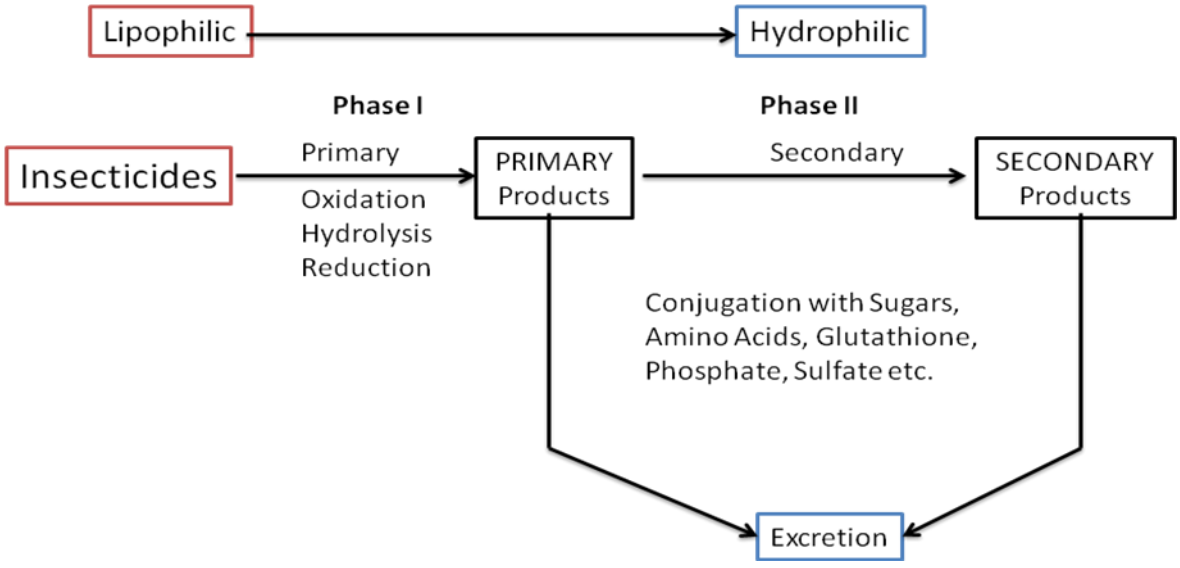

Figure 2.1 Insecticide detoxification pathways.

Phase I reactions consists of oxidation, hydrolysis and reduction. The phase I metabolites are sometimes polar enough to be excreted, but are usually further converted by phase II reactions. In phase II reactions, the polar products are conjugated with variety of endogenous compounds such as sugars, sulfate, phosphate, amino acids or glutathione and subsequently excreted. Phase I reactions are usually responsible for decreasing biological activity of the toxins and therefore the enzymes involved are rate limiting with respect to toxicity. The most important function of biotransformation is to decrease the lipophilicity of insecticides, so that they can be quickly excreted (Li *et al.*, 2007).

A. Phase I reactions:

i) Cytochrome P450 monooxygenases (E.C. 1.14.-.-):

Oxidation is considered the most important among the phase I reactions. The oxidative reactions are carried out mainly by a group of enzymes called cytochrome P450 monooxygenases [also known as Mixed Function Oxidases (MFO) or Polysubstrate monooxygenases (PSMO), microsomal oxidase, P450 enzymes]. Cytochrome P450, or *CYP* genes, constitute one of the largest family of genes, with representatives in virtually all living organisms, from bacteria to protists, plants, fungi, and animals (Werck-Reichhart and Feyereisen, 2000). An ever-growing number of P450 sequences are available, and the role of P450 enzymes is being documented in an increasing number of physiological processes (Feyereisen, 2012).

In insects, P450-monooxygenases are involved in many processes including roles in the metabolism of plant allelochemicals by herbivores, and in detoxification of insecticides. However, insecticides may also be metabolised to more active forms by the

P450-monooxygenases; bioactivation of some phosphorothioates to their corresponding "oxons" and cyclodiences to their epoxides is accomplished by P450- monooxygenases. Furthermore, some classes of insecticide synergists are also activated by P450-monooxygenases, ***e.g.*** the monooxygenase inhibitor piperonyl butoxide (PBO) (Feyereisen, 1999). The human genome carries about 57 CYP genes, and insect genomes can carry from 36 CYP genes in the body louse *Pediculus humanus* (Lee *et al.*, 2010) to 170 CYP genes in a mosquito (Arensburger *et al.*, 2010). Each P450 protein is the product of a distinct CYP gene, and P450 diversity is the result of successive gene (or genome) duplications followed by sequence divergence (Feyereisen, 2012).

The typically 45- to 55-kDa P450 proteins are heme-thiolate enzymes. Their essential common feature is the absorbance peak near 450 nm of their Fe^{II}-CO complex for which they are named (Omura and Sato, 1964). P450 enzymes are best known for their monooxygenase role, catalyzing the transfer of one atom of molecular oxygen to a substrate and reducing the other to water. The simple stoichiometry commonly describes the monooxygenase or mixed-function oxidase reaction of P450.

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP^+$$

However, oxygen atom transfer is not the only catalytic function of P450 enzymes. They also show activity as oxidases, reductases, desaturases, isomerases, etc., and collectively are known to catalyze at least 60 chemically distinct reactions (Table 2.5) (Ortiz de Montellano, 1995; Mansuy, 1998; Guengerich, 1991, 2001). The first insect P450s cloned and sequenced were CYP6A1 from *Musca domestica* (Feyereisen *et al.*, 1989); CYP4C1 from *Blaberus discoidalis* (Bradfield *et al.*, 1991); and CYP4D1 and

CYP6A2 from *Drosophila melanogaster* (Gandhi *et al.*, 1992; Waters *et al.*, 1992), as well as CYP6B1 from *Papilio polyxenes* (Cohen *et al.*, 1992). The P450 gene complement (CYPome) size of an insect genome is not a definite number (Feyereisen, 2011). Insects can survive with small CYPomes even in toxic environments. The human body louse *Pediculus humanus*, with 36 CYP genes, is highly resistant to many classes of insecticides (Lee *et al.*, 2010), and the honey bee, with just 46 CYP genes (Claudianos *et al.*, 2006), is not more sensitive than other species in a comparison to the toxicity of 62 insecticides (Hardstone and Scott, 2010). The main driver of CYPome evolution is of course gene duplication, followed by divergence (by neofunctionalization or subfunctionalization) or death (pseudogenization or deletion) (Feyereisen, 2012).

Table 2.5 Enzymatic Reactions Catalyzed by Insect P450 Enzymes (adapted from Feyereisen, 2005).

Reaction catalyzed	***P 450***
Oxidase activity O_2 to H_2O, H_2O_2, O_2^-	CYP6A1(and probably most P450 enzymes)
Aliphatic hydroxylation C-H hydroxylation	CYP4C7, CYP6A1, CYP6A2, CYP6A8, CYP6G1, CYP6M2, CYP6CM1vQ, CYP9T2, CYP12A1, CYP18A1, CYP302A1 CYP306A1, CYP312A1, CYP314A1, CYP315A1
O-dealkylation	CYP6A1, CYP6D1, CYP6A5, CYP6B4, CYP6B17, CYP6B21, CYP6G1, CYP6Z2, CYP6CM1vQ, CYP9A12, CYP9A14, CYP12A1, CYP321A1,
dehalogenation	CYP6G1
Epoxidation	CYP6A1, CYP6A2, CYP6B8, CYP6B27, CYP6AB3, CYP6 CYP6AB11, CYP9E1, CYP12A1, CYP15A1, CYP321A1
Aromatic hydroxilation	CYP6D1, CYP6G1, CYP6M2

Heteroatom oxidation and dealkylation Phosphorothioate ester oxidation	 CYP6A1, CYP6A2, CYP6D1, CYP12A1
N-dealkylation	CYP6A5, CYP12A1
N-oxidation	+(nicotine)
S-oxidation	+(phorate)
Aldehyde oxidation	CYP18A1
Complex and atypical reactions Cyanogenic glucoside biosynthesis: (1) Val/Ile to oximes (2) Oximes to cyanohydrins Aryl ether cleavage Carbon-carbon cleavage Decarbonylation with c-c cleavage Aromatization Reduction Endoperoxide isomerization	 CYP405A2 CYP332A3 CYP6M2 +?(sterols, ecdysteroid) CYP4G1 +(defensive steroids) - -

ii) Carboxylesterases (EC 3.1.1.1):

Carboxyl-esterase or esterase is a collective term for the enzymes which hydrolyses carboxylic esters (Hemingway and Karunaratne, 1998). Classification of these enzymes is difficult because of their overlapping substrate specificity (Heymann and Jakoby, 1980). However, the esterase classification of Aldridge (1953) is generally recognized. According to that classification, esterases inhibited by paraoxon in a progressive and temperature-dependent manner are called B esterases and those which are not inhibited are A esterases (Aldridge, 1993). Some A esterases can hydrolyse OPs, through an acylated cysteine in their active site, and are termed phosphoric triester hydrolases (EC 3.1.8.) (Reiner, 1993; Walker, 1993). The term carboxylesterase is now mainly attributed to B esterases (Reiner, 1993; Walker, 1993). These enzymes have an active site serine residue, hence the terms B esterase and serine hydrolase are synonymous. Insecticides

such as organophosphate, carbamates, pyrethroids and some juvenoids which contain ester linkages are susceptible to hydrolysis. Estrerases are hydrolases that split ester compounds by the addition of water to yield an acid and alcohol.

$$R^1COOH + H_2O \longrightarrow R^1COOH + ROH$$

Esterases that metabolize organophosphates can be divided into three groups: A-esterases which are not inhibited by organophosphates but hydrolyze them; B-esterases, which are susceptible to organophosphate inhibition; and C-esterases which are uninhibited by organophosphates and do not degrade them (Yu, 2008).

There are two types of esterases that are important in metabolizing insecticides, namely, carboxylesterases and phosphatases (also called phosphorotriester hydrolases or phosphor-triesterases). Carboxyl esterases, which are B-esterases, play a significant role in degrading organophosphates, carbamates, pyrethroids, and some juvenoids in insects. The best example is malathion hydrolysis, which yields both α- and β-monoacids and ethanol (Yu, 2008). Phosphatases are A-esterases that detoxify many organophosphorous insecticides especially phosphates in insects. In houseflies, paraoxon can be hydrolyzed to diethyl phosphoric acid and *p*-nitrophenol. Phosphatases also hydrolyze the alkyl groups of organophosphates. Paraoxon is hydrolyzed by the enzyme in houseflies. Several amide containing organophosphorous insecticides such as dimethoate and acephate have been shown to be hydrolyzed by carboxylamidases to their corresponding carboxylic acid derivatives (Yu, 2008).

B. Phase II Reactions:

Phase I reactions with xenobiotics in the addition of functional groups such as hydroxyl, carboxyl and epoxide. These phase I products can further undergo conjugation reactions with endogenous molecules. These reactions are called phase II reactions. The endogenous molecules include sugars, amino acids, glutathione, phosphate and sulfate. Conjugation products are usually more polar, less toxic and more readily excreted than their parent compounds. Thus the process with only a few exceptions results in detoxifications.

Three types of conjugation reactions occur in insects. Type I requires an activated conjugating agent that then combines with the substrate to form the conjugated product. Type II involves the activation of the substrate to form an activated donor that combines with an endogenous molecule to yield a conjugated product. In the Type III, conjugation can proceed directly between the substrate and the conjugating agent without involving activation. Thus, Type I and II require formation of high-energy intermediates before conjugation reactions proceed. The chemical groups required for Type I are –OH, NH_2, COOH and SH (glucose conjugation, sulfate conjugation and phosphate conjugation); for Type II COOH (amino acid conjugation); and for Type III, halogens, alkenes, NO2, epoxides, ethers and esters (glutathione conjugation).

i) Glutathione S-transferases (EC 2.5.1.18.):

Glutathione conjugations are performed by a group of multifunctional enzymes known as glutathione S-transferases (GSTs) and are involved in detoxification mechanisms of many molecules. GSTs are involved in the transport of physiologically important

lipophilic compounds. These enzymes catalyze reactions in which the sulfur atom of glutathione provides electron for nucleophilic attack on a second electrophilic substrate the later can be endogenous natural substrates such as epoxides, organic hydroperoxides, or activated alkenals resulting from oxidative metabolism. These enzymes catalyze the conjugation of reduced glutathione (GSH) with electrophilic substrates. Glutathione S-transferases perform a variety of reactions including…

1. The S-alkylation of GSH by alkyl halides and related compounds;
2. The replacement of labile aryl halogen or nitro groups by GSH;
3. The replacement of labile arakyl halogen and ester groups by GSH;
4. The addition of GSH to various epoxides;
5. The addition of GSH to α, β-unsaturated compounds including aldehydes, ketones, lactones, nitriles and nitro compounds; and
6. The *O*-alkyl and *O*-aryl conjugation of phosphorothiotes and phosphates with GSH.

The glutathione conjugate is subsequently transformed to mercapturic acid through the stepwise loss of glutamic acid and glycine to a cysteine conjugate, which is finally acetylated before excretion. Because of their broad substrate specificities, glutathione S-transferases are responsible for the detoxification of numerous xenobiotics (Yu, 1996). More than 40 GST genes have been identified in insects (Ranson *et al*, 2002). Mammalian GSTs have been classified into eight cytosolic classes (Alpha, Mu, Pi, Theta, Sigma, Zeta, Kappa, and Omega) and a microsomal class on the basis of their amino acid sequence, immunological properties, and substrate specificity. Each class share 40% or higher amino acid identity (Chelvanayagam *et al.,* 2001). The classification of insect

GST is not clear. The majority of insect GSTs do not belong to mammalian classes. Insect glutathione S-transferases consist of two subunits (homodimers and heterodimers) of molecular weight between 19 and 35 kd. Two class of insect GSTs (Class I and Class II) were reported (Fournier *et al.,* 1992) which have been referred to as the Delta and Sigma class respectively. Recently a new class of insect GSTs, referred to as Epsilon has been described in several species of insects including *Anopheles gambiae* (Ranson *et al.,* 2001). Purified cytosolic and microsomal glutathione S-transferases isozymes from fall armyworm larvae all possessed cumene hydrperoxide peroxidase (Yu, 2002). A Delta class GST purified from German cockroaches also showed high peroxidase activity (Ma and Chang, 2007). The name of each GST is composed of the initials of the species scientific name, followed by the acronym GST, a capital letter to designate the class name and an Arabic number for the individual protein, such as AgGSTD2.

Glutathione S-transferases are important in the metabolism of organophosphorous insecticides resulting in detoxification (Ranson and Hemingway, 2005; Che-Mendoza *et al.,* 2009). For example methyl parathion is dealkylated by glutathione S-tranferases to form desmethyl parathion and methyl glutathione (Yu, 2008). On the other hand parathion can be de arylated by glutathione S-transferases to produce diethyl phosphorothioic acid and *S*-(*p*-nitrophenyl) glutathione (Yu, 2008). Interestingly, a glutathione S-transferase isozyme from the housefly exhibits DDT-dehydrochlorinase activity, showing that DDT-dehydrochlorinase (DDTase) is one of glutathione S-tranferase (Clark and Shamaan, 1984). DDT-dehydrochlorinase converts DDT to DDE, resulting in detoxification (Yu, 2008).

Detoxifying Enzymes and Insecticide Metabolism:

The metabolism of insecticides by P450 enzymes is very often a key factor in determining toxicity to insects and to non-target species. The importance of monooxygenases in insecticide resistance became evident in the early 1960s, when it was shown that resistance to carbaryl could be abolished by the P450 inhibitor sesamex (Eldefrawi *et al.*, 1960). Additional evidence of monooxygenase based resistance quickly amassed (Georghiou and Metcalf, 1961; Schonbrod *et al.*, 1965). Monooxygenase-mediated detoxification is frequently found as a major mechanism of resistance, and unlike target site resistance, detoxification has the potential to confer cross-resistance to toxins independent of their target sites (Agosin, 1985; Oppenoorth, 1985; Scott, 1991). Most cases of monooxygenase-mediated resistance result from an increase in detoxification. However, in cases where the parent insecticide must undergo monooxygenase-mediated bioactivation, as is the case for many organophosphates, it is also possible that resistance could be achieved through decreased activation. Although this has been reported (Konno and Dauterman, 1989), it does not appear to be a common mechanism of resistance. This may explain why esterases are relatively more common than monooxygenases in resistance to some organophosphates (Oppenoorth, 1985; Scott, 1991). The classical example is probably the metabolism of phosphorothioate insecticides. In many cases, the active ingredients of organophosphorus insecticides are phosphorothioate (P=S) compounds (also known as phosphorothionates), whereas the molecule active at the acetylcholinesterase target site is the corresponding phosphate (P=O) (Figure 2.2).

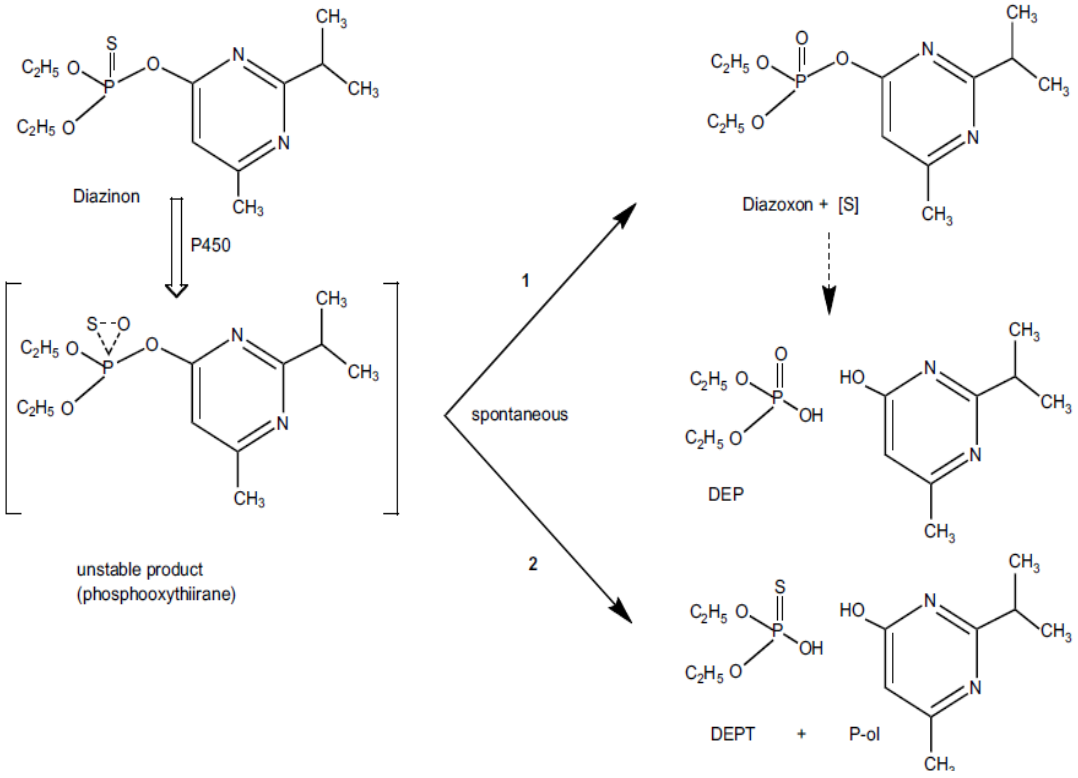

Figure 2.2 Metabolism of diazinon by cytochrome P450. Following an insertion of oxygen into the substrate, a reactive intermediate collapses (1) by desulfuration, or (2) by cleavage of the ester linkage. DEP, diethylphosphate; DEPT, diethylphos-phorothioate; P-ol, 2-isopropoxy-4-methyl-6-hydroxypyrimidine; [S], reactive form of sulfur released during the reaction. Adapted from Feyereisen, 2012.

P450 enzymes that metabolize OPs can metabolize other insecticides as well, and this sometimes leads to potentially useful interactions. Thus enhanced detoxification of dicofol in spider mites can lead to enhanced chlorpyriphos activation, and hence negative cross-resistance (Hatano *et al.*, 1992). Similarly, permethrin resistance in horn flies is suppressible by piperonyl butoxide, and negatively related to diazinon toxicity (Cilek *et al.*, 1995). In *H. armigera* populations from West Africa, triazophos shows negative cross-resistance with pyrethroids, and in this case the synergism shown by the OP towards the pyrethroid appears due to an enhanced activation to the oxon form (Martin *et*

al., 2003). Organophosphorus compounds such as disulfoton and fenthion can also be activated by thioether oxidation (formation of sulfoxide and sulfone). Pyrethroid metabolism by P450 enzymes is a well known in insects. Hydroxylations and further metabolism make pyrethroid metabolism and has shown for the single enantiomer of deltamethrin (Figure 2.3).

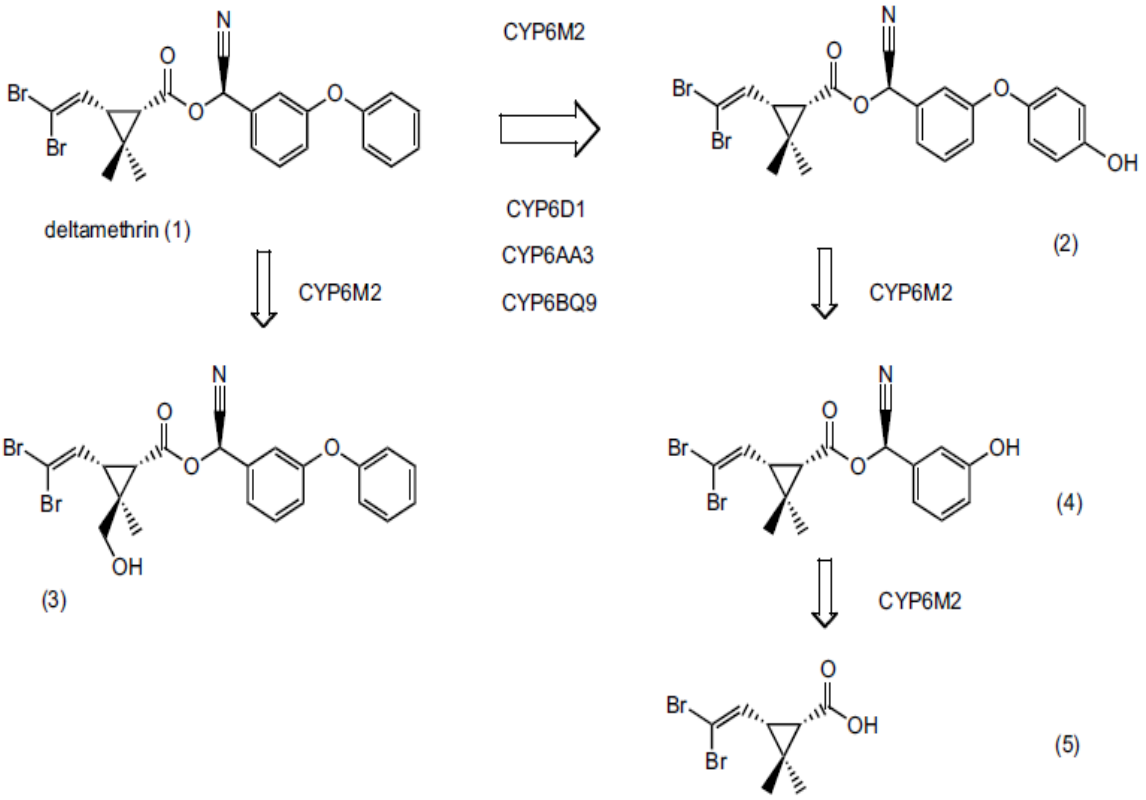

Figure 2.3 Metabolism of deltamethrin by insect P450 enzymes: (1) deltamethrin; (2) 4′ hydroxydeltamethrin; (3) *trans*hydroxymethyl-deltamethrin; (4) cyano (3-hydroxyphenyl) methyl deltamethrate; (5) deltamethric acid. Adapted from Feyereisen, 2012.

The now banned cyclodiene insecticides aldrin, heptachlor, and isodrin are epoxidized by P450 enzymes to the environmentally stable, toxic epoxides, dieldrin, heptachlor epoxide, and endrin respectively (Drabek and Neumann, 1985). Recombinant CYP6A1, -A2, -A8, -B8, and -B27, CYP12A1, and CYP321A1 can catalyze these epoxidations. Examples of

pro-insecticide metabolism include the activation of chlorfenapyr by N-dealkylation (Black *et al.*, 1994), and of diafenthiuron by S-oxidation (Kayser and Eilinger, 2001). In each case, the insect P450-dependent activation is a key in the selective toxicity of these pro-insecticides that target mitochondrial respiration. Recombinant house fly CYP6A1 catalyzes the activation of chlorfenapyr (Figure 2.4).

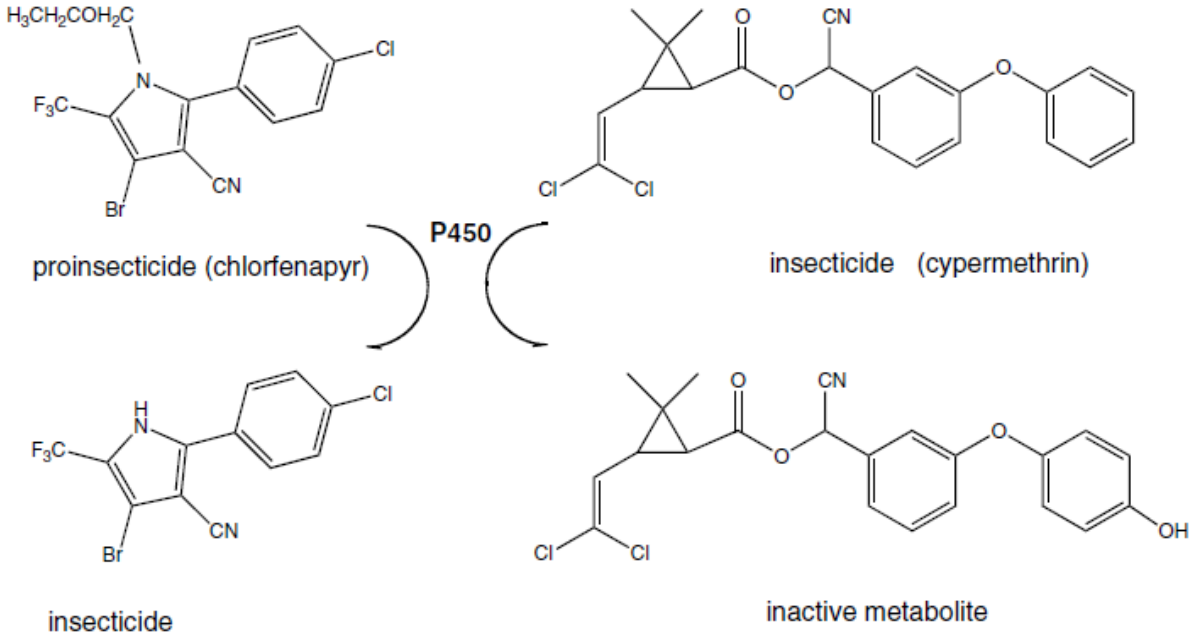

Figure 2.4 Chlorfenapyr and cypermethrin metabolism. The same P450 in *Heliothis virescens* probably activates the pyrrole and inactivates the pyrethroid, resulting in negative cross-resistance. Adapted from Feyereisen, 2012.

In *H. virescens*, toxicity of chlorfenapyr is negatively correlated with cypermethrin toxicity (Pimprale *et al.*, 1997). The metabolism of imidacloprid is also of interest, particularly in relation to resistance. Piperonyl butoxide can synergize the toxicity of imidacloprid, and two P450 enzymes, CYP6G1 of *D. melanogaster* and CYP-6CM1vQ of *Bemisia tabaci*, have been shown to metabolize this neonicotinoid (Joussen *et al.*, 2008; Karunker *et al.*, 2009) (Figure 2.5).

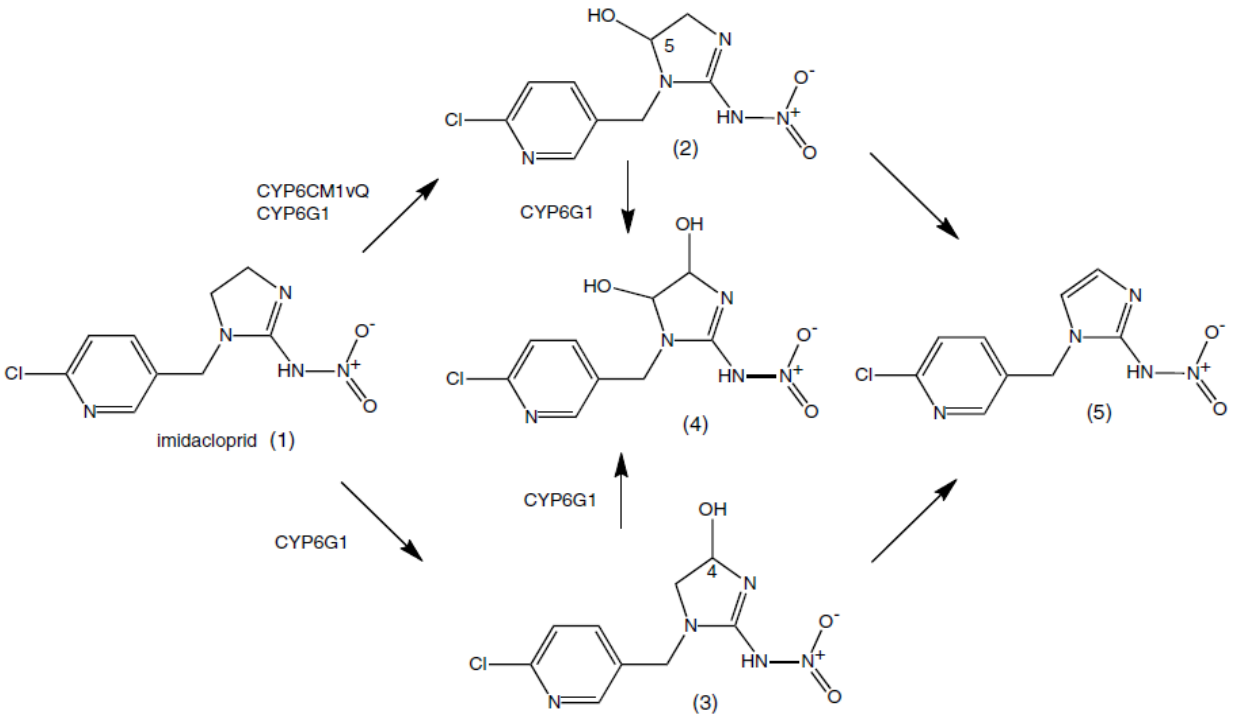

Figure 2.5 Metabolism of imidacloprid by insect P450 enzymes: (1) imidacloprid; (2) 5-hydroxyimidacloprid; (3) 4-hydroxyimidacloprid; (4) dihydroxyimidacloprid; (5) Non-enzymatically derived dehydroimidacloprid. Adapted from Feyereisen, 2005.

Hydroxylations at the 4 and 5 positions can lead to the olefinic metabolite or to the dihydroxylated metabolite. In the whitefly the 5-hydroxy metabolite is not toxic, but the 4-hydroxy metabolite is as toxic as the parent compound, so region selectivity may be of importance.

Table 2.6 Over expressed CYP genes in Insecticide-Resistant Strains.

Species	P450 Overexpressed	Resistance Pattern	Reference
Musca domestica	CYP6A1	OP, Carbametes	Feyereisen *et al.*,1989
		IGR	Carino *et al.*, 1992; Sabourault *et al.*, 2001
	CYP6A5v2, CYP6A36	Pyrethroids	Zhu and Liu, 2008; Zhu *et al.*, 2008
	CYP6D1, CYP6D3	Pyrethroids	Liu and Scott, 1996; Kasai and Scott, 2001a,b
	CYP6D1	Pyrethroids	Kasai and Scott, 2000
	CYP6D1, CYP6D3v2	Pyrethroids	Kamiya *et al.*, 2001
	CYP6A24	Pyrethroids	Shono *et al.*, 2002
	CYP12A1	Pyrethroids	Guzov *et al.*, 1998
Drosophila melanogaster	CYP6A2	DDT, Malathion	Waters *et al.*, 1992; Maitra *et al.*, 2000
	CYP6A8	Malathion	Maitra *et al.*, 1996;
		Malathion	Maitra *et al.*, 2000
		DDT	Le Goff *et al.*, 2003
	CYP6G1	DDT	Daborn *et al.*, 2001,2002
		DDT	Catania *et al.*, 2004
		Lufenuron, propoxur	Daborn *et al.*, 2002
		Imidacloprid	Daborn *et al.*, 2002
		Imidacloprid	Catania *et al.*, 2004
		DDT	Brandt *et al.*, 2002
		Diazinon	Pyke *et al.*, 2004
	CYP12D1/2	DDT	Brandt *et al.*, 2002
		DDT	Le Goff *et al.*, 2003
	CYP12A4	Lufenuron	Bogwitz *et al.*, 2005
Drosophila simultans	CYP6G1	DDT, Imidacloprid, Malathion	Le Goff *et al.*, 2003
Anopheles gambiae	CYP6Z1	Pyrethroids	Nikou *et al.*, 2003
	CYP325A3	Pyrethroids	David *et al.*, 2005
	CYP6M2, CYP6P3	Pyrethroids	Djouaka *et al.*, 2008
	CYP6P3	Permethrin	Muller *et al.*, 2008a,b
	CYP6M2, CYP6Z2	Permethrin	Muller *et al.*, 2007
	CYP4C27, CYP4H15	DDT	Vontas *et al.*, 2005
	CYP6Z1,2, CYP12F1, CYP314A1	DDT	David *et al.*, 2005
		DDT	
A. stephensi	CYP325C1	Pyrethroids	Vontas *et al.*, 2007
A. funetus	CYP6P4, CYP6P9	Pyrethroids	Amenya *et al.*, 2008; Wondji *et al.*, 2009
Aedes aegypti	CYP9J10,27,32	Pyrethroids	Strode *et al.*, 2008
	CYP9M10	Permethrin	Hardstone *et al.*, 2010

Culex pipiens quinquefasciatus	CYP6F1	Permethrin	Kasai *et al.*, 2000
	CYP4H34, CYP6Z10, CYP9M10	Permethrin	Komagata *et al.*, 2010
	CYP4H21, H22, H23, CYP4J4, CYP4J6	Deltamethrin	Shen *et al.*, 2003
Heliothis virescens	CYP9A1	Thiodicarb	Rose *et al.*, 1997
Helicoverpa zea	CYP6B8,B9	Cypermethrin	Hopkins *et al.*, 2010
H. armigera	CYP4G8	Pyrethroids	Pittendrigh *et al.*, 1997
	CYP6B7	Pyrethroids	Ranasinghe and Hobbs, 1998
	CYP6B7, CYP9A12, CYP9A14	Pyrethroids	Yang *et al.*, 2006
	CYP4S1, CYP337B1	Fenvalerate	Wee *et al.*, 2008
	CYP4L5,11, CYP4M6,7, CYP6AE11, CYP9A14, CYP332A1, CYP337B1	Deltamethrin	Brun-Barale *et al.*, 2010
Plutella xylostella	CYP6BG1,	Cypermethrin	Bautista *et al.*, 2007
	CYP4M20	Cypermethrin	Baek *et al.*, 2009
Lygus lineolaris	CYP6X1	Permethrin	
Bemisia tabaci	CYP6CM1vQ	Imidacloprid	Karunker *et al.*, 2008
Nilaparvata ligens	CYP6ER1	Imidacloprid	Bass *et al.*,2011
Myzus persicae	CYP6CY3	Neonicotinoids	Puinean *et al.*, 2010
Diabrotica virgifera	CYP4	Me-parathion	Scharf *et al.*, 2001
Tribolium castaneum	CYP6BQ8,9,10, CYP436B1, B2	Deltamethrin	Zhu *et al.*, 2010
Blattella germanica	P450MA,	Chlorpyriphos	Scharf *et al.*, 1999
	CYP4G19	Pyrethroids	Pridgeon *et al.*, 2003

Despite the continuous use of insecticides there are repeated failures in controlling the sucking insect pest species in recent years (Rahaman *et al.*, 2005; Gurusubramanian *et al.* 2008a; Roy *et al.* 2008b) in different conventional tea plantations of Terai, the Dooars

and Darjeeling foothill regions. Such a failure occurs due to change in the susceptibility level of the pest species to the applied insecticides. Susceptibility level changes mainly due to metabolic detoxification of the insecticides through higher level of activity of some insecticide detoxifying enzymes under the stress of different management practices (Sarker and Mukhopadhyay, 2003, 2006a,b; Saha *et al.*,2008, 2010, 2012a,b,c,f; Saha and Roy, 2012). In another mirid pest, *Lygus lineolaris*, metabolic resistance to insecticides due enhanced level of activity has been reported (Zhu *et al.*, 2004, 2011).

In Western Flower Thrips, *Frankniella occidentalis*, metabolic detoxification of insecticides has been reported (Ferrari *et al.*, 1993, Immaraju *et al.*, 1992). In *Bemisia tabaci*, metabolic resistance due to enhanced activity of insecticide resistance related enzymes has been reported (Kang *et al.*, 2006; Alon *et al.*, 2008; Vassiliou *et al.*, 2011).

Host Allelochemicals, Induction of Detoxifying Enzymes and Insecticide Resistance:

Understanding the diversity of insect responses to chemical pressures (plant allelochemicals and insecticides) in their local ecological context represents a key challenge in developing sustainable pest control strategies. Plants and insects have had coexisting relationships for a long time. Insects were suppressed either by other insects or toxins or by plant defense mechanisms in order to create a balance between the insect pest population and host. Each plant species has a unique set of defense traits ranging from morphological to phytochemical parameters that have behavioral and physiological ramifications for a potential herbivore consumer (Wink and Waterman, 1999; Wink, 2003). Therefore the resistance mechanisms evolved by insects to deal with the chemical

defences of plants are similar to those mechanisms that have evolved to resist synthetic insecticides. The chemical structure of some synthetic insecticides is comparable to that of some plant produced compounds (e.g. pyrethroids and nicotinoids). Insect resistance to plant allelochemicals interferes with their resistance to synthetic insecticides (Yu and Ing, 1984). From the evolutionary perspective, despite the key role of the chemical 'arms race' in driving the co-evolution of plants and insects, much research has focused so far on describing the diversity of plant chemicals and their effects on herbivores. So, the understanding of the insecticide resistance mechanisms as well as taking into account other ecological parameters is important in predicting the spread of insecticide resistance in natural pest populations, and in choosing the optimum strategy for managing insect pest populations. Less is known about the multiple mechanisms evolved by insects to overcome these chemical defences (Table 2.7). These mechanisms include contact and ingestion avoidance, excretion, sequestration, degradation of the toxin and target site mutation.

Table 2.7. Plant allelochemicals and associated resistance mechanisms in insects.

Plant Allelochemicals	Target (Mechanism of effect)	Resistance mechanisms	Species (Reference)
Alkaloids	Neuroreceptors (inhibition); ion channels (antagonists);nucleic acids (disruption of DNA synthesis); feeding (deterrent owing to bitterness); enzymes (inhibition)	Modification of nicotine synthesis by salivery glucose oxidase	*Helicoverpa zea* (Lep.) (Musser *et al.*, 2002)
Cardenolides	Nervous system (depressing activity); Na^+,K^+-ATPase (specific inhibitor);	Canal trenching behavior Target-site mutation	*Danaus plexippus* (Lep.) (Helmus and Dussourd, 2005) *Chrysochus sp* (Col.) (Labeyrie and Dobler, 2004)
Cyanogenic glycosides	Electron transport (inhibition of mitochondrial cytochrome oxidase)	Ingestion avoidance, sequestration and detoxification	*Schistocerca americana* (Ort.); *Hypera brunneipennis* (Col.); *Zygaena sp.* (Lep.); *Clossiana euphrosyne* (Lep.); *Heliconius sara* (Lep.) (Zagrobelny *et al.*, 2004)
Glucosino-lates	Respiration (inhibition)	Detoxification by GSTs, Detoxification by a glucosinolate sulfatase, formation of nitriles instead of isothiocyanate detoxification by P450s, detoxification by N-oxidation and sequestration	*Myzus persicae* (Hem.) (Francis *et al.*, 2005); *Plutella xylostella* (Lep.) (Ratzka *et al.*, 2002); *Pieris rapae* (Lep.) (Wittstock *et al.*, 2004); *Drosophila melanogaster* (Dip.) (Fogleman, 2000); *Estigmene acrea* (Lep.) (Hartmann *et al.*, 2005); *Tyria jacobaeae* (Lep.) (Naumann *et al.*, 2002).

Flavonoids and phenolic acids	Respiration (inhibition); growth (inhibition)	Ingestion avoidance, Decease of toxin levels in gall tissue, glycosylation by UDP-glycosyl-transferase; sequestration and/or excretion	*Maduca sexta* (Lep.) (Glendinning, 2002); *Potania sp* (Hym) (Nymann and Julkunen-Tiitto, 2000); *Bombyx mori* (Lep.) (Luque *et al.,* 2002).
Iridoid glycosides	feeding (deterrent owing to bitterness); nucleic acids (inhibition of DNA polymerase); proteins (denaturant and cross-linking activities)	sequestration	*Longitarsus sp*. (Col.) (Willinger and Dobler, 2001)
Coumarins and Furanocouma-rins	Nucleic acids (photoactivie DNA bonding); Pro-oxidant activity	Detoxification by P450s, Detoxification by GSTs,	*Papilio polyxenes* (Lep.) (Peterson *et al.,* 2001); *Despressaria pastinacella* (Lep.) (Nitao *et al.,* 2003); *Spodoptera frugiperda* (Lep.) (Yu, 2002).
Protease inhibitors	Digestive system (inhibition of protease)	Overexpression of insensitive protease	*Callosobruchus maculates* (Col.) (Moon *et al.*, 2004);
Terpinoids	Nervous system (inhibition of acetyl – choline esterases); feeding (deterrent owing to physical barrier and bitterness);groth and development inhibitor (pheromone analog)	Repression of genes involved in biosynthetic pathways	*Spodoptera exigua* (Lep.) (Bede *et al.,* 2006).
Tannins	Feeding (complexation of salivary and gut proteins); pro-oxidant activity	Synthesis of anti-oxidant compounds	*Orgyia leucostigma* (Lep.) ((Barbehenn *et al.*, 2003).

Lep.: Lepidoptera; Col.: Coleoptera; Ort.: Orthoptera; Hym.: Hymenoptera; Dip.: Diptera; Hem.: Hemiptera.

Biotransformation of plant toxins is one of the major weapons that insects have evolved in their co-evolutionary arms race with plants (Berenbaum, 2002). To date metabolic resistance to plant chemicals has been identified not only in herbivorous insect (Glendinning, 2002), but also in detrivorous insects such as mosquito larvae feeding plant debris (Meyran *et al.*, 2002). Metabolic resistance often results from overproduction of 'detoxification enzymes' that can metabolize plant xenobitics (allelochemicals). This mechanism is often associated with phenotypic plasticity, as the production of detoxification enzymes is usually induced by the presence of plant xenobitics in the diet of the insect.

Induction of insect detoxifying enzyme activities by plant allelochemicals is a clear manifestation of biochemical phenotypic plasticity and has been documented in several instances. Many of the theories and some of the experiments implicitly or explicitly deal with the insect's ability to metabolize plant secondary metabolites by P450 and other enzymes. In the case of detoxification, however, the landmark paper of Krieger *et al.* (1971) can be seen as echoing the Fraenkel (1959) paper, by exposing the role of P450 enzymes. They stated that "higher activities of midgut microsomal oxidase enzymes in polyphagous species than in monophagous species indicates that the natural function of these enzymes is to detoxify natural insecticides present in the larval food plants." In that 1971 study, aldrin epoxidation was measured in gut homogenates of last instar larvae from 35 species of Lepidoptera. Polyphagous species had on average a 15-times higher activity than monophagous species. This trend was seen in sucking insects as well. A 20-fold lower aldrin epoxidase activity was found in the oleander aphid *Aphis nerii* when compared to the potato aphid *Myzus euphorbiae* or to the green peach aphid *Myzus*

persicae (Mullin, 1986). The former is a specialist feeder on two plant families, Asclepiadaceae and Apocyanaceae, whereas the latter two are generalists found on 30–72 plant families. The concept extended to other detoxification enzymes– for example, in mites, where the predatory mite has a five times lower aldrin epoxidase activity than its herbivorous prey (Mullin *et al.,* 1982). The toxicity of the natural phototoxin α-terthienyl is inversely proportional to the level of its metabolism in Lepidoptera, and is related to diet breadth. Metabolism is highest in *Ostenia nubilalis*, which feeds on numerous phototoxic Asteraceae; lower in *Helicoverpa virescens*, which has a broad diet, including some Asteraceae that are non-phototoxic; and lowest in *Manduca sexta*, a specialist of Solanaceae (Iyengar *et al.*, 1990).

H. theivora, E flavescens and *S. dorasalis* all are polyphagous in nature. *H. theivora* feeds on at least sixteen different plant families (Roy, 2010; Gogoi *et al.,* 2011; Saha *et al.*, 2012d). Similarly, *E flavescens* is also polyphagous (DeLong, 1971; Saha *et al.*, 2012e). *S. dorasalis* has been documented to attack more than 150 hosts from at least 40 different plant families (Mound and Palmer, 1981; Bournier, 1999; Saha *et al.*, 2012b; Saha and Mukhopadhyay, 2013). So, these pests are exposed to wide variety of plant allelochemicals of diverse groups having potential to induce the activity of this resistance related enzymes. Saha *et al.* (2012d) reprted higher level of detoxifying enzymes activity in *H. theivora* when reared on two alternative hosts i.e. *Mikania micrantha* (Asteraceae) anmd *Psidium guajava* (Myrtaceae), than on tea.

The conceptual framework of Krieger and colleagues has been challenged (Gould, 1984) and defended (Ahmad, 1986). An alternative view (Berenbaum *et al.,* 1992) proposes that aldrin epoxidation represents "P450s with broad substrate specificity [that]

is most abundant in insects that encounter a wide range of host plant metabolites." The ability to induce P450 enzymes and deal with a wide range of toxic chemicals in the diet has been thought to present a "metabolic load" for polyphagous species, with specialists restricting their "detoxification energy" to one or a few harmful substrates (Whittaker and Feeny, 1971). However, careful studies in both oligophagous and polyphagous species have refuted the concept of induction as imposing a metabolic load (Neal, 1987; Appel and Martin, 1992). Global approaches to the comparison of generalist and specialist herbivores feeding on plants with higher or lower levels of chemical defenses are now available, as shown by the elegant studies of Govind *et al.* (2010) where the transcriptional responses of neonates of the specialist *M. sexta* and generalist *H. zea* fed for 24 hours on *Nicotiana attenuata* plants, wild type or progressively suppressed in their jasmonate response, were compared by microarrays. The metabolism of plant toxins by insects has been reviewed extensively (Brattsten, 1979a; Ahmad, 1986; Ahmad *et al.*, 1986; Mullin, 1986; Yu, 1986; Li *et al.*, 2007). Many P450 inducers are known in insects (Agosin, 1985; Hodgson, 1985; Scott *et al.*, 1998) and it appears that several of these inducers alter the expression of different P450s (Scott *et al.*, 1998).

In addition to insecticides, insect hydrolases also metabolize allelochemicals glycosides. B-glucosidase is active toward a variety of glucosides in fall armyworms, corn earworms, cabbage loopers, and valvetbean caterpillars. The *p*-nitrophenyl β-D-glucoside, 4-methyl umbelliferyl β-D-glucoside, D (+)-cellobiose, D-amygdalin, and helicon were preferred substrates whereas sinigrin, phloridzin, α-solanine, tomatine and linamarin were poor substrates. There was no correlation between degree of herbivore polyphagy and β-glucosidase activity among these species (Yu, 1989). B-Glycosidases

have been shown to play important roles in the survival of certain phytophagous insects. The ability of peach tree borer, *Synanthedon exitiosa* larvae to survive well on prunasin containing peach tree is because they can metabolize cyanogenic glycosides through β-glucosidase and detoxify the released cyanide by β-cyanoalanine synthase, thereby allowing them to utilize peach trees (Reilly *et al.*, 1987). Another example is the larvae of the tiger swallowtail, *Papilio glaucus*, which feed on quacking aspen, which contain various phenolics glycosides (e.g. salicortin). These larvae hydrolyze the glycosides by β-glucosidase and detoxify the released phenolics aglycone by a highly active esterase, thereby allowing them to survive on aspen (Lindroth *et al.*, 1988; Lindroth, 1989).

Glutathione S-trnaferases are also involved in the metabolism of toxic allelochemicals including α, β-unsaturated carbonyl compounds (e.g. *trans*-cinnamaldehyde, trans-2-hexanal), isothio-cyanates (e.g. allyl isothiocyanate, benzyl isothiocyanate), and organothiocyanates (e.g. benzyl thiocyanate) in lepidopteran insects. These transferase activities in the specialist velvetbean caterpillar are lower than in the generalist fall armyworm; the activity towards the isothiocyanates in the crucifer-adapted cabbage looper was 2- to 6-fold higher than that in the fall armyworm. The results suggest that glutathione S-transferases play an important role in allelochemicals resistance in phyto-phagous Lepidoptera (Wadleigh and Yu, 1987, 1988). Many allelochemicals are found to be potent inhibitors of glutathione S-transferases in insects (Yu and Abo-Elghar, 2000). Many flavonoids, otherphenols, and α, β-unsaturated carbonyl compounds are also found to be potent inhibitors of the enzymes.

Over the four hundred million years of co-evolution with plants, phytophagous insects have developed diverse resistance mechanisms to cope with plant chemical

defences. Because insects face a geographical mosaic of chemical environments, from non-toxic to highly toxic plants, the costs associated with resistance traits vary with the probability of encountering a toxin. Moreover, other selection pressures, such as the presence or absence of competitors and predators, can also influence the costs and selection of particular resistance traits. Thus, the complexity of the local community composition is a key factor in maintaining the diversity of adaptive mechanisms to plant xenobiotics. These mechanisms are more plastic and complex compared with those involved in resistance to insecticides, perhaps because environments in which insecticides are heavily used also tend to have communities of low diversity and complexity. However, because some detoxification enzymes are involved in plant toxins and insecticides metabolism, cross-resistance mechanisms can be predicted to be observed under specific environmental conditions. Deciphering the impact of allelochemicals in cross-resistance mechanisms with insecticides at a local scale, and comparing the molecular and evolutionary mechanisms of resistance to phytotoxins and synthetic insecticides, represents promising areas of research for developing longterm sustainable insect control strategies for the effective management of pest concern.

Genetic Variability in Sucking Pests:

Earlier, common visible markers including mophometrices, eye colour, body spots or bands and hairs or spines, wing venation were used as phenotypic markers in studying pattern of dispersal, mating behaviour, population variability and inheritance of genetic traits in insects (Bartlett *et al.*, 1968; Bartlett and Butler, 1975). Although the phenotypic markers are found at all time of life span of the organism and can be readily used for

studies in field conditions, they suffer from many practical limitations. The major drawback is that these visible phenotypes are relatively infrequent and often hard to score. Because the phenotype markers are rare, use of these markers in mapping a trait is difficult. For all such difficulties and with the concurrent advancement in biochemical methodologies, protein markers then became more popular. Protein markers made a significant contribution in the early periods when DNA technologies were not so much advanced as it is now (Loxdale and Lushai, 1998). A diverse range of novel molecular (DNA) markers are now available for entomological investigations. Now a day's both DNA and protein markers have revolutionized the biological sciences and have enhanced many fields of insect study, especially ecological entomology.

Insecticide resistance is the result of an increase in the ability of individuals of an insect species to survive insecticide application and is an important example of man-driven evolution (Daly, 1993). Alleles conferring resistance may arise and spread in populations and to other populations with variable success, depending on factors such as selective forces, genetic variability, gene flow, population size and environmental conditions. Studies that map the population structure of pest insects, as well as the potential for gene flow between populations, are needed to understand the development of resistance and prevention of its spread (Roush and Daly, 1990; Labbe *et al.*, 2005). Development of resistance is often rapid in isolated populations that have been treated by insecticides (Denholm *et al.*, 1985). The rate of development of insecticide resistance may, however, be influenced by gene flow between treated and untreated populations by maintaining the frequency of resistance alleles at a low level (Lenormand, 2002). Whitehead *et al.* (2003) showed that contaminant-exposure was a poor predictor of

population structure and the level of gene flow was a better predictor of relatedness. Gene flow may balance divergence by opposing the effect of selection pressures (Lenormand, 2002). Population genetic patterns should therefore be investigated with reference to geographical variability, as well as selection pressure. Detoxification resistance occurs when enhanced levels or modified activities of biotransformation enzymes prevent the insecticide from acting on its site of action because the metabolites produced have little or no activity compared with the original substance (Brogdon and McAllister, 1998). These changes may be due to mutations resulting in a protein with slightly different properties or altered expression.

As chemical control is frquently used to avoid economic damage, the sucking insects have been subjected to major selection pressure. Insecticides will probably continue to be the main control method in the near future and therefore it is important to study the sucking insects population structure and change in insecticide susceptibility. There are several techniques for estimating genetic diversity such as randomly amplified polymorphic DNA analysis, microsatellites, minisatellites, restriction fragment length polymorphism analysis, and amplified fragment length polymorphism (AFLP) analysis. DNA markers are also suitable for use with small amounts of insect material and can be used with stored, dry or old samples. Some have complex multi-locus banding patterns which may be of a non-Mendelian nature (e.g. RAPDs, randomly amplified polymorphic DNA). They have an expanding range of applications, many involving intra- and interspecific discriminations (Beeman and Brown, 1999).

Objectives and Scope of Study

OBJECTIVES:

- *Biochemical variability in the populations of sucking insect pests of tea plantation such as Assam tea thrips, tea jassid and tea mosquito bug will be studied at subcellular level which would include activity of principal detoxifying enzymes.*
- *Intra-population variability of the pest species at different altitudes, on alternate host plants and the species populations with or without stress of insecticides will be studied.*
- *The enzymes that are mainly to be studied are general esterase or carboxylesterases, glutathione S-transferases and cytochrome P450 monooxygenases.*
- *Genetic variability will be studied by Randomly Amplified Polymorphic DNA Polymerase Chain Reactions (RAPD-PCR) from the species of above mentioned sucking insect pest populations with or without stress of insecticides.*
- *Once variations are established it would help to develop a population specific control statregy for effective managemrnt of the sucking insect pest populations in sub Himalayan tea plantations of North Bengal.*

SCOPE OF THE STUDY:

- *In sub-Himalayan tea plantations of northern part of West Bengal, tea is cultivated in three distinct geographical regions, Terai, the Dooars and the Darjeeling hill slopes.*
- *These landscapes having unique phyto-geographic climate and soil, harbor tea plantations often interspaced by river courses, forest patches, low hills, highways and agricultural lands.*
- *In different tea plantations different management practices are followed to protect the tea crop against different insect pests.*
- *Most plantations are managed conventionally routinely using different synthetic insecticides, whereas some organic plantations use herbal and microbial insecticides round the year to keep insect pest populations under control.*
- *This difference in management practices and continuous routine application of synthetic insecticides in conventional plantations may create variability in pest populations which then may become unmanageable by similar treatment.*
- *Once variations among populations of pest species will be established would help to develop population specific control measures or management practices that would minimize undue loss of manpower and insecticides which is a burden to the environment, ensure supply of residue free made tea.*
- *At the same time it would become easier to manage any resistant or tolerant pest populations including those having biotypes.*

Materials and Methods

Collection and Rearing of three sucking pest species:

1. Collection and Laboratory Rearing of *Helopeltis theivora*:

Helopeltis theivora were collected periodically from different sub Himalayan conventional and organic tea plantations of Terai, the Dooars and Darjeeling hill slopes. These landscapes with their unique agro-geographic climate and soil, supports vast tea plantation running over 250 km across. The plantation is dissected at places by river courses, interspersed with forest patches, low hills, highways and agricultural lands.

A laboratory culture of *H. theivora* was set up (at 25 ± 2 ^{0}C and 70-80% RH with 12 hour light and 12 hour darkness) on TV1 clone. The specimens were collected from organically maintained tea plantations of Mokaibari Tea Estate of Darjeeling foothills and were maintained in the laboratory for five generations to be used as control (insecticide unexposed). Adult male and female *H. theivora* collected from tea garden by hand were kept in glass chimney (10x20cm) at a ratio of 1 female: 2 males per chimney. After mating, females laid eggs singly on internodes of tender shoots, petioles of leaves and often on buds. Shoots with eggs were kept upright by passing through manually made perforations on a thermacol sheet which was fitted in a petri dish containing water. Fresh tender shoots of tea having few tender leaves were kept on the thermacol sheet in such a way that their shoots touched the water present in the petri dish below the thermacol sheet. The petri dishes were kept under a glass chimney and the open mouth end of which was covered with fine nylon mesh. After incubation period is over hatching took place. Earlier nymphal (1st and 2nd) stages were reared on petridishes covered by another petridish of same size. Later nymphal stages were reared to adults in the glass

chimney by replacing old shoots with fresh daily. The adults emerging were allowed to mate and oviposit on fresh tea shoots collected from the North Bengal University experimental garden maintained organically by the Department of Zoology. After oviposition the whole process was repeated for five generations.

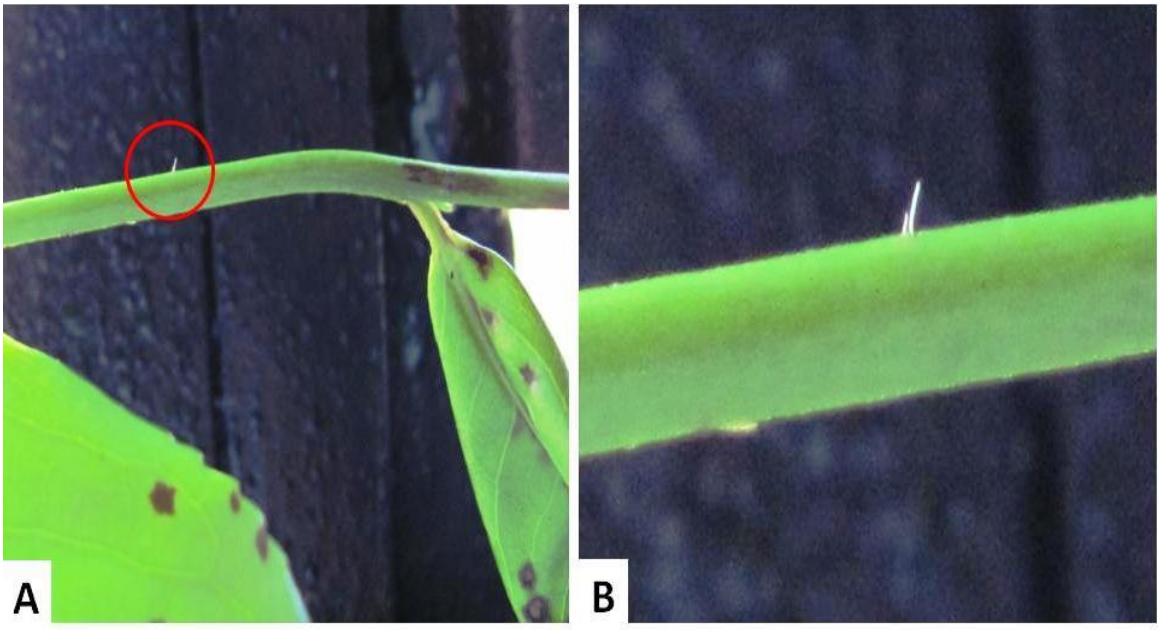

Figure 3.1 A. Inserted egg of *H. theivora* on tender stem of *C. sinensis*. B. Enlarged view

2. Rearing of *Helopeltis theivora* on alternative hosts:

Helopeltis theivora was also reared on two alternative hosts, mikania (*Mikania micrantha*) and guava (*Psidium guajava*), beside tea, for five - six generations following the above mentioned method. The host-specific adult males and females of fifth generation were used for enzyme assays.

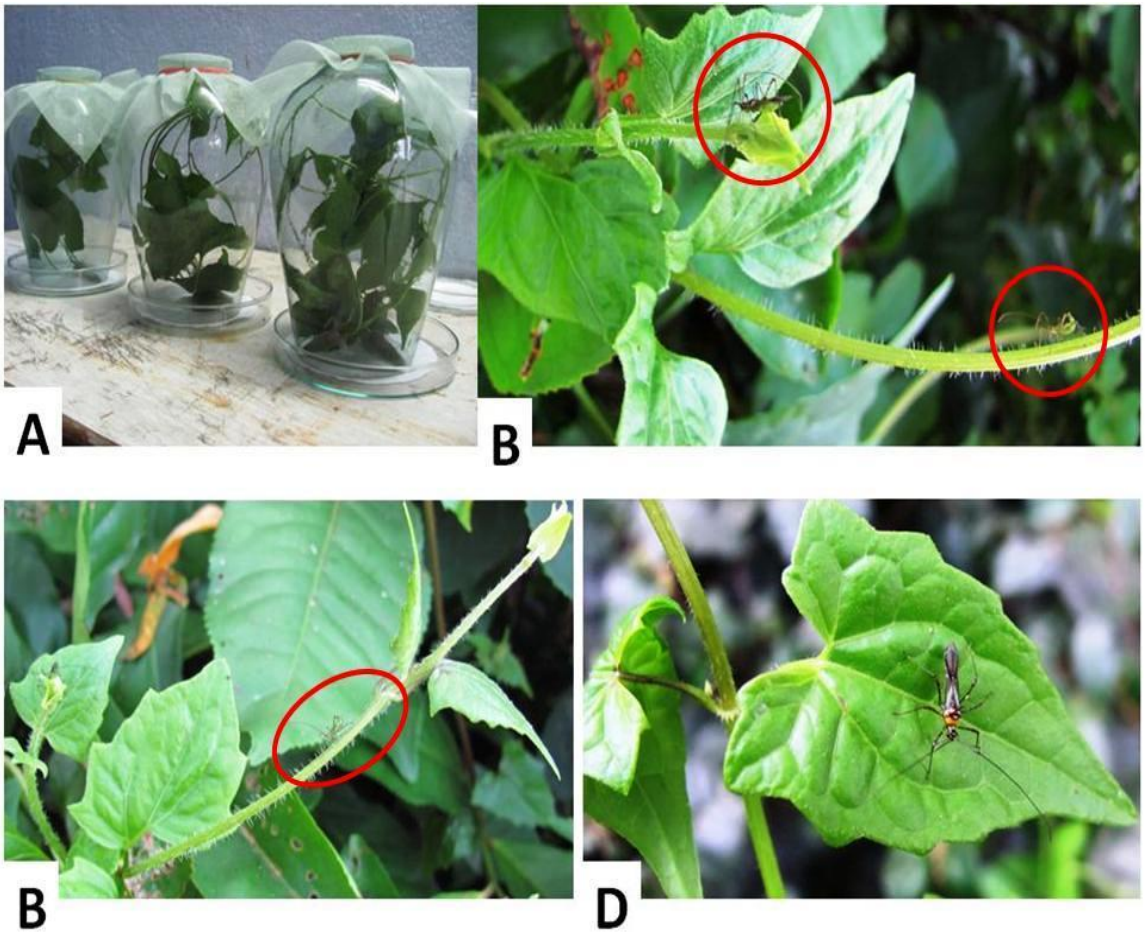

Figure 3.2 Rearing of *H. theivora* on *Mikania micrantha*. A. Laboratory rearing. B. Adults and nymphs feeding on *M. micrantha* leaves. C. *M. micrantha* leaves showing trichomes. D. Female *H. theivora* on *M. micrantha* leaf.

Data on the pre-oviposition period, oviposition period, fecundity, hatchability percent, total nymphal duration and total developmental time, sex ratio, adult longevity on each host plant (tea, mikania and guava) were recorded.

Insecticide exposed specimens were collected from different pesticide/insecticide managed plantations of Terai, the Dooars and Darjeeling foothills (field populations) for biochemical assays.

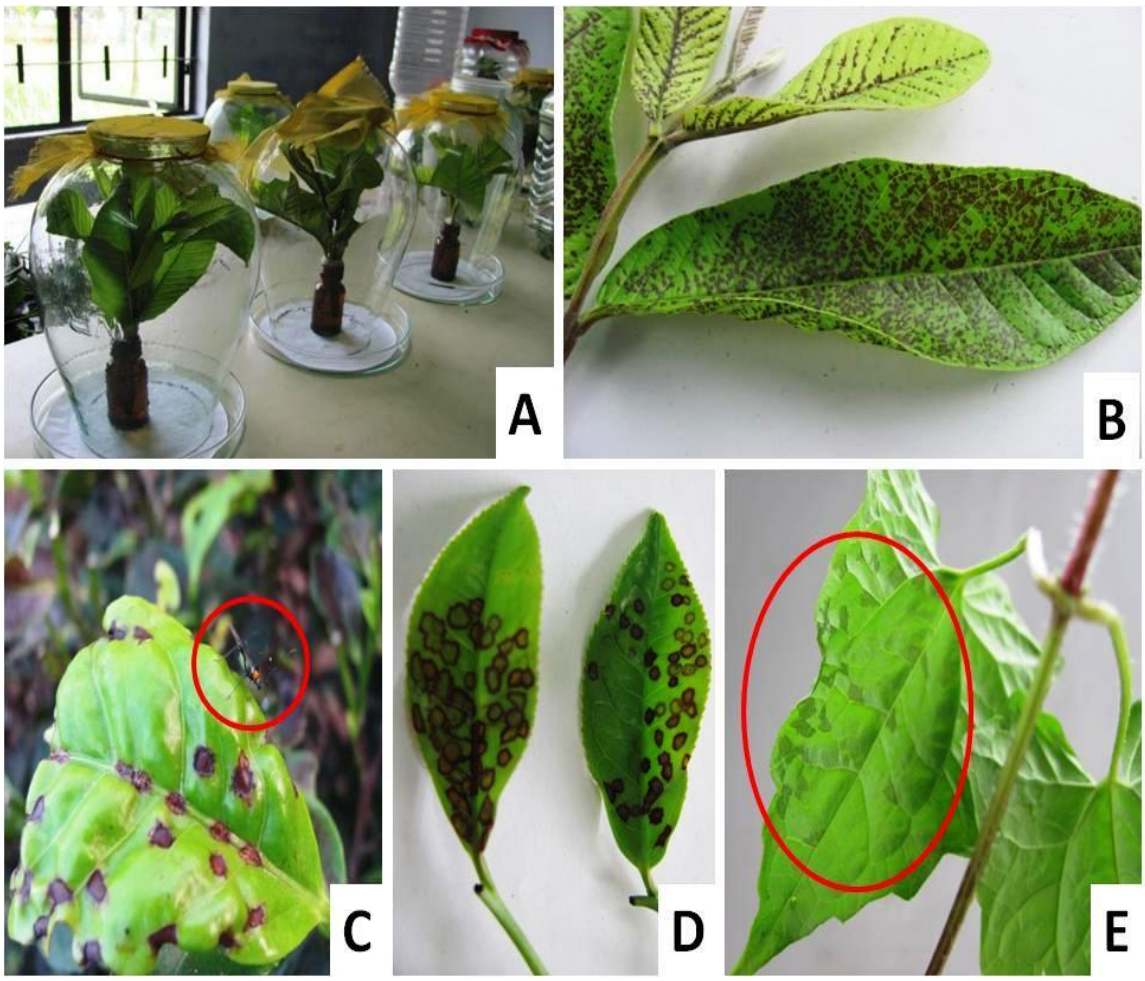

Figure 3.3 Rearing of *H. theivora* on *Psidium guajava*. A. Laboratory rearing. B. Feeding spots on *P. guajava* leaves. C. *H. theivora* feeding on *C. sinensis* leaves. D. Feeding spots by adults and nymphs of *H. theivora* on *C. sinensis* leaves. E. Feeding spots on *M. micrantha* leaves.

1. Collection and Rearing of *Empoasca flavescens*:

A laboratory culture of *E. flavescens* was set up (at 25 ± 2 ^{0}C and 70-80% RH) on tea, collected from organically maintained plantations of Mokaibari Tea Estate of Darjeeling foothills and was maintained in the laboratory for five generations to be used as control (insecticide unexposed). Adult male and female *E. flavescens* were collected from tea plantations by aspirator and kept in plastic container (10x10x30cm). After mating,

females laid eggs on tender leaves mainly on the lower side in crevices of veins, tender shoots. Leaves and tender shoots with eggs were kept upright by passing through a perforation made manually on a thermacol sheet fitted in a petri dish containing water. Fresh tender shoots, having few tender leaves were kept separately in such a way that their shoots touched the water present in the petri dish below the thermacol. The petri dishes were kept under a glass chimney and the open mouth was covered with fine nylon mesh. Nymphs were reared to adults by replacing old with fresh tea shoots daily. The adults emerging were allowed to mate and oviposit on fresh tea shoots. Rearing was done using same stock through five to six generations.

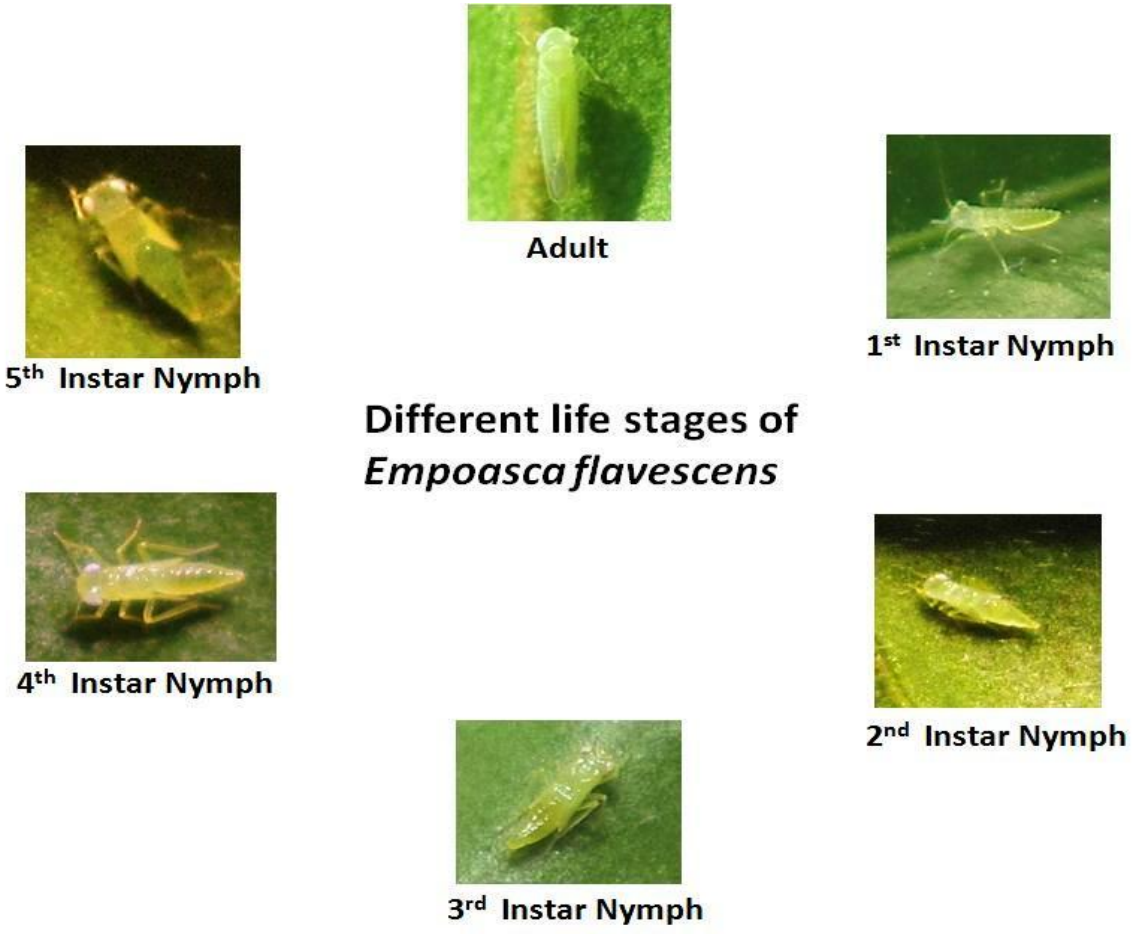

Figure 3.4 Different Life stages of *Empoasca flavescens.*

Figure 3.5 Rearing of *Empoasca flavescens* on C. sinensis.

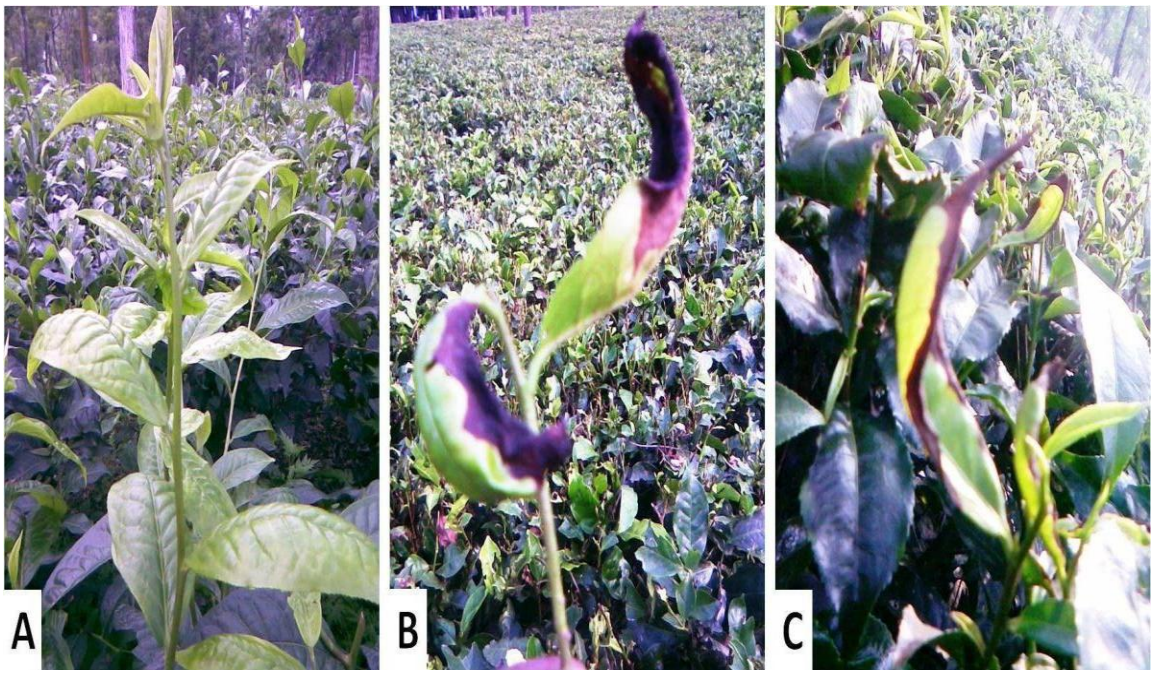

Figure 3.6 A. *E. flavescens* susceptible tea variety. B. Extreme damage symptom (Rim Blight) on tea leaves.C. Part of tea plantation severely attacked by *E. flavescens.*

2. Rearing of *Empoasca flavescens* on alternative hosts:

E. flavescens was also reared on two alternative hosts, potato (*Solanum tuberosum* L.) and castor (*Ricinus comunis* L.) beside tea (*C. sinensis*) for five to six generations following by the process mentioned as above. In case of rearing on potato potted plants were used. The host-specific males and females of fifth generation were also used for enzyme assays.

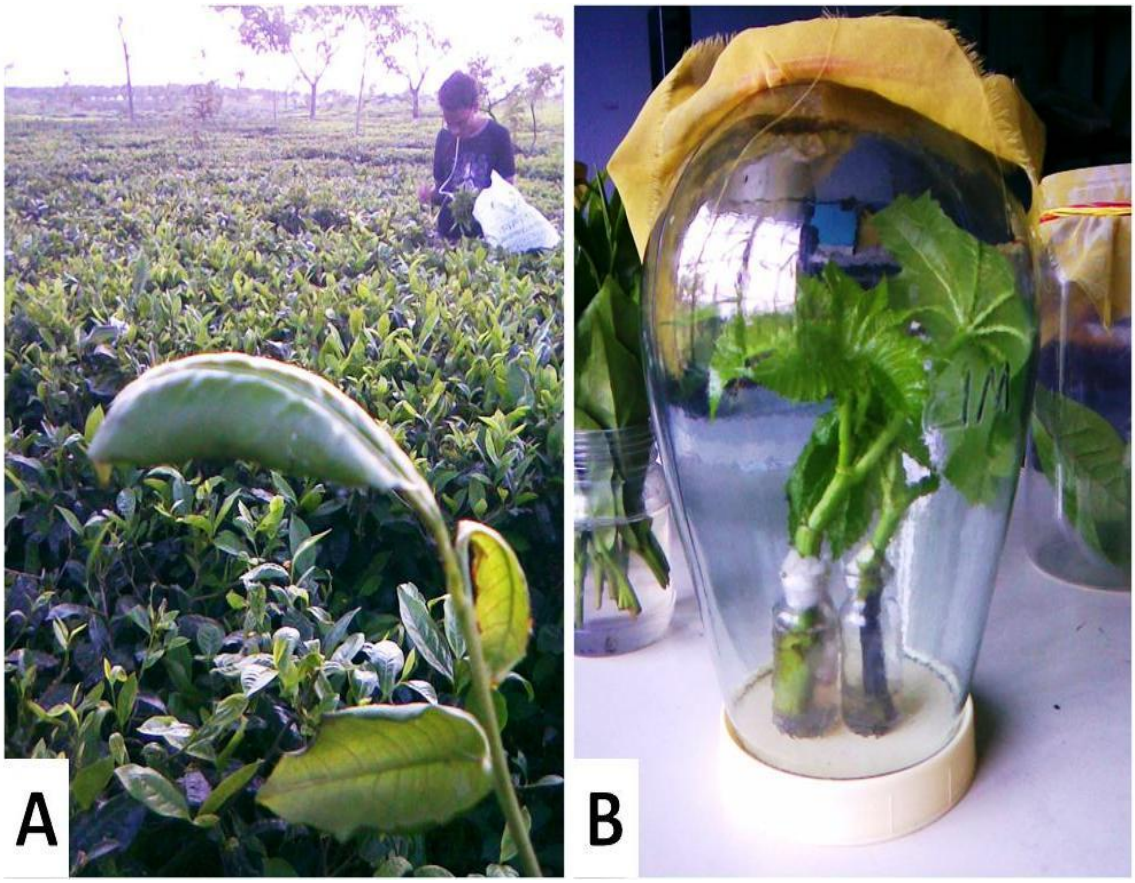

Figure 3.7 A. Collection of adult *Empoasca flavescens* from attacked part of tea plantation by mouth aspirator showng the symptom of attack on tea leaves. B. Rearing of *E. flavescens* on *Ricinus communis* (Castor) leaves in laboratory.

Data on the pre-oviposition period, oviposition period, fecundity, hatchability percent, total nymphal duration and total developmental time and adult longevity on each host plant (tea, potato and castor) were recorded.

Empoasca flavescens collected periodically from different sub Himalayan conventional and organic tea plantations of Terai, the Dooars and Darjeeling hill slopes were also used for enzyme extraction.

1. Collection and Rearing of *Scirtothrips dorsalis*:

A laboratory culture of *Scirtothrips dorsalis* was set up (at 25 ± 2 ^{0}C and 70-80% RH) on TV1 clone, collected from organically maintained tea plantations and was maintained in the laboratory for five generations to be used as control (insecticide unexposed). Infected tea shoots containing larval stages, prepupal, pupal and adult *S. dorsalis* were collected by hand from organic tea plantations and kept in plastic container (10x10x20cm). Next day, in the laboratory, fresh tea shoots were placed in glass containers, the adult thrips were then shifted to fresh shoots kept in the containers. The open mouth of the container was covered by fine nylon mesh. After mating, females laid eggs on tender leaves mainly in crevices on the lower side of veins, tender shoots. Leaves and shoots with eggs were kept upright by passing through a perforation made manually on a thermacol sheet fitted in a petri dish containing water. Fresh tender shoot, having few tender leaves were kept separately in such a way that their shoots touched the water present in the petri dish below the thermacol. The petri dishes were kept under a glass chimney, which was covered with fine nylon mesh. Nymphs were reared to adults by replacing old with fresh

shoots daily. The adults emerging were allowed to mate and oviposit on fresh tea shoots. The whole rearing process was continued for five - six generations.

2. Rearing of *Scirtothrips dorsalis* on alternative hosts:

S. dorsalis was also reared on two alternative hosts, Chilli (*Capsicum annuum*) and castor (*Ricinus comunis*) beside tea (*C. sinensis*), for five to six generations following the method mentioned above. In case rearing on chilli potted plants were used. The host-specific adult males and females of fifth generation were also used for enzyme assays.

Data on the pre-oviposition period, oviposition period, fecundity, hatchability percent, total nymphal duration and total developmental time and adult longevity on each host plant (tea, chilli and castor) were recorded.

S. dorsalis collected periodically from different sub Himalayan conventional and organic tea plantations of Terai, the Dooars and Darjeeling hill slopes were used for enzyme extraction.

Figure 3.8 Rearing of *Scirtothrips dorsalis* on *C. sinensis*. A. Early stages i.e. First, second Instar larva and Prepupa of *S. dorsalis* on *C. sinensis* leaf. B. Enlarged view.

Figure 3.9 Different life stages of *Scirtothrips dorsalis*.

Figure 3.10 A. Damage symptoms caused by *Scirtothrips dorsalis* on tea leaves. B. & C. Damage causing life stages of *Scirtothrips dorsalis* i.e. B. Adult C. First and second instar larva.

Enzyme extraction from three sucking pests:

Helopeltis theivora:

Adult (male and female) *H. theivora* was homogenized individually in 500µl ice cold 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 12,000g for 20 min at 4^0C in high speed refrigerated centrifuge (SIGMA 3K30). The resultant post mitochondrial

supernatant were aliquoted 100µl each in 0.5ml centrifuge tube and was stored at -80^{0}C to be used as enzyme source.

Empoasca flavescens:

Adult *E. flavescens* was homogenized individually in 200µl ice cold 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 12,000g for 20 min at 4^{0}C in high speed refrigerated centrifuge (SIGMA 3K30). The resultant post mitochondrial supernatant were aliquoted 40µl each in 0.2ml centrifuge tube and was stored at -80^{0}C to be used as enzyme source.

Scirtothrips dorsalis:

A pool of ten adult *S. dorsalis* was homogenized in 200µl ice cold 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 12,000g for 20 min at 4^{0}C in high speed refrigerated centrifuge (SIGMA 3K30). The resultant post mitochondrial supernatant were aliquoted 40µl each in 0.2ml centrifuge tube and was stored at -80^{0}C to be used as enzyme source.

Detoxifying Enzyme Activity Assay

General Esterase Activity (α-Esterase):

General esterase activity was measured using α-naphthyl acetate (α-NA) as substrate according to the method of van Asperen (1962) with minor modifications for using in microplate reader. Twenty microliters of supernatant was taken in each well of the microplate reader (Opsys MR, DYNEX Technologies) in duplicate. Two hundred microliters of freshly prepared 30mM α-NA was added to each well for reaction to occur. The reaction was stopped after 15 min by adding 50µl of staining solution prepared fresh by mixing two parts 0.1% Fast Blue BB salt with five parts of 5% sodium dodecyl

sulphate (SDS). The plate was left for 5 min for equilibration and absorbance was recorded at 590 nm. The change in absorbance was converted to end product (α-naphthol) using the standard curve of α-naphthol (0.05-1.00 µM). Blanks were set at the same time using a reaction mixture without protein extracts.

Glutathione S-transferase Activity:

GST activity was estimated using the method of Kao *et al.* (1989) with minor modifications. Fifty microliters of 50mM 1-chloro-2,4-dinitrobenzene (CDNB) and 150µl of 50mM reduced glutathione (GSH) were added to 4ml 2.78ml of sodium phosphate buffer (100mM, pH 6.5). Twenty microliters of enzyme stock were then added. The contents were shaken gently, incubated 2-3 minutes at 20^0C and then transferred to a cuvette in the sample cuvette slot of UV-Visual Spectrophotometer (Rayleigh UV-2601). The reaction was carried out in duplicate. Reaction mixture (3ml) without enzyme was placed in the reference slot for zeroing. Absorbance at 340nm was recorded for 10-12 min employing kinetics (time scan) menu. A unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 µ mol of 2,4-dinitrophenylglutathione per minute at 30^0C using 1 mM concentrations of GSH and CDNB. Specific activity is defined as units per mg of protein. 9.6is the difference in the milimolar extinction co-efficient between CDNB-GSH conjugate and CDNB. Changes in absorbance per minute were converted into µ mol CDNB conjugated $min^{-1}mg^{-1}$ protein using the formula,

$$\text{CDNB-GSH conjugates (µmol } min^{-1} \text{ mg } ^{-1} \text{ protein)} = \frac{\text{Abs. increase (in 5 min) x 3 x 1000}}{\text{9.6* x 5 x protein in mg}}$$

(*9.6mM/cm- is the extinction coefficient for CDNB-GSH conjugate at 340 nm) (Habig *et al.* 1974; Nehare *et al.* 2010).

Cytochrome P450 Monooxygense Activity

Most of the classic methods to evaluate oxidase activity with chromogenic substrates require the purification of microsomal fractions. But these methods cannot be used to estimate the differences in oxidase activity in single small insects (Martin *et al.*, 2002). The alternative method is to measure the level of heam-containing enzymes, which includes the cytochrome oxidase enzymes (Brogdon *et al.*, 1997). Peroxidation of tetra-methyl benzidine (TMBZ) is catalyzed by microsomal proteins with hydrogen peroxide as co-substrate. The amount of oxidase enzymes is correlated with the peroxidase activity of the heam groups. Such a technique would be helpful for estimating large scale differences in oxidase activity characteristic of insecticide resistance and oxidase induction (Martin *et al.*, 2002). Cytochrome P450 activity was estimated by measuring heam peroxidase activity (Penilla *et al.*, 2007; Tiwari *et al.*, 2011). As heam constitutes the majority of cytochrome P450 of non-blood-fed (herbivorous) insects, quantification of heam activity can be expressed as cytochrome P450 (Brogdon *et al.*, 1997). For cytochrome P450 activity, 20µl supernatant of the enzyme extract was taken in the well of microplate containing 200 µl of 6.3 mM TMBZ solution [10mg TMBZ dissolved in 5ml absolute methanol mixed with 15 ml of 0.25M sodium acetate buffer ($NaC_2H_3O_2$), pH 5.0, prepared fresh daily]. Then to each well of the microplate 80 µl of 0.0625M Potassium Phosphate buffer (KHPO4) pH 7.2 and 25 µl of 3% hydrogen peroxide (H_2O_2) was added. Two controls per plate were prepared each with 20µl of homogenizing buffer and with all the ingredients except the enzyme source. Absorbance was recorded at 630 nm after 30 min of incubation in a microplate reader (Opsys MR, DYNEX Technologies) against blanks at 25^0C. A standard curve for heam peroxidise activity was prepared using

different concentrations of cytochrome C (0.0025 nmol - 0.02 nmol) from horse heart type VI (Sigma Aldrich). Total cytochrome P450 activity was expressed as n mol of cytochrome P450 equivalent units (EUs) mg $^{-}$1 protein min^{-1}.

Electrophoretic analysis of General esterases:

Electrophoresis of different sucking pest populations i.e. field collected populations from conventional and organic tea plantations of Terai, the Dooars and Darjeeling hill slopes as well as from laboratory reared control populations were carried out in 8% polyacrylamide gels using equal amount of protein in tris-glycine (pH 8.3) at 200V for 6-7 hours at 4^0C. The gels were stained for esterase isozymes according to Georghiou and Pasteur (1978) with slight modifications. Esterase isozymes were designated as EST I, II, III, IV, V, VI etc based on mobility from anode to cathode. The Rm (relative mobility) values of esterase isozyme bands were determined according to the standard method. Densitometric analysis of the isozyme bands was performed using Image Aide Analysis software. The increase in esterase isozymes (Est I, II, III, IV, V, VI etc) was quantified as the relative pixel density of the bands for the specimens collected from field (organic and insecticide managed i.e. conventional) tea plantations along with laboratory reared control specimens.

Protein quantification

Enzyme activities were corrected for protein concentration. The total protein content of the homogenate was determined by Folin-Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as the standard.

RAPD-PCR Analysis:

Genomic DNA Isolation:

Genomic DNA isolation, purification and quantification From *Helopeltis theivora*

DNA was isolated by homogenization of the whole body of adult *H. theivora* with a sterile Teflon made micro pestle in a 1.5 ml centrifuge tube in 20 µl homogenization buffer (0.1 M NaCl, 0.2 M Tris-HCl pH 9.0, 0.05 M EDTA and 5% SDS) following the process of Latif *et al.* (2008). The micro-pestle was washed with an additional 40 µl homogenization buffer and the homogenate was incubated at 65^0C for 40 min. To the homogenate 10 µl 5 M potassium acetate was added to a final concentration of 0.7 M and the tube was placed on ice for 40 min. The homogenate was then centrifuged at 14,000 rpm for 20 min. The supernatant was transferred to a fresh 1.5 ml centrifuge tube. 100 µl chilled (-20^0C) 100% ethanol was added to it and, the DNA was allowed to precipitate at room temperature for 10 min. The tube was centrifuged at 14,000 rpm for 20 min, and the ethanol was carefully removed with a pipette. The DNA pellet was washed with 100 µl chilled 70% ethanol and centrifuged for 10 min. the DNA pellet was dried by first pouring off the ethanol and keeping the tube for 20 min at room temperature. The dried DNA was suspended in 50 µl TE [Tris-EDTA (10:1), pH 8.0] buffer and gently mixed for few minutes. The DNA was purified by DNA purification kit from Genei, Bangalore, India.

The purity and quantity was checked by taking OD (Optical density) in UV-Visual spectrophotometer (Rayleigh UV-2601) at 260 and 280 nm (Sambrook *et al.*, 1989).

Genomic DNA isolation, purification and quantification From *Empoasca flavescens*

DNA was isolated individually following the method of Coen *et al.* (1982). Each individual were homogeniged in 120 µl of extraction buffer (0.1 M NaCl, 0.2 M sucrose, 0.01 M Na_2 EDTA, 0.03 M Tris-HCl pH 8.0) in 1.5 ml microcentrifuge tube with the

help of Teflon homogeniger. 30 µl of lysis buffer (0.25 M Na_2 EDTA, 2.5% SDS, 0.5 M tris-HCl pH 9.2) and diethyl pyrocarbonate, were added to the homogenate and the tubes were incubated at 65^0C for 40 minutes. 50 µl of 5 M potassium acetate (pH 5.0) was added to the suspension and the tubes were vortexed briefly. The crude extract was incubated on ice for 30 minutes, and then centrifuged at 15000x g for 15 minutes. The supernatant was then transferred to new tubes and two volumes of chilled 95% ethanol were added to these tubes. DNA was pelleted by centrifugation at 15000x g for 15 minutes. DNA pellet was washed with chilled 70% ethanol, and then rinsed with 100% ethanol. Extracted DNA was re-precipitated in one volume of 100% ethanol and one-tenth volume of 7.5 M ammonium acetate and then recovered as above, and washed with chilled 100% ethanol. The recovered DNA pellet was air dried and re-dissolved in 50 µl of TE [Tris-EDTA (10:1), pH 8.0] buffer. The purity and quantity was checked by taking OD (Optical density) in UV-Visual spectrophotometer (Rayleigh UV-2601) at 260 and 280 nm (Sambrook *et al.*, 1989).

Genomic DNA isolation, purification and quantification From *Scirtothrips dorsalis*

DNA was extracted from a pool of 20 individuals following the method of Coen *et al.* (1982). Each individual was homogeniged in 120 µl of extraction buffer (0.1 M NaCl, 0.2 M sucrose, 0.01 M Na_2 EDTA, 0.03 M Tris-HCl pH 8.0) in 1.5 ml microcentrifuge tube by Teflon homogeniger. 30 µl of lysis buffer (0.25 M Na_2 EDTA, 2.5% SDS, 0.5 M tris-HCl pH 9.2) and diethyl pyrocarbonate, were added to the homogenate and the tubes were incubated at 65^0C for 40 minutes. 50 µl of 5 M potassium acetate (pH 5.0) was added to the suspension and the tubes were vortexed briefly. The crude extract was

incubated on ice for 30 minutes and then centrifuged at 15000x g for 15 minutes. The supernatant was transferred to new tube and two volumes of chilled 95% ethanol was added to these tubes. DNA was pelleted by centrifugation at 15000x g for 15 minutes. DNA pellet was washed with chilled 70% ethanol and rinsed with 100% ethanol. Extracted DNA was re-precipitated in one volume of 100% ethanol and one-tenth volume of 7.5 M ammonium acetate and then recovered as above and washed with chilled 100% ethanol. The recovered DNA pellet was air dried and re-dissolved in 50 µl of TE [Tris-EDTA (10:1), pH 8.0] buffer. The purity and quantity was checked by taking OD (Optical density) in UV-Visual spectrophotometer (Rayleigh UV-2601) at 260 and 280 nm (Sambrook *et al.* 1989).

Primer Selection:

A total of 40 RAPD primer, 20 primers from each Kit 'A' and Kit 'D' (Operon Technologies Inc.) were screened to find strongest bands, polymorphism and reproducibility. Based on band reproducibility and polymorphism a set of eight RAPD primers four from Kit A (OPA-02, OPA-08, OPA09 and OPA-11) and four from Kit D (OPD-05, OPD-08, OPD-12, OPD-20) were used for genomic DNA amplifications of *H. theivora* and *E. flavescens*. In case of *S. dorsalis* OPD 09 was additionally selected from Kit D.

Table 3.1 Primers available in Kit 'A' and 'D' from Operon Technologies Inc.

KIT 'A'	
PRIMER NAME	**SEQUENCE(5'-3')**
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-12	TCGGCGATAG
OPA-13	CAGCACCCAC
OPA-14	TCTGTGCTGG
OPA-15	TTCCGAACCC
OPA-16	AGCCAGCGAA
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC

KIT 'D'	
PRIMER NAME	**SEQUENCE(5'-3')**
OPD-01	ACCGCGAAGG
OPD-02	GGACCCAACC
OPD-03	GTCGCCGTCA
OPD-04	TCTGGTGAGG
OPD-05	TGAGCGGACA
OPD-06	ACCTGAACGG
OPD-07	TTGGCACGGG
OPD-08	GTGTGCCCCA
OPD-09	CTCTGGAGAC
OPD-10	GGTCTACACC
OPD-11	AGCGCCATTG
OPD-12	CACCGTATCC
OPD-13	GGGGTGACGA
OPD-14	CTTCCCCAAG
OPD-15	CATCCGTGCT
OPD-16	AGGGCGTAAG
OPD-17	TTTCCCACGG
OPD-18	GAGAGCCAAC
OPD-19	CTGGGGACTT
OPD-20	ACCCGGTCAC

Table 3.2 Selected primers from Kit 'A' and 'D' from Operon Technologies Inc.

PRIMER		SEQUENCE (5'-3')
KIT 'A'	OPA-02	TGCCGAGCTG
	OPA-08	GTGACGTAGG
	OPA-09	GGGTAACGCC
	OPA-11	CAATCGCCGT
KIT 'D'	OPD-05	TGAGCGGACA
	OPD-08	GTGTGCCCCA
	OPD-12	CACCGTATCC
	OPD-20	ACCCGGTCAC

RAPD-PCR amplifications for *H. theivora* genomic DNA

PCR amplifications were performed using a set of eight RAPD primers, four from Kit A (OPA-02, OPA-08, OPA09 and OPA-11) and four from Kit D (OPD-05, OPD-08, OPD-12, OPD-20) after screening 20 set of primer from each kit from Operon Technologies Inc., Alamada, California. Amplifications were carried out in PeqLab (Gradient) Thermal cycler with an initial denaturation of 180 sec at 94 ^{0}C followed by 45 cycles consisting of 60 sec denaturation at 93 ^{0}C, 60 sec annealing at 35 ^{0}C and 120 sec extension at 72 ^{0}C with a final extension of 300 sec at 72 ^{0}C. To reduce possibility of variation in amplification reaction, master mixing of the reaction constituents was always used. PCR reaction mixture of 25 µl comprised of 1x buffer, 0.2 mM d ATP, dCTP, dGTP, dTTP, 2 mM MgCl2, 0.2 µM of primer, 50 ng of template DNA and 0.25 U of Taq DNA polymerase. Amplified products were electrophoresed on 1.8% agarose gel with 100 bp DNA ladder as size marker. Gels were photographed using transmitted UV light. Presence and absence of fragments were recorded as 0 or 1. Reproducibility of the RAPD fragments were tested by independently repeating the amplification reaction three times with selected primer and comparing the resulted fragments.

RAPD-PCR amplifications for *E. flavescens and S. dorsalis* genomic DNA:

Amplifications were carried out in PeqLab (Gradient) Thermal cycler with an initial denaturation of 120 sec at 94 ^{0}C followed by 50 cycles consisting of 60 sec denaturation at 92 ^{0}C, 60 sec annealing at 35 ^{0}C and 120 sec extension at 72 ^{0}C with a final extension of 300 sec at 72 ^{0}C. To reduce possibility of variation in amplification reaction, master mixing of the reaction constituents was always used. PCR reaction mixture of 25 µl

comprised of 1x buffer, 0.05 mM each dATP, dCTP, dGTP, dTTP, 2 mM $MgCl_2$, 0.2 µM of primer, 50 ng of template DNA and 0.3 U of Taq DNA polymerase. Amplified products were separated by electrophoresis in 1.8% agarose gel in 0.5x TAE (Tris-EDTA) and 0.05mg ml^{-1} ethidium bromide with 100 bp DNA ladder as size marker. Gels were photographed using transmitted UV light. Presence and absence of fragments were recorded as 0 or 1. Reproducibility of the RAPD fragments were tested by independently repeating the amplification reaction three times with selected primer and comparing the resulted fragments.

Statistical analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. Allele frequencies were estimated based on the square root of the frequency of the null (recessive) genotype. All calculations were carried out using the Population Genetic Analysis Software, PopGene 1.32 (Yeh *et al.*, 2000). The UPGMA dendrogram of population was constructed based on Nei's (1978) genetic distances using TFPGA (Tools for Population Genetics Analysis) software (Miller, 1997). Calculation of means and standard deviation/errors of enzyme activities among different field collected, laboratory reared control and host specific populations were done by using Graph Pad Instat, Graph Pad Software Inc., USA and SPSS 16.0 for Windows, SPSS Inc., USA.

Results and Discussion

Rearing of three sucking pests on tea and two alternative hosts:

1. Laboratory Rearing of *Helopeltis theivora* on tea and two other hosts

Feeding preference and stance:

Helopeltis theivora was reared on three alternative host plants i.e. *Camellia sinensis*, *Mikania micrantha* and *Psidium guajava*. In choice test, *H. theivora* was offered to feed *C. sinensis* in combination with *M. micrantha* in one set, and *C. sinensis* in combination with *P. guajava* in another set. *H. theivora* produced more number of feeding spots on *C. sinensis* than other two alternative hosts. The number of feeding spots was always higher on *C. sinensis* both in male and female *H. theivora* than that on the non- tea hosts (Table 4.1). The diameter of the feeding spot was also higher in *C. sinensis* than other two hosts in both sexes of *H. theivora*. Compared with *C. sinensis*, the number and diameter of feeding spots on *M. micrantha* and *P. guajava* leaves were few and smaller in diameter (Table 4.1).

Table 4.1 Feeding preference (choice test) of *H. theivora* for *C. sinensis, M. micrantha* and *P. guajava* (Values are Mean ± SD).

Combination of host plants	Host plants	No. of feeding spot		Feeding spot diameter (mm)	
		Male	Female	Male	Female
C. sinensis + *M. micrantha*	*C. sinensis*	72.6±5.43[a*]	128.9±6.32[a]	2.18±0.07[a]	2.80±0.05[a]
	M. micrantha	3.7±0.82[b]	4.3±0.81[b]	1.29±0.03[b]	1.83±0.02[b]
C. sinensis + *P. guajava*	*C. sinensis*	59.8±3.06[a]	118.75±4.12[a]	2.18±0.07[a]	2.80±0.05[a]
	P. guajava	18.6±1.73[c]	23.083±1.93[c]	0.69±0.01[c]	1.13±0.01[c]

[*]Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Similar trend was evident in both sexes of *H. theivora*. In a non-choice test, being forced to feed, *H. theivora* fed comparably on all the studied hosts with no significant difference in the number of feeding puncture marks made on the different host plants (Table 4.2). However, the feeding stance of *H. theivora* on *C. sinensis* was significantly less than that on *M. micrantha* and *P. guajava* (Table 4.3). Female *H. theivora* spent more time in each feeding spot than the males.

Table 4.2 Feeding preference (non-choice test) of *H. theivora* for *C. sinensis*, *M. micrantha* and *P. guajava* (Values are Mean ± SD).

Host plants	Number of feeding spot	
	Male	Female
C. sinensis	145.6±4.35[a*]	188.6±7.54[a]
M. micrantha	133.4±3.53[a]	174.3±6.85[a]
P. guajava	129.8±4.26[a]	168. 5±5.32[a]

[*]Within columns, means followed by the same letter do not differ significantly ($P \leq 0.05$).

Table 4.3 Feeding stance (in minute) of *H. theivora* in three alternative hosts.

Host Plants	Feeding Stance (in minute) (Mean± SD of 10 observation in each host plants)	
	Male	Female
C. sinensis	4.8±0.61[a*]	7.0±0.17[a]
M. micrantha	5.2±0.56[b]	7.2±0.48[b]
P. guajava	5.4±0.44[c]	7.4±0.88[c]

[*]Within columns, means followed by the same letter do not differ significantly ($P \leq 0.05$).

The complicated associations of plant and insect have played an important part in understanding ecological and evolutionary processes (Whitham *et al.*, 2006). The

efficient exploitation of available food is a vital requirement of all organisms. Polyphagous herbivorous insects encounter dietary nutritional variation in plants, which impact their growth and development (Bae and Sicher, 2004; Roberts and Paul, 2006) thus they need to make feeding decisions to achieve a nutritionally balanced food intake that leads to optimal performance. Suboptimal nutritional content of the diet can adversely affect survival, growth, development, and fecundity of insects (Barton, 1993; Auger, 1995; Awmack and Leather, 2002). The study of feeding and food preference is therefore most important. Food preference depends on many factors such as the total food abundance, the relative abundance of food types and "risk" in each food i.e. the role of toxic substances or secondary metabolites present in them (Emlen, 1966).

Female *Helopeltis theivora* always produced significantly high number of feeding spots on *C. sinensis* than male implying that the former acquires more nutrition from leaf tissue. Reduced number of feeding spots on *M. micrantha* and *P. guajava* leaves in comparison to *C. sinensis* may be due to the insect's low preference or may be due to poor availability of nutrients in the alternative hosts or due to 'presence of toxic allelochemicals'. In the study of feeding stance, *H. theivora* spends less time on *C. sinensis* (Male: 4.8±0.61 minute, Female: 7.0±0.17) than on the other two non-tea hosts, *M. micrantha* (Male: 5.2±0.56 minute, Female: 7.2±0.48) and *P. guajava* (Male: 5.4±0.44 minute, Female: 7.4±0.88). This also advocates for poor availability of nutrients to exploit in the alternative hosts. Expenditure of more time in each sucking stance by female than male on all the three host plants further substantiated the female's 'requirement of a higher energy input' possibly to produce viable eggs and to establish sustainable second generation.

Fitness traits of H. theivora on different alternative hosts:

Life history traits (fitness traits) of *H. theivora* varied significantly on three studied alternative hosts (Table 4.4). The total development period (in days) was significantly high on *P. guajava* (22.5±0.4) than on *C. sinensis* (20.0±0.3) and *M. micrantha* (21.9±0.4) (Table 4.4). The total nymphal period (in days) on *C. sinensis* was significantly low than on the other two alternative hosts. The nymph development period of *H. theivora* was significantly shorter on *C. sinensis* (13.3±0.16 days) (Table 4.4; Table 4.5) than on the other two hosts, *M. micrantha* (14.2 ± 0.22 days) and *P. guajava* (14.7 ± 0.23 days) (Table 4.4; Table 4.5). The fecundity, hatchability percent and oviposition period were significantly higher on *C. sinensis* than on the other two host plants (Table 4.4).

The fecundity (*C. sinensis*: 172.6 ± 4.5 no. egg / female, *M. micrantha*: 128.6 ± 4.4 no. egg / female, *P. guajava*: 118.7 ± 3.3 no. egg / female), oviposition period (*C. sinensis*: 24.1 ± 0.7 days, *M. micrantha*: 22.5 ± 0.6 days, *P. guajava*: 21.7 ± 0.8 days) and hatchability (*C. sinensis*: 80.9 ± 1.9 %, *M. micrantha*: 69.4 ± 1.6 %, *P. guajava*: 64.1 ± 1.7 %) were noted to be significantly higher on *C. sinensis* (Table 4.4). The age at reproductive maturity and egg incubation periods were lower on *C. sinensis* than on the other two host plants. The stadial duration of different nymphal stages of *H. theivora* on different studied hosts recorded (Table 4.5). These stadial periods of *H. theivora* were always higher on two non tea hosts that tea (Table 4.5).The morphometrics (length in mm) of different life stages and body parts of *H. theivora* when reared on *C. sinensis* has been presented in Table 4.6. The body length of the female *H. theivora* was 7.54±0.24 mm and was greater than the male which measures 6.42±0.16 mm. The body length of the different instars progressively increased (Table 4.6). The scutellar horn (=dumstick) was first noticeable in second instar which progressively increases in advancing instars. The wing development as wing pads started from third instar. The antenna was quite longer in adults (Male: 10.38±0.21 mm; Female: 11.24±0.12 mm) (Table 4.6).

Table 4.4 Life history traits of *H. theivora* on three different alternative hosts i.e. *C. sinensis*, *M. micrantha* and *P. guajava*.

Host plants	ARM*	OP	PoOP	FD	IP	HC (%)	NDP	TDP	SR	AL	
										Female	Male
C. sinensis	4.2±0.07^{b*} (3.6-5.2)	24.1±0.7a (17-32)	2.6±0.09^{a} (1.7–3.3)	172.6±4.5^{a} (108-212)	6.7±0.18^{b} (6-9)	80.9±1.9^{a} (55-94)	13.3±0.16^{b} (12-15)	20.0±0.3^{b} (18-24)	1:0.94^{a}	40.7±0.92^{a} (28-46)	28.5±0.84^{a} (18-44)
M. micrantha	4.5±0.08^{a} (3.8-5.4)	22.5±0.6^{b} (17-30)	2.4±0.06^{a} (1.6–2.8)	128.6±4.4^{b} (75-172)	7.7±.19^{a} (6-9)	69.4±1.6^{b} (45-81)	14.2±0.22^{a} (13-17)	21.9±0.4^{a} (19-26)	1:0.92^{a}	37.3±0.73^{a} (26-42)	30.4±0.69^{a} (22-37)
P. guajava	4.6±.0.09^{a} (3.9-5.5)	21.7±0.8^{b} (13-27)	2.2±0.06^{b} (1.6–2.7)	118.7±3.3^{b} (78-145)	7.8±.20^{a} (6-9)	64.1±1.7^{b} (44-78)	14.7±0.23^{a} (13-17)	22.5±0.4^{a} (19-26)	1:0.92^{a}	36.9±0.84^{a} (26-42)	30.9±0.79^{a} (22-37)

*(Means± Standard Error), Figures in parenthesis represent the range.

*Within columns, means followed by the same letter do not differ significantly ($P <$ 0.05).

- ARM: Age at reproductive maturity (days);
- OP: Oviposition period (days);
- PoOP: Post oviposition period (days),
- FD: Fecundity (no. of eggs laid/female);
- IP: Incubation (egg) period (days);
- HC: Hatchability (% of eggs hatched);
- NDP: Nymph development period (days);
- TDP: Total developmental period (days);
- SR: Sex ratio (female: male);
- AL: Average longevity (days).

Table 4.5 Stadial period (in days) of different nymphal instars of *Helopeltis theivora* on *C. sinensis, M. micrantha* and *P. guajava.*

Nymphal instars	*Duration (Days)*		
	C. sinensis	*M. micrantha*	*P. guajava*
First Instar	**3.4±0.52**	**3.5±0.46**	**3.6±0.51**
Second Instar	**2.8±0.22**	**3.0±0.34**	**3.1±0.42**
Third Instar	**2.3±0.26**	**2.5±0.31**	**2.6±0.36**
Fourth Instar	**2.4±0.42**	**2.6±0.38**	**2.7±0.45**
Fifth Instar	**2.4±0.28**	**2.6±0.34**	**2.7±0.42**

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Table 4.6 Morphometrics (length in mm) of different life stages of *Helopeltis theivora.* Mean±SD of ten observations.

Body / Body Part length (in mm)	***Nymphal Instars***					***Adult***	
	First Instar	***Second Instar***	***Third Instar***	***Fourth Instar***	***Fifth Instar***	***Male***	***Female***
Body	**1.48±0.12**	**1.98±0.14**	**2.89±0.16**	**4.24±0.15**	**5.04±0.21**	**6.42±0.16**	**7.54±0.24**
Body width (Thorax region)	**0.33±0.01**	**0.39±0.04**	**0.46±0.02**	**0.58±0.05**	**0.72±0.02**	**0.72±0.04**	**0.76±0.02**
Antenna	**1.88±0..13**	**2.62±0.18**	**3.96±0.16**	**5.54±0.21**	**6.58±0.18**	**10.38±0.21**	**11.24±0.12**
Rostrum	**0.52±0.12**	**0.84±0.16**	**3.22±0.08**	**4.52±0.18**	**5.38±0.21**	**3.92±0.16**	**4.22±0.16**
Scutellar horn	_	**0.15±0.02**	**0.23±0.02**	**0.31±0.04**	**0.38±0.07**	**1.34±0.08**	**1.48±0.06**
Fore Wing	_	_	**0.17±0.03**	**0.24±0.02**	**0.30±0.04**	**5.16±0.14**	**5.66±0.13**
Hind Wing	_	_	**0.09±0.05**	**0.18±0.07**	**0.22±0.09**	**3.96±0.16**	**4.48±0.14**
Foreleg	**0.98±0.11**	**1.42±0.13**	**2.09±0.08**	**2.92±0.16**	**3.48±0.16**	**5.22±0.21**	**5.38±0.16**
Interleg	**1.18±0.08**	**1.52±0.11**	**2.26±0.14**	**3.16±0.12**	**3.78±0.22**	**5.42±0.19**	**5.48±0.12**
Hindleg	**1.24±0.11**	**1.76±0.09**	**2.66±0.16**	**3.66±0.24**	**4.42±0.19**	**6.84±0.24**	**7.14±0.14**

2. Laboratory Rearing of *Empoasca flavescens* on tea and two other hosts

Life history traits (fitness traits) of *E. flavescens* varied significantly on three alternative host i.e. *Camellia sinensis, Solanum tuberosum* and *Ricinus communis* considered in the present work (Table 4.7). The total development period (in days) was significantly high on *R. communis* (21.4±0.38) than on *C. sinensis* (18.2±0.35) and *S. tuberosum* (19.2±0.32) (Table 4.7). The total nymphal period (in days) on *C. sinensis* was significantly low than other two alternative hosts. The stadial duration of different nymphal stages of *E. flavescens* on different studied hosts recorded (Table 4.8). The stadial periods on non tea hosts were always higher than on tea (Table 4.8). The nymph development period (in days) of *E. flavescens* is significantly shorter on *C. sinensis* (11.7±0.38) (Table 4.7; Table 4.8) than on the other two hosts, *S. tuberosum* (12.8 ± 0.26) and *R. communis* (13.6 ± 0.35) (Table 4.7; Table 4.8). However, the fecundity, hatchability percent and oviposition period were significantly higher on *C. sinensis* than on the other two host plants (Table 4.7).

The fecundity (*C. sinensis*: 20.3 ± 2.6 no. egg / female, *S. tuberosum*: 16.3 ± 2.8 no. egg / female, *R. communis*: 17.3 ± 3.2 no. egg / female), oviposition period (*C. sinensis*: 9.5 ± 1.12 days, *S. tuberosum*: 8.7 ± 1.22 days, *R. communis*: 8.4 ± 1.18 days) and hatchability (*C. sinensis*: 82.4 ± 4.4 %, *S. tuberosum*: 73.2 ± 5.3 %, *R. communis*: 68.6 ± 6.2 %) were recorded to be significantly higher on *C. sinensis* (Table 4.7). The age at reproductive maturity and egg incubation periods were lower on *C. sinensis* than the other two host plants (*C. sinensis*: 3.3 ± 0.35 days, *S. tuberosum*: 3.6 ± 0.35 days, *R. communis*: 3.8 ± 0.35 days). The morphometrics (length in mm) of different life stages and body parts of *E. flavescens* has been presented in Table 4.9. The body length of the female *E. flavescens* was 2.74±0.04 mm and was larger than the male which measures 2.52±0.06 mm. The length of the different instar progressively increased (Table 4.9). The wing development started from third instar.

Table 4.7 Life history traits of *E. flavescens* on three different alternative hosts i.e. *C. sinensis*, *S. tuberosum* and *R. communis*.

Host plants	ARM*	OP	PoOP	FD	IP	HC (%)	NDP	TDP	AL	
									Male	Female
C. sinensis	3.3±0.35a (3-4)	9.5±1.12a (8-11)	3.8±0.63a (3-5)	20.3±2.6a (17-24)	6.5±0.32a (6-8)	82.4±4.4a (76-88)	11.7±0.38a (7-18)	18.2±0.35a (19-36)	15.6±1.62 (12-18)	16.4±1.80 (12-20)
S. tuberosum	3.6±0.35b (3-4)	8.7±1.22b (7-10)	3.4±0.34b (3-5)	16.3±2.8b (13-22)	7.6±0.38b (6-9)	73.2±5.3b (68-76)	12.8±0.26b (7-18)	19.2±0.32b (19-36)	14.2±1.44 (11-17)	15.2±1.62 (12-18)
R. communis	3.8±0.35c (3-5)	8.4±1.18c (7-10)	3.1±0.26c (2-4)	17.3±3.2c (14-22)	7.8±0.46b (6-9)	68.6±6.2c (61-73)	13.6±0.35c (7-18)	21.4±0.38c (19-36)	13.1±1.32 (11-17)	14.6±1.54 (12-18)

*(Means± SE), Figures in parenthesis represent the range.

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

- ARM: Age at reproductive maturity (days);
- OP: Oviposition period (days);
- PoOP: Post oviposition period (days),
- FD: Fecundity (no. of eggs laid/female);
- IP: Incubation (egg) period (days);
- HC: Hatchability (% of eggs hatched);
- NDP: Nymph development Period (days);
- TDP: Total developmental Period (days);
- AL: Average longevity (days).

Table 4.8 Duration of different nymphal instars of *Empoasca flavescens* on three reared hosts i.e. *C. sinensis, S. tuberosum* and *R. communis*

Nymphal instars	*Duration (Days)*		
	C. sinensis	*S. tuberosum*	*R. communis*
First instar	**1.88±0.64**	**2.16±0.56**	**2.26±0.24**
Second instar	**2.08±0.72**	**2.26±0.62**	**2.38±0.28**
Third instar	**2.21±0.96**	**2.36±0.38**	**2.48±0.44**
Fourth instar	**2.29±0.62**	**2.42±0.46**	**2.58±0.52**
Fifth instar	**3.28±0.88**	**3.62±0.52**	**3.88±0.42**

Table 4.9 Morphometrics (length in mm) of different life stages of *Empoasca flavescens.* Mean±SD of ten observations.

Body / Body Part length (in mm)	***Nymphal Instars***					***Adult***	
	First Instar	***Second Instar***	***Third Instar***	***Fourth Instar***	***Fifth Instar***	***Male***	***Female***
Body	**0.68±0.02**	**1.12±0.18**	**1.35±0.14**	**1.64±0.12**	**2.30±0.10**	**2.52±0.06**	**2.74±0.04**
Body width (Thorax region)	**0.22±0.01**	**0.29±0.04**	**0.33±0.02**	**0.55±0.05**	**0.65±0.02**	**0.72±0.04**	**0.76±0.02**
Antenna	**0.07±0.01**	**0.08±0.01**	**0.10±0.01**	**0.11±0.02**	**0.12±0.02**	**0.13±0.02**	**0.15±0.02**
Head width	**0.03±0.01**	**0.04±0.01**	**0.05±0.02**	**0.06±0.01**	**0.07±0.01**	**0.07±0.01**	**0.09±0.01**
Inter ocular distance	**0.02±0.01**	**0.02±0.02**	**0.03±0.01**	**0.04±0.01**	**0.04±0.01**	**0.04±0.02**	**0.06±0.02**
Labial	**0.02±0.01**	**0.02±0.01**	**0.03±0.01**	**0.03±0.01**	**0.04±0.01**	**0.04±0.01**	**0.06±0.01**
Wing Legth	-	-	**0.15±0.02** Wing pad	**0.26±0.03** Wing pad	**0.36±0.02** Wing pad	**0.53±0.02**	**0.58±0.02**

3. Laboratory Rearing of *Scirtothrips dorsalis* on tea and two other hosts

The biology of *S. dorsalis* was studied on three alternative hosts i.e. tea (*C. sinensis*), Chilli (*C. annuum*) and Castor (*R. communis*). Six developmental stages were recorded in *S. dorsalis*: the egg, two larval instars (first and second), two pupal (Prepupa and pupa) stages and the adult. The duration of different life stages of *S. dorsalis* on three host plants recorded (Table 4.10). These stadial periods of *S. dorsalis* were always higher on two non tea hosts that on tea (Table 4.10). *S. dorsalis* preferred oviposition site was on tender leaves. The eggs were kidney-shaped and glossy white in colour. They laid 2-3 eggs per day. The fecundity varied greatly on three hosts. In *C. sinensis* (11.4±0.36) significantly high fecundity was observed than on *C. annuum* (9.7±0.24) and *R. communis* (8.6±0.32). The egg incubation period also varied significantly on three alternative hosts. In *C. sinensis,* egg incubation period was 6.2±0.23 which was significantly less than other two hosts, *C. annuum* (7.1±0.34) *and R. communis* (7.5±0.52).The total development period (TDP) was significantly less in *C. sinensis* (13.6±0.52) than other two alternative hosts i.e. *C. annuum* (15.5±0.36) *and R. communis* (16.7±0.14).The larval development period (LDP) also varied significantly on three alternative hosts, The larval development period was 4.3±0.64 days on *C. sinensis,* 4.9±0.46 days on *C. annuum* and on 5.6 ±0.36 days on *R communis*. The pupal development period (PDP) (in days) showed significant variation on three alternative hosts. On *C. sinensis* pupal development period was 3.1±0.42 days which is significantly less than on other two non tea hosts, *C. annuum* (3.5±0.34 days) and *R. communis* (3.6±0.22 days).

The oviposition period (*C. sinensis*: 3.1 ± 0.52 days, *C. annuum*: 2.6 ± 0.36 days, *R. communis*: 2.5 ± 0.34 days) and hatchability (*C. sinensis*: 92.6 ± 1.14 %, *C. annuum*: 82.5 ± 1.16 %, *R. communis*: 74.6 ± 1.22 %) were noted to be significantly higher on *C. sinensis* (Table 4.11). The age at reproductive maturity and egg incubation periods were lower on *C. sinensis* than the other two host plants (*C. sinensis*: 1.4 ± 0.29 days, *C. annuum*: 1.6 ± 0.21 days, *R. communis*: 1.9 ± 0.32 days). The morphometrics (length in mm) of different life stages and body parts of *S. dorsalis* studied on *C. sinensis* presented in Table 4.12. The body length of the female *S. dorsalis* is 1.06±0.06 mm and was larger than the male which measured 0.72±0.04 mm. The length of the different instar progressively increased (Table 4.12).

Table 4.10 Duration of different life stages of *Scirtothrips dorsalis* on three reared hosts i.e. *C. sinensis, C. annuum* and *R. communis*

Life stages	*Duration (Days)*		
	C. sinensis	*C. annuum*	*R. communis*
First instar larva	**2.22±0.24**	**2.62±0.36**	**2.96±0.24**
Second instar larva	**2.12±0.32**	**2.32±0.22**	**2.62±0.28**
PrePupa	**1.72±0.32**	**1.96±0.34**	**1.98±0.44**
Pupa	**1.42±0.26**	**1.62±0.36**	**1.64±0.52**

Table 4.11 Life history traits of *S. dorsalis* on three different alternative hosts i.e. *C. sinensis*, *C. annuum* and *R. communis*.

Host plants	ARM*	OP	PoOP	FD	IP	HC (%)	LDP	PDP	TDP	AL	
										Male	Female
C. sinensis	1.4±0.29a (1-2)	3.1±0.52a (2-5)	1.4±0.62a (1-2)	11.4±1.36 a (10-14)	6.2±0.23a (6-7)	92.6±1.14a (80-94)	4.3±0.64a (3-5)	3.1±0.42a (3-4)	13.6±1.52a (13-16)	5.2±0.68a (2-3)	5.8±0.39a (4-6)
C. annuum	1.6±0.21b (1-2)	2.6±0.36b (2-4)	1.2±0.35b (1-2)	9.7±1.24b (8-11)	7.1±0.34b (6-8)	82.5±1.16b (70-86)	4.9±0.46b (3-5)	3.5±0.34b (3-4)	15.5±1.36b (13-18)	4.4±0.36b (2-3)	5.4±0.52b (4-6)
R. communis	1.9±0.32b (1-2)	2.5±0.34b (2-4)	1.0±0.26c (1-2)	8.6±1.32c (7-10)	7.5±0.52b (6-8)	74.6±1.22c (60-84)	5.6±0.36c (3-5)	3.6±0.22b (3-4)	16.7±1.14c (14-18)	4.2±0.48b (2-3)	5.4±0.36b (4-6)

*(Means± SE), Figures in parenthesis represent the range.

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

- ARM: Age at reproductive maturity (days);
- OP: Oviposition period (days);
- PoOP: Post oviposition period (days),
- FD: Fecundity (no. of eggs laid/female);
- IP: Incubation (egg) period (days);
- HC: Hatchability (% of eggs hatched);
- LDP: Larval development period (days);
- PDP: Pupal developmental period (days);
- TDP: Total developmental period (days);
- AL: Average longevity (day).

Table 4.12 Morphometrics (length in mm) of different life stages *Scirtothrips dorsalis* on *C. sinensis.* Mean±SD of ten observations.

Life Stages	Length (mm)	Width (mm)
First instar larva	0.30±0.02 (0.28-0.32)*	0.10±0.01 (0.08-0.12)
Second instar larva	0.49±0.02 (0.48-0.52)	0.15±0.02 (0.16-0.18)
Pre-pupa	0.59±0.03 (0.58-0.62)	0.22±0.02 (0.21-0.24)
Pupa	0.60±0.03 (0.55-0.62)	0.23±0.02 (0.22-0.25)
Adult (Male)	0.72±0.04 (0.70-0.74)	0.14±0.02 (0.13-0.15)
Adult (Female)	1.06±0.06 (1.04-1.08)	0.21±0.02 (0.20-0.22)

*Figures in parenthesis represent the range.

The knowledge of population growth parameters of any pest would facilitate the designing of a comprehensive pest management programme for its host plants. So, an understanding of the biology and life history traits of a pest on alternative hosts are essential for the planning and development of effective management strategies. In addition to that how do chemical cues affect host choice and how do insects deal with the plant allelochemicals need to be understood, along with their adaptation to host chemical compounds in constraining or expanding their the ability to cope with other compounds (Berenbaum *et al.*, 1986; Lindroth *et al.*, 1988). The term host plant quality describes the components of the host plant (e.g., the levels of nitrogen, carbon, trace elements, defensive compounds) that positively or negatively affect the performance of herbivorous insects. The host plant quality is a key determinant of the fecundity of herbivorous insects at both the individual and the population scale (Awmack and Leather, 2002). The low quality of plants can perform as a defence mechanism against herbivorous pests and cause a decline in their fecundity and an increase in developmental time (Legrand and Barbosa, 2000).

Host plants have significant effects on the development, survival and reproduction on herbivorous insects (Murphy and Briscoe, 1999; Farazmand *et al.*, 2000; Ju *et al.*, 2011). Host Plant chemistry may also influence the energy budget of herbivorous insects and life history traits (Giles *et al.*, 2002; Castañeda *et al.*, 2010). A female insect encountering a poor-quality host plant may modify her oviposition behavior either by reducing the number of eggs she lays on each plant or, in some cases, adjusting the size or nutritional content of the eggs (Leather and Burnand, 1987). In extreme cases, where the quality of the host plant is too low to support adult survival, female insects may resorb eggs or embryos and use the nutrients gained to increase their longevity and thus their potential to find better-quality host plants for their offspring (Brough and Dixon, 1990; Ward and Dixon, 1982). Plant quality may also affect sex ratios in some insect orders and the importance of the male insect's contribution to reproduction, via the quality of nuptial gifts.

The duration of nymphal development is affected by the quality of the food source (Awang *et al.*, 1988; Ju *et al.*, 2011; Saeed *et al.*, 2010). Significantly shorter nymphal duration (days) of *H. theivora* on *C. sinensis* than on the other two alternative host plants clearly indicates its preference (=acceptability), better adaptation and utilization of *C. sinensis*. The conjecture is further supported by higher fecundity, hatchability and longer oviposition period on *C. sinensis* than on the two other hosts. Physical properties of the plant such as leaf pubescence (Foster *et al.*, 1999), smoothness (Kanno and Harris, 2000), toughness and the presence of trichomes (Pffannenstiel and Yeargan, 1998), and shape and color (Alonso-Pimentel *et al.*, 1998) can also be used by the ovipositing female to assess host plant quality. On a poor-quality host plant a female insect may either lay few good-quality eggs or a large number of poor-quality eggs (Rossiter, 1991a,b; Rossiter *et al.*, 1993). The reduced fecundity, hatchability on *M. micrantha*

and *P. guajava* may be due to high titer of phenol and low ratio of protein to phenol in these host leaves (Banerjee and Hoque, 1985) in addition to difference in structure of egg laying surface as evident in alternative hosts (Presence of trichomes in *M. micrantha*) (Figure 4.1). The differences in these fitness traits are likely due to the nutritional quality of these hosts and incorporation of greater amount of phenolics into ovary and insect system from *M. micrantha* and *P. guajava* hosts (Banerjee and Hoque, 1985; Awmack and Leather, 2002).

II. Detoxifying enzymes activity in Field Collected populations:

Helopeltis theivora

General Esterase Activity (GEs) in H. theivora:

Activities of general esterases (GEs), in *H. theivora* populations collected from different Organic and Conventional tea plantations of the study area showed significant variations (Figure 4.1) and are summarized in Tables 4.13. In male *H. theivora*, the GEs activity was 6.6–11.2-folds higher in insecticide-exposed (conventional) populations from Terai as compared to organic plantations of Terai. Similarly, female *H. theivora*, showed 6.2-10.3-folds higher in insecticide-exposed (conventional) populations than organic plantations from Terai (Tables 4.13)

In the Dooars, GEs activity ranged from 10.5–11.4-folds and 8.3-9.6-folds higher in insecticide-exposed (conventional) male and female *H. theivora* populations respectively than the organic plantations from the Dooars (Tables 4.13).

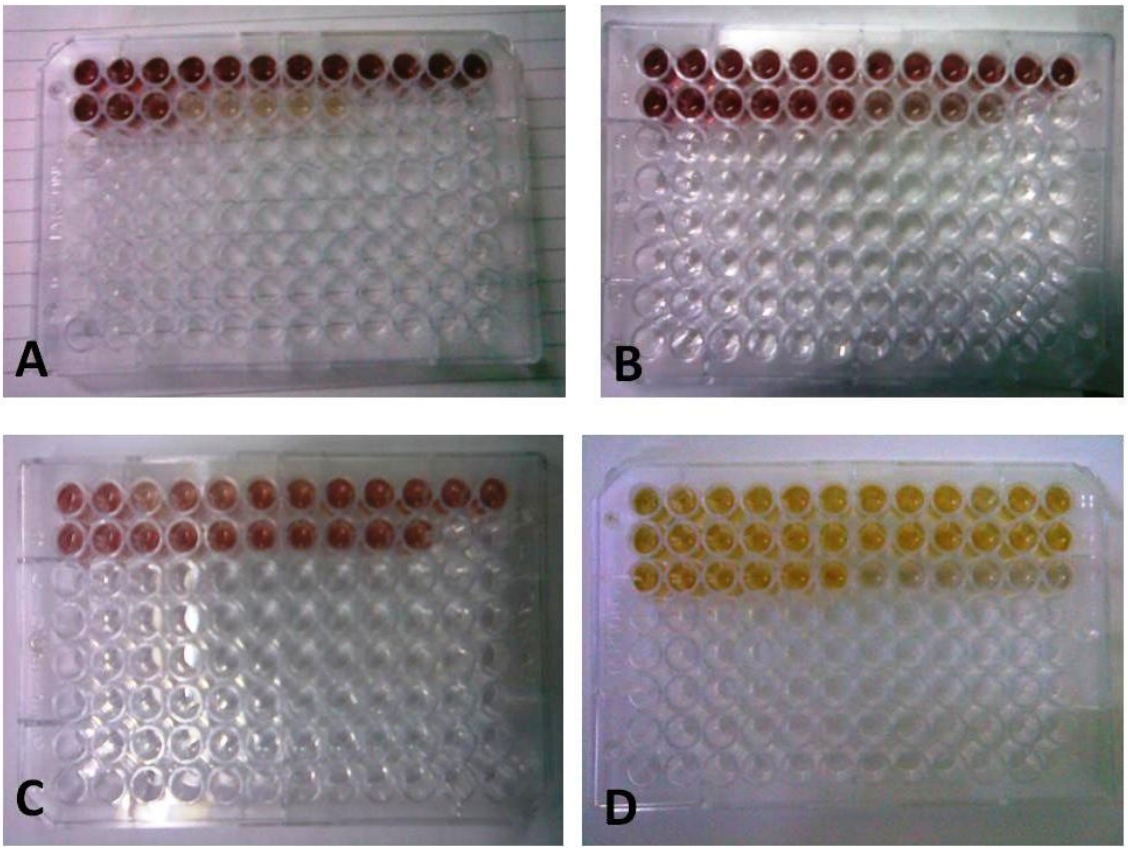

Figure 4.2 General Esterase activity of *H. theivora* from Conventional and organic tea plantations evident through different degree of colour development in the microplate wells (A: Conventional tea plantations of Terai, B: Conventional tea plantations of Dooars, C: Conventional tea plantations of Terai D: Organic tea plantations from Darjeeling hill slopes).

Table 4.13 Activities of general esterases (GEs) in *Helopeltis theivora*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill slopes (Values are Means ± SE).

Populations			No. of specimens tested (n)	General Esterases(GEs) (μ mol min^{-1} mg $protein^{-1}$)	
				Male	Female
Organic T.E.	Darjeeling hill		62	0.11±0.04a[#]	0.12±0.02a
	Terai		70	0.16±0.06a	0.18±0.05a
	Dooars		75	0.17±0.09a	0.22±0.07a
Conventional T.E.	TERAI	Northern Terai	62	1.06±0.08b	1.11±0.09b
		Western Terai	72	1.27±0.16c	1.33±0.14c
		Central Terai_1	63	1.80±.012c	1.85±0.13c
		Central Terai_2	75	1.71±0.11c	1.80±0.11c
		Eastern Terai	70	1.73±0.09c	1.85±0.17c
	DOOARS	Eastern Dooars	66	1.93±0.09d	2.11±0.12d
		Central Dooars_1	72	1.81±0.11c	1.94±0.13d
		Central Dooars_2	75	1.76±0.14c	1.78±0.12c
		Western Dooars	70	1.78±0.09c	1.82±0.11c

[*]Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Cytochrome P450 Monooxygenase Activity (CYPs) in H. theivora:

Cytochrome P450 monooxygenase activity (CYPs) activity also varied among organic and different conventional tea plantations of Terai and the Dooars (Figure 4.3; Table 4.14). CYPs activity ranged from 2.0–3.2 and 1.9–3.2-folds higher in male and female *H. theivora* respectively from the organic tea plantations of Terai. Similarly, in the Dooars, CYPs activity ranged from and 3.0–3.2 and 3.0–3.3-folds higher in male and female *H. theivora* populations respectively compared to its organic plantation population (Table 4.14).

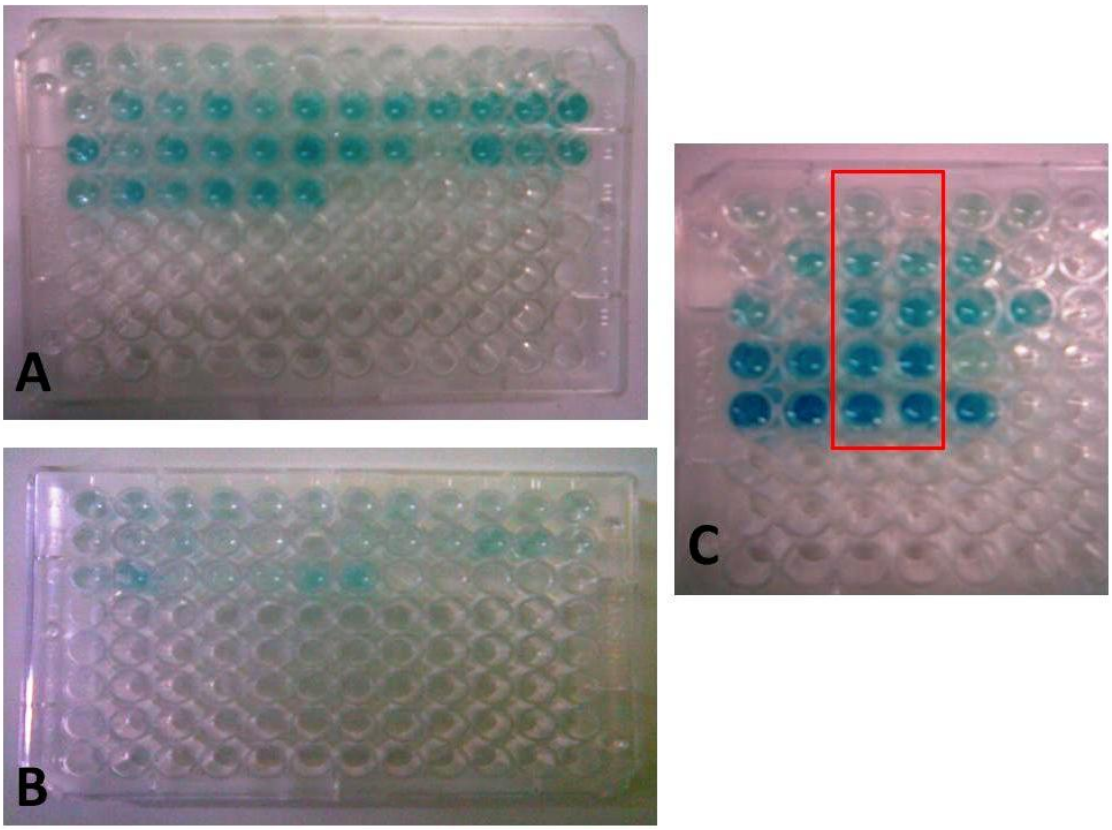

Figure 4.3 Cytochrome P450 monooxygenase activity of *H. theivora* from Conventional and organic tea plantations (A: Conventional tea plantations of Terai, B: Organic tea plantations from Darjeeling hill slopes, C: Standard).

Table 4.14 Activities of cytochrome P450 monooxygenases (CYPs) in *Helopeltis theivora* collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill slopes (Values are Means ± SE).

Populations			No. tested (N)	Monooxygenases (CYPs) (n mol min^{-1} mg $protein^{-1}$)	
				Male	Female
ORGANIC T.E.	Darjeeling hill		62	0.51±0.05a	0.56±0.11a
	Terai		70	0.59±0.04a	0.62±0.07a
	Dooars		75	0.62±0.06a	0.63±0.05a
CONVENTIONAL T.E.	TERAI	Northern Terai	62	1.18±0.06b	1.21±0.09b
		Western Terai	72	1.27±0.14b	1.32±0.16b
		Central Terai_1	63	1.88±0.05c	1.92±0.12c
		Central Terai_2	75	1.89±0.08c	1.94±0.06c
		Eastern Terai	70	1.78±0.06c	1.87±0.06c
	DOOARS	Eastern Dooars	66	1.98±0.09c	1.97±0.08c
		Central Dooars_1	72	1.87±0.11c	1.89±0.09c
		Central Dooars_2	75	1.81±0.12c	1.84±0.12c
		Western Dooars	70	1.91±0.12c	2.06±0.09c

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Glutathione S-transferase Activity (GSTs) in H. theivora:

In *H. theivora*, GSTs activity was 5.2–8.3 and 3.5–5.4-folds higher in Terai and 6.4–8.7 and 4.4–6.0-folds higher in the Dooars in male and female populations respectively (Table 4.15). The activities of all the enzymes were found significantly low in organic populations from tea plantations of Darjeeling foothills (field control specimen).

Table 4.15 Activities of glutathione S-transferases (GSTs) in *Helopeltis theivora* collected from organic and insecticide-managed tea plantations of Terai, Dooars and organic plantations of Darjeeling hill slopes (Values are means ± SE).

Populations			No. tested (N)	Glutathione S-transferases (GSTs) (μ mol min^{-1} mg $protein^{-1}$)	
				Male	Female
ORGANIC T.E.	Darjeeling hill		62	0.16±0.03a	0.24±0.05a
	Terai		70	0.17±0.04a	0.27±0.08a
	Dooars		75	0.19±0.04a	0.29±0.07a
CONVENTIONAL T.E.	TERAI	Northern Terai	62	0.89±0.06b	0.94±0.08b
		Western Terai	72	1.36±0.06c	1.39±0.06c
		Central Terai_1	63	1.35±0.04c	1.38±0.05c
		Central Terai_2	75	1.38±0.04c	1.42±0.04c
		Eastern Terai	70	1.41±0.03c	1.45±0.04c
	DOOARS	Eastern Dooars	66	1.65±0.05d	1.74±0.08d
		Central Dooars_1	72	1.43±0.11c	1.52±0.09c
		Central Dooars_2	75	1.58±0.14d	1.62±0.11d
		Western Dooars	70	1.23±0.13c	1.29±0.11c

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Empoasca flavescens:

General Esterase Activity (GEs) of E. flavescens:

In *E. flavescens*, general esterase (GEs) activity was 3.0-5.2 folds higher in insecticide exposed (conventional) populations from Terai tea plantations than that of laboratory reared control specimens. Similarly, in the Dooars, the enzyme activity was found to be 3.0-9.7 folds higher in comparison to the laboratory reared specimens (Table 4.16).

Table 4.16 Activities of general esterases (GEs) in *Empoasca flavescens*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill region, and in laboratory-reared control specimens (Values are means ± SE).

POPULATIONS		No. specimen tested (n)	General Esterases (GEs) (μ mol min^{-1} mg $protein^{-1}$)
	Laboratory reared (L_R)	60	0.18±0.02a*
TERAI	Organic T.E. (O_T)	60	0.22±0.01a
	Insecticide managed T.E. (T_1)	60	0.54±0.02b
	Insecticide managed T.E. (T_2)	60	0.93±0.03c
	Insecticide managed T.E. (T_3)	60	0.88±0.03c
	Insecticide managed T.E. (T_4)	60	0.64±0.02b
	Insecticide managed T.E. (T_5)	60	0.62±0.03b
DOOARS	Organic T.E. (O_D)	60	0.31±0.03a
	Insecticide managed T.E. (D_1)	60	1.17±0.03d
	Insecticide managed T.E. (D_2)	60	1.80±0.04e
	Insecticide managed T.E. (D_3)	60	0.55±0.02b
	Insecticide managed T.E. (D_4)	60	0.83±0.03c
	Insecticide managed T.E. (D_5)	60	1.74±0.04e

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Cytochrome P450 Monooxygenase Activity (CYPs) of E. flavescens:

In *E. flavescens*, cytochrome P450 monooxygenase (CYPs) activity was 1.5-4.8 folds higher in insecticide exposed (conventional) populations from Terai tea plantations than that of laboratory reared control specimens. Similarly, in the Dooars, the enzyme activity was found to be 3.6-5.3 folds higher in comparison to the laboratory reared specimens (Table 4.17).

Table 4.17 Activities of cytochrome P450 monooxygenases (CYPs) in *Empoasca flavescens*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill region, and in laboratory-reared control specimens (Values are means ± SE).

POPULATIONS		No. specimen tested (n)	Cytochrome P450 (CYPs) (n mol min^{-1} $mg\ protein^{-1}$)
	Laboratory reared (L_R)	60	0.21±0.02a
TERAI	Organic T.E. (O_T)	60	0.24±0.02a
	Insecticide managed T.E. (T_1)	60	1.01±0.03b
	Insecticide managed T.E. (T_2)	60	0.93±0.03b
	Insecticide managed T.E. (T_3)	60	0.78±0.04c
	Insecticide managed T.E. (T_4)	60	0.86±0.02c
	Insecticide managed T.E. (T_5)	60	0.62±0.03d
DOOARS	Organic T.E. (O_D)	60	0.26±0.03a
	Insecticide managed T.E. (D_1)	60	0.81±0.02c
	Insecticide managed T.E. (D_2)	60	0.88±0.02b
	Insecticide managed T.E. (D_3)	60	0.76±0.02c
	Insecticide managed T.E. (D_4)	60	0.97±0.03b
	Insecticide managed T.E. (D_5)	60	1.12±0.04e

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Glutathione S-transferase Activity (GSTs) of E. flavescens:

In *E. flavescens*, glutathione S-transferases (GSTs) activity was 1.2 - 3.5 folds higher in insecticide exposed (conventional) populations from Terai tea plantations than that of laboratory reared control specimens. Similarly, in the Dooars, the enzyme activity was found to be 1.5 - 2.5 folds higher in comparison to the laboratory reared specimens (Table 4.18).

Table 4.18 Activities of glutathione S-transferase (GST) in *Empoasca flavescens*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill region, and in laboratory-reared control specimens (Values are means ± SE).

POPULATIONS		No. specimen tested (n)	Glutathione S-transferase (μ mol min^{-1} mg $protein^{-1}$)
	Laboratory reared (L_R)	60	1.35±0.03a
TERAI	Organic T.E. (O_T)	60	1.38±0.02a
	Insecticide managed T.E. (T_1)	60	1.92±0.06b
	Insecticide managed T.E. (T_2)	60	4.67±0.12c
	Insecticide managed T.E. (T_3)	60	4.29±0.13d
	Insecticide managed T.E. (T_4)	60	1.60±0.02a
	Insecticide managed T.E. (T_5)	60	2.18±0.06b
DOOARS	Organic T.E. (O_D)	60	1.42±0.03a
	Insecticide managed T.E. (D_1)	60	2.37±0.05b
	Insecticide managed T.E. (D_2)	60	2.19±0.05b
	Insecticide managed T.E. (D_3)	60	2.07±0.05b
	Insecticide managed T.E. (D_4)	60	3.41±0.04e
	Insecticide managed T.E. (D_5)	60	2.25±0.05b

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Scirtothrips dorsalis:

General Esterase Activity (GEs) of S. dorsalis:

Similar, results were obtained for general esterase activity for *S. dorsalis* which was higher by 2.0-6.0 folds in Terai and 2.3-5.6 folds in the Dooars respectively. There was no significant difference in GEs activity in populations among the two organically-managed tea plantations and laboratory-reared control specimens (Table 4.19).

Table 4.19 Activities of general esterases (GEs) in *Scirtothrips dorsalis*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill region, and in laboratory-reared control specimens (Values are means ± SE).

POPULATIONS		No. tested (N)	General Esterase (GEs) (μ mol min^{-1} mg $protein^{-1}$)
	Laboratory reared (L_R)	60	0.53±0.07a*
TERAI	Organic T.E. (O_T)	60	0.58±0.04a
	Insecticide managed T.E. (T_1)	60	1.10±0.02b
	Insecticide managed T.E. (T_2)	60	1.21±0.05b
	Insecticide managed T.E. (T_3)	60	1.29±0.03b
	Insecticide managed T.E. (T_4)	60	3.16±0.07c
	Insecticide managed T.E. (T_5)	60	1.41±0.05b
	Insecticide managed T.E. (T_6)	60	3.20±0.10c
DOOARS	Organic T.E. (O_D)	60	0.68±0.03a
	Insecticide managed T.E. (D_1)	60	1.24±0.06b
	Insecticide managed T.E. (D_2)	60	1.33±0.05b
	Insecticide managed T.E. (D_3)	60	1.44±0.03b
	Insecticide managed T.E. (D_4)	60	2.87±0.05c
	Insecticide managed T.E. (D_5)	60	1.26±0.06b
	Insecticide managed T.E (D_6)	60	2.97±0.04c

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Cytochrome P450 Monooxygenase Activity (CYPs) of S. dorsalis:

Cytochrome P450 monooxygenase (CYPs) activity was 1.5- 2.3 and 1.6-2.4 folds higher in *S. dorsalis* collected from insecticide-managed tea plantations of Terai and the Dooars respectively (Table 4.20).

Table 4.20 Activities of cytochrome P450 monooxygenases (CYPs) in *Scirtothrips dorsalis*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill region, and in laboratory-reared control specimens (Values are means ± SE).

POPULATIONS		No. tested (N)	Cytochrome P450 (CYPs) (n mol min^{-1} mg $protein^{-1}$)
	Laboratory reared (L_R)	60	0.83±0.06a
TERAI	Organic T.E. (O_T)	60	0.89±0.04a
	Insecticide managed T.E. (T_1)	60	1.27±0.02b
	Insecticide managed T.E. (T_2)	60	1.35±0.06b
	Insecticide managed T.E. (T_3)	60	1.76±0.05c
	Insecticide managed T.E. (T_4)	60	1.22±0.04b
	Insecticide managed T.E. (T_5)	60	1.31±0.06b
	Insecticide managed T.E. (T_6)	60	1.88±0.05c
DOOARS	Organic T.E. (O_D)	60	0.99±0.03a
	Insecticide managed T.E. (D_1)	60	1.34±0.07b
	Insecticide managed T.E. (D_2)	60	1.48±0.07b
	Insecticide managed T.E. (D_3)	60	1.88±0.04c
	Insecticide managed T.E. (D_4)	60	1.26±0.05b
	Insecticide managed T.E. (D_5)	60	1.37±0.04b
	Insecticide managed T.E (D_6)	60	1.93±0.05c

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

Glutathione S-transferase Activity (GSTs) of S. dorsalis:

GST activity was 2.6-3.7 and 2.3-3.6 folds higher in *S. dorsalis* populations from insecticide managed tea plantations of Terai and Dooars respectively than that of laboratory reared control specimens (Table 4.21). There were no significant differences in the activities in populations obtained from organic tea plantations and the one reared in laboratory

Table 4.21 Activities of Glutathione S-transferases (GSTs) in *Scirtothrips dorsalis*, collected from organic and insecticide-managed tea plantations of Terai, Dooars and Darjeeling hill region, and in laboratory-reared control specimens (Values are means ± SE).

POPULATIONS		No. tested (N)	Glutathione S-transferase (GSTs) (μ mol min^{-1} mg $protein^{-1}$)
	Laboratory reared (L_R)	60	0.87±0.09a
TERAI	Organic T.E. (O_T)	60	0.93±0.06a
	Insecticide managed T.E. (T_1)	60	2.25±0.07b
	Insecticide managed T.E. (T_2)	60	2.98±0.08c
	Insecticide managed T.E. (T_3)	60	2.34±0.06b
	Insecticide managed T.E. (T_4)	60	2.19±0.04b
	Insecticide managed T.E. (T_5)	60	3.11±0.05c
	Insecticide managed T.E. (T_6)	60	3.18±0.18c
DOOARS	Organic T.E. (O_D)	60	0.99±0.03a
	Insecticide managed T.E. (D_1)	60	1.98±0.08b
	Insecticide managed T.E. (D_2)	60	2.78±0.06c
	Insecticide managed T.E. (D_3)	60	3.05±0.07c
	Insecticide managed T.E. (D_4)	60	2.94±0.06c
	Insecticide managed T.E. (D_5)	60	1.75±0.05b
	Insecticide managed T.E (D_6)	60	3.12±0.09c

*Within columns, means followed by the same letter do not differ significantly ($P < 0.05$).

III. Densitometric analysis of electro-phoregram of General Esterases:

Helopeltis theivora:

The electrophoretic analysis for general esterases revealed six isozyme bands, Est I-VI (Figure 4.4). The Rm values of the esterase bands (Est I-VI) were 0.25, 0.35, 0.47, 0.64, 0.76 and 0.89 respectively. The staining intensity (level of expression) was found to be higher as evident from the densitometric study of the populations of *H. theivora* in specimens from conventional tea plantations of Terai and the Dooars as compared to susceptible specimens from organic tea plantations from Darjeeling foothills (field control specimens) (Figure 4.4). The susceptible specimens of organic tea plantations showed a very low level of expression for all the esterase bands (Est I-VI). Such a low profile was found true for specimens of the Dooars (organic) and Darjeeling hill (organic) except for Est I and II bands (Figure 4.4).

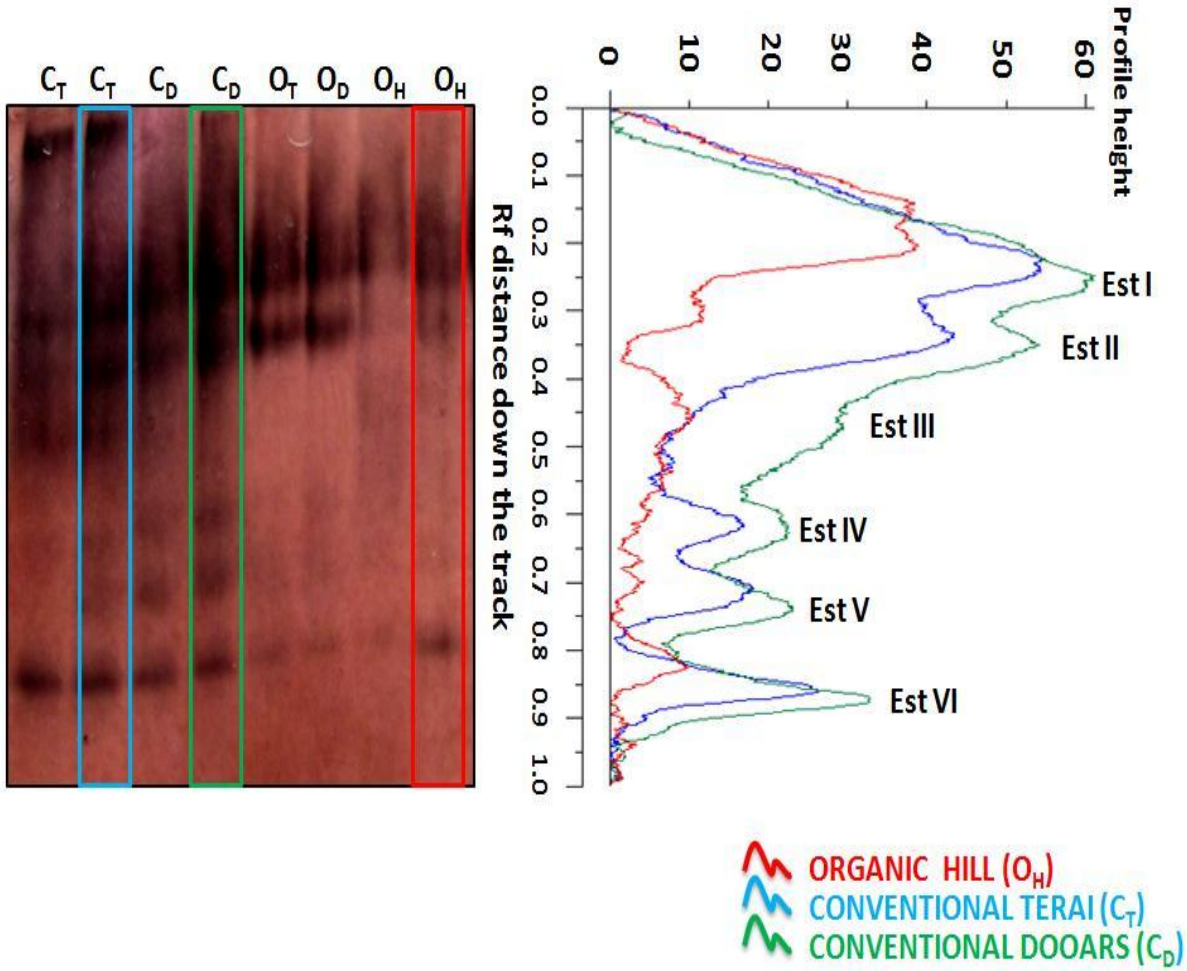

Figure 4.4 Densitometric analysis of electro-phoregram of general esterase of *H. theivora* from Conventional and organic tea plantations (C_T: Conventional tea plantations of Terai, C_D: Conventional tea plantations of Dooars, O_T: Organic tea plantations of Terai, O_D: Organic tea plantations of Dooars and O_H: Organic tea plantations of Darjeeling foothills.

Compared to the populations of *H. theivora* from organic tea plantations of Darjeeling foothills, the conventional field populations from the Dooars and Terai varied considerably in the level of activity of three principal detoxifying enzymes. The conventional field collected strain was expected to have developed tolerance against the regularly sprayed different synthetic insecticides. The development of tolerance may be related to the history of the commonly sprayed insecticides or other pesticides of similar groups (Fragoso *et al.*, 2002; Reyes *et al.*, 2009; Zhu *et al.*, 2011).

Kind and quality of chemical applications for the tea cultivation area can be assessed from the marketing statistics of the local outlet of chemical companies and the survey report on use pattern of insecticides in tea plantations of the North Bengal (Sannigrahi and Talukdar, 2003 and Roy *et al.*, 2008b). The literature reveals that in conventional tea plantations different insecticides of same or different functional groups or mode of actions are routinely sprayed to control insect and mite tea pests. The target sites and mode of actions are different for each group of insecticides such as organophosphates and carbamates have the target site acetylcholinesterase, pyrethroids and DDT have the sodium channel, Chlorinated cyclodiens have the GABA receptor, nicotinyl insecticides have nicotinic acetylcholine receptor (Yu, 2008). At the same time general esterase and cytochrome P450s are the phase I (Primary) detoxifying enzymes which mainly by oxidation, hydrolysis and reduction detoxify insecticides, and GSTs are the phase II detoxifying enzymes which conjugate the polar products with various endogenous compounds such as sugars, sulphate, phosphate amino acids or glutathione (Yu, 2008).

Metabolic detoxification of insecticides is an important toxico-kinetic mechanism in insects to reduce the toxic effects of insecticides. Esterases have great versatility and are

generally involved in the metabolism of organophosphorous insecticides (Cao *et al.*, 2008a).Overproduction of esterase can result from up-regulation of the transcription of a single copy of the esterase gene (Berrada and Fournier, 1997) or accumulation of multiple copies of the esterase genes (Siegfried *et al.,* 1997; Hawkes and Hemingway, 2002). Elevated levels of esterase gene expression may reach range from 4- to 80-fold higher in resistant strains (Field *et al.,* 1999). The expression difference of carboxylesterase gene has been sufficiently reflected by a difference in carboxylesterase activity. Compared with susceptible strain, 5.1-fold higher expression level of esterase gene causes more than 6-, 3- and 10-fold higher activities using α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), and p-nitrophenyl acetate (p-NPA) as subtracts in malathion resistant strain of tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae), respectively (Zhu *et al.,* 2004). The same trend was also observed in omethoate resistant strain of *Aphis gossypii* (Hemiptera: Aphididae). Compared with the susceptible strain, the relative transcription levels and gene copy numbers of the carboxylesterase were 4.54- and 0.97-fold in the resistant strain which led to significantly higher carboxylesterase activity in resistant strain than in susceptible strain (Cao *et al.*, 2008a). In the deltamethrin-resistant strain of *A. gossypii*, carboxylesterase mRNA expression level was 6.61-folds higher than the susceptible strain. However, carboxylesterase activity in the deltamethrin-resistant strain of *A. gossypii* showed 3.67-, 2.02- and 1.16-folds increase over susceptible strain when different substrates like α-NA, β-NA and α-NB were used respectively for assay (Cao *et al.*, 2008b). In malathion-resistant *A. gossypii* over expression of carboxylesterase was determined (Pan *et al.*, 2009).The relative transcript level of carboxylesterase gene was 1.99-folds in the resistant strain compared with the susceptible strain of the cotton aphids. Compared with the susceptible strain the relative gene copy number was 4.42-fold in the resistant strain indicating a significant

difference in the gene copy number of carboxyl esterase between the malathion-resistant and susceptible strains. The expression difference in carboxyl esterase gene has been reflected by a difference in the activity with 3.7-, 3.0-, 2.0-, 2.9-, and 1.6-fold more carboxyl esterase activity with α-NA, β-NA, α-NPr, α-NB and α-naphthyl caprylate (α-NC) as substrates in malathion-resistant strain than in susceptible strain respectively. These results showed that the increased transcription levels of carboxylesterase mRNA and gene copy number were associated with the malathion resistance in cotton aphids.

Zhu and Snodgrass (2003) demonstrated different structures of cytochrome P450 cDNAs and corresponding gene expression differences between pyrethroid-susceptible and resistant strains of *Lygus lineolaris*. This P450 gene expression was inducible by pyrethroid treatment. Multiple applications of malathion to cotton, done for boll weevil eradication in Mississippi during 1999–2001, were found to cause increases in malathion resistance as high as 30.5-fold in plant bug populations collected from wild hosts in the fall after the malathion treatments to cotton ended (Snodgrass and Scott, 2003). Malathion resistance is usually associated with increased carboxylesterase activity (Liu and Han, 2003; Wool and Front, 2003; Perez-Mendoza *et al.*, 2000; Smyth *et al.*, 2000; Raghavendra *et al.*, 1998). Single-gene point mutation was also observed in many resistant insects (Campbell *et al.*, 1998a; Zhu *et al.*, 1999; Smyth *et al.*, 2000). Resistant insects were able to survive malathion treatment by sequestering (Karunaratne and Hemingway, 2001) or metabolizing malathion to non-toxic malathion acids and malaoxon acids (Campbell *et al.*, 1998b).

There was significant enhancement in different detoxifying enzymes activity in *H. theivora* from the conventional tea plantations. In resistant pest species due to insecticide selection higher level of activity of general esterase (Yu *et al.*, 2003; Hemingway *et al.*, 2004; Wu *et al.* 2011) has

been noticed. Detoxifications by cytochrome P450-monooxygrnase are generally considered to be an important mechanism in conferring resistance in insects (Hodgson and Kulkarni 1983; Feyereisen 1995, 1999). Oxidation mediated by cytochrome P450 monooxygenases is considered to be the major pathway for insecticide detoxification (Feyereisen, 1999). When piperonyl butoxide was synergisted with the insecticides there was increase in the susceptibility level of *H. theivora* indicating the involvement of cytochrome P450 mediated metabolism of insecticides (Roy *et al.* 2009b). A strong correlation between fenvalerate toxicity and cytochrome P450 activity in *Helicoverpa armigera* has also been reported by Chen *et al.* (2005). Such a correlation involving cytochrome P450 indicates strongly its role as main detoxifying enzymes in *H. theivora*.

Glutathione-S-transferase enzymes (GST) play an important role in detoxification of xenobiotic compounds including insecticides. GSTs can produce resistance to a range of insecticides by conjugating reduced glutathion (GSH) to the insecticide or by its primary toxic metabolic products (Hemingway *et al.*, 2000; Enayati *et al.*, 2005). In the insecticide resistant strains of housefly, the OP resistance is associated with elevated levels of GST activity compared with that of susceptible flies (Zhou and Syvanen, 1997).GST activity also increases with the insecticide resistance (Nehare *et al.*, 2010). Increased GST activity was associated with organophosphate resistance (Cheng *et al.*, 1983; Yu and Ngugen, 1992) have been reported indicating their role in detoxifying mechanisms. Perera *et al.* (2008), Sarker *et al.* (2009) and Zhu *et al.* (2011) have shown that in insecticide-treated field populations of insects, the activity of all three principal detoxifying enzymes (general esterase, GSTs and cytochrome P450) may be enhanced.

Qualitative changes of esterases may also give rise to resistance (van Asperen and Oppenorth, 1959; Devonshire and Field, 1991; Wu *et al.*, 2011). Esterase based resistance due to its qualitative changes is recorded in *Musca domestica* (van Asperen and Oppenoorth, 1960), *Myzus persicae* (Devonshire, 1977) and *Blattella germanica* (Prabhakaran and Kamble, 1993). The electrophoretic analysis for general esterases in *H. theovora* revealed six isozyme bands, Est I-VI. The expression levels of bands (colour intensity) were higher in specimens from conventional tea plantations of Terai and the Dooars as compared to that of *H. theivora* populations of organic tea plantations from Darjeeling foothills. The susceptible specimens showed a very low level of expression for all the esterase isozymes, particularly Est III-VI, which was also found true in quantitative estimation (Table 4.13). These differential expressions of GEs indicate their relation with insecticide-tolerance levels in *H. theivora.* The increase in the activity of detoxifying enzymes in *H. theivora* populations from conventional plantations strongly advocates that continuous exposure to different insecticides has selected of *H. theivora* populations through several decades to become more tolerant to the commonly sprayed insecticides. In pest management programs, treatment decisions are based on economic thresholds or aesthetic injury levels. However, insecticide-resistance reduces the efficacy of insecticide treatments (Kawai, 1997), thereby influencing the decision process by reducing the number of viable treatment options. An enzyme-based early detection of resistant or tolerant populations of *H. theivora* may help in taking decision on the insecticides selection and spray operations. Similarly, an understanding of the susceptibility levels of the pest to insecticides can be helpful in monitoring the process of development of insecticide-tolerant strains of its populations spread over Terai and the Dooars, hence opening up opportunities of better planning of management.

Densitometric analysis of electrophoregram of General Esterases of Empoasca flavescens and Scirtothrips dorsalis:

The electrophoretic analysis for GEs revealed six isozyme bands, Est I-VI in *E. flavescens* (Figure 4.5) and five isozyme bands, Est I-V, in *S. dorsalis* (Figure 4.6). The Rf values of the esterase bands of *E. flavescens* (Est I-VI) were 0.17, 0.21, 0.26, 0.34, 0.41 and 0.48 respectively. Similarly the Rf values of the esterase bands of *S. dorsalis* (Est I-V) were 0.10, 0.26, 0.31, 0.36 and 0.47 respectively. The expression level was found to be higher in specimens from insecticide-exposed as compared to organic and laboratory-reared control specimens. In *E. flavescens* Est I-VI were found to be constitutively expressed in insecticide-exposed tea plantations from Terai and the Dooars, whereas negligible expression was observed in specimen of organic plantations (Figure 4.5). The laboratory-reared control specimens showed a very low level of expression for all the bands of esterases (Est I-V) (Figure 4.5) which is also evident from quantitative estimation of general esterases (Table 4.16). Similarly a very low expression was observed in *S. dorsalis* obtained from organic plantations (Figure 4.6). The laboratory reared control specimens also showed a very low intensity for all the esterase bands (Est I-V) (Figure 4.6) which is also corroborated by the low value registered in quantitative estimation (Table 4.19).

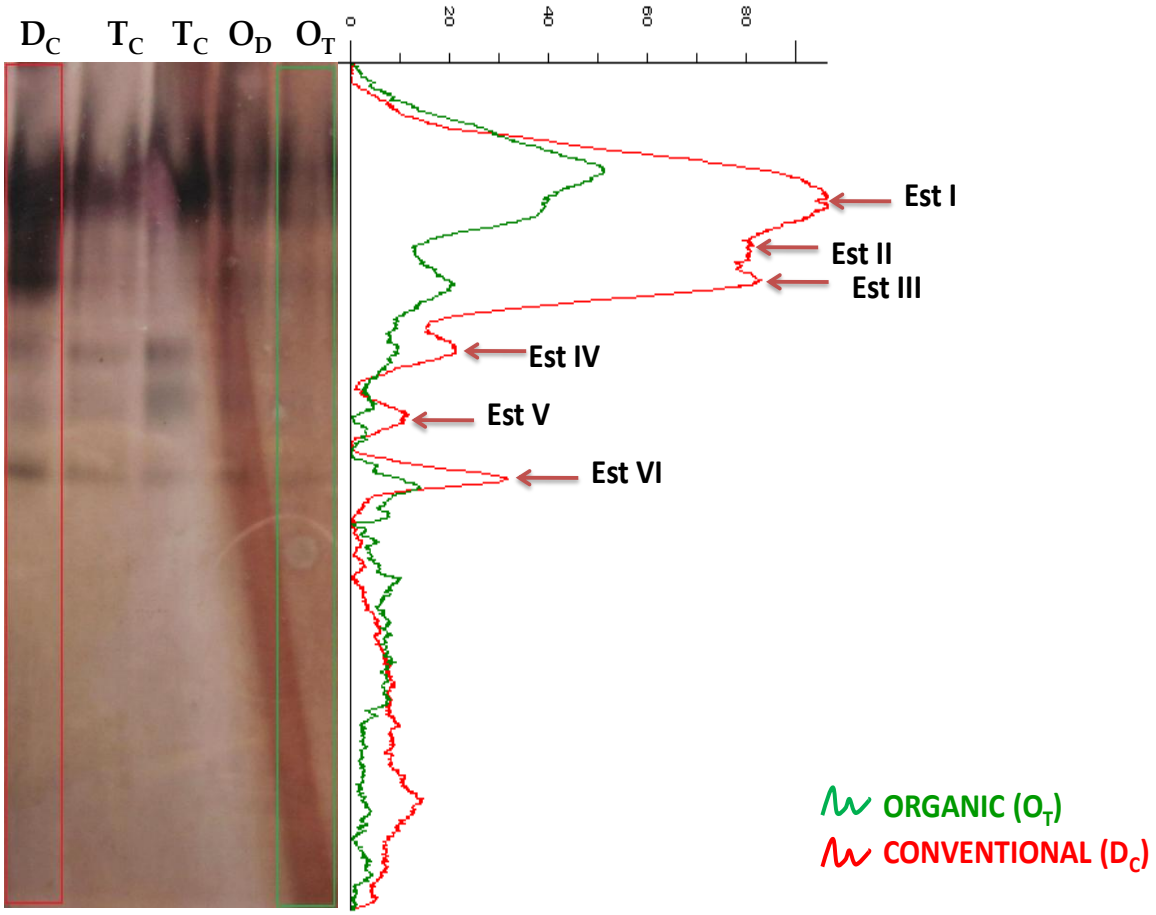

Figure 4.5 Densitometric analysis of electro-phoregram of general esterases of *E. flavescens* from Organic and Conventional Tea Plantations. (T_C= Conventional tea plantations of Terai, D_C= Conventional tea plantations of Dooars, O_T= Organic tea plantations of Terai, O_D= Organic tea plantations of Dooars).

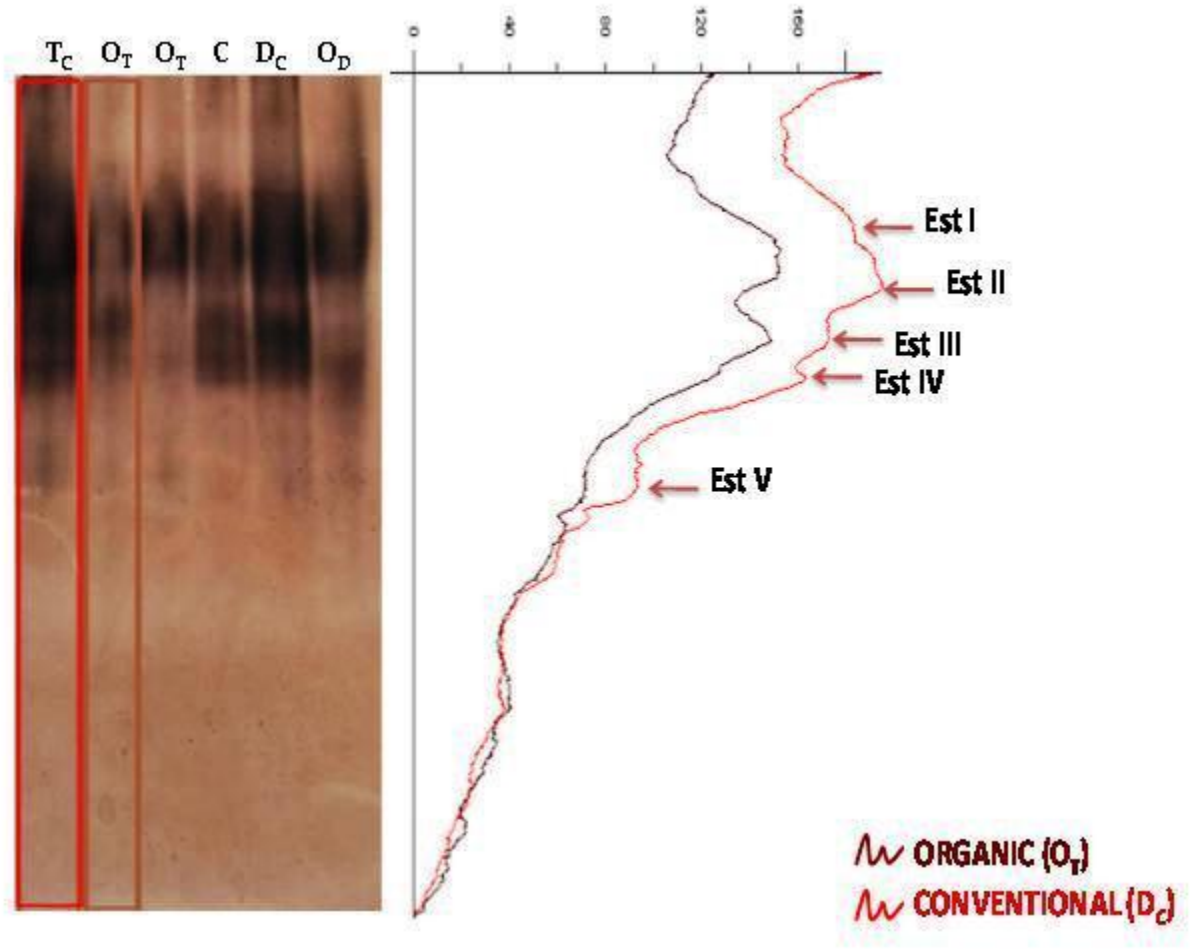

Figure 4.6 Densitometric analysis of electro-phoregram of general esterases of *S. dorsalis* from Conventional and Organic Tea Plantations. (T_C= Conventional tea plantations of Terai, C= Laboratory control, D_C= Conventional tea plantations of Dooars, O_T = Organic tea plantations of Terai and O_D= Organic tea plantations of Dooars).

In conventional tea plantations of Terai and the Dooars, *E. flavescens* and *S. dorsalis* are the two upcoming sucking pests exposed continuously to spray of different kinds of synthetic insecticides such as organochlorines, organophosphates, synthetic pyrethroids and neonicotinoids for many generations (Gurusubramanian *et al.*, 2008a, 2009) evenwhen the sprays areapplied for the control of other major sucking and chewing pests. Many insecticides contain carboxyl, phosphate, or carbamate ester bonds. Hydrolytic cleavage of ester bonds is an important mechanism in detoxification of insecticides. The enzymes hydrolyzing these bonds are esterases that comprise a diverse group of enzymes. The hydrolytic degradation of organophosphorus (OPs) insecticides has long been implicated in the development of resistance to these compounds in treated insect populations (Oppenoorth, 1985). The above esterase-based resistance mechanisms are not mutually exclusive; both a modified gene structure and an increase in expression of carboxylesterase may be responsible for the high level of malathion resistance have been found in *Anisopteromalus calandrae* (Zhu *et al.*, 1999). The esterases have great versatility and broad substrate specificity and are generally involved in metabolism of organophosphorous insecticides. Esterases involved in detoxification of insecticides have often been reported to be present in enhanced quantity in resistant insect populations (Oppenoorth, 1985; Soderlund and Bloomquist, 1990; Nehare *et al.*, 2010; Wu *et al.*, 2011). It has been reported that high levels of GST and esterase activities are present in resistant thrips (Immaraju *et al.*, 1990, 1992; Ferrari, 1993; Zhao *et al.*, 1994, 1995; Jensen, 2000). The sucking pests, *S. dorsalis* and *E. flavescens* both have a short generation time and high fecundity (Ananthakrisnan, 1993; DeLong, 1971). Such populations get selected under insecticide-exposed condition with fast development of tolerance/resistance in them. The higher activity of the general esterases occurs through overproduction of enzymes. In the cotton aphid, *Aphis gossypii* (Glover)

(Hemiptera: Aphididae), esterase-mediated OPs resistance has been reported in many instances (O'Brien *et al.,* 1992; Saito, 1993; Suzuki *et al.,* 1993; Owusu *et al.*,1996; Suzuki and Hama, 1998). The elevation of esterase activity through (i) gene amplification or (ii) up-regulate transcription and (iii) point mutations within the esterase structural genes changing their substrate specificity were predominantly the molecular basis of esterase-mediated resistance in target insects (Hemingway, 2000). Over-production of esterases as an evolutionary response to organophosphorus insecticide selection pressure has been well documented in numerous arthropod species including *Myzus persicae* (Field and Devonshire, 1997; Field *et al.,* 1999; Bizzaro *et al.,* 2005), *A. gossypii* (Cao *et al.,* 2008a), *Culex pipiens quinquefasciatus* (Karunaratne, 1994; Karunaratne *et al.*,1995; Vaughan *et al.*,1997; Paton *et al.*, 2000), other mosquitoes in Culicine (Devonshire and Field, 1991; Vaughan and Hemingway,1995; Hemingway *et al.*,1998, 2004; Mouches *et al.*,1986), and the brown planthopper, *Nilaparvata lugens* (Small and Hemingway, 2000). Over production of esterases that detoxify or sequester insecticides has also been noticed in resistant populations of *Culex pipiens* (Diptera: Culicidae) mosquito complex (Feyereisen, 1999). Using five different substrates, α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), α-naphthyl propionate (α-NPr), α-naphthyl butyrate (α-NB), α-naphthyl caprylate (α-NC) and S-methyl thiobutyrate (S-MTB), Pan *et al.*(2009) has reported that carboxylesterase activity in malathion resistant *Aphis gossypii* was 3.7-fold for α-NA, 3.0-fold for β-NA, 2.0-fold for α-NPr, 2.9-fold for α-NB and 1.6-fold for α-NC, So, higher activity of the GEs and GSTs in insecticide-exposed populations of these two pests under study compared to the control specimens indicates the involvement of enzyme based detoxifying mechanism. The activity of GEs and GSTs was similar and at a low level in both the pest specimens collected from organic tea plantations and the laboratory-reared ones. The results therefore, signify that

GEs and GSTs both play the most important role in the metabolism of insecticides in the insecticide-exposed *S. dorsalis* and *E. flavescens* in conventional tea plantations in this region.

Detoxifications by CYPs are generally considered to be an important mechanism in conferring tolerance/resistance to insects (Hodgson and Kulkarni, 1983; Agosin, 1985; Oppenoorth, 1985; Soderlund and Bloomquist, 1990). Possible involvement of CYPs in insecticide metabolism of western flower thrips has been established by using synergist piperonyl butoxide (Immaraju *et al.*, 1992; Jensen, 2000). An elevated level of activity of CYP in insecticide-exposed populations compared to organic and laboratory-reared control populations of *S. dorsalis* and *E. flavescens* also imply the involvement of the same mechanism of detoxification in these two pests of tea.

Esterase based resistance due to qualitative changes in esterases have been recorded in *Musca domestica* (van Asperen and Oppenoorth, 1959, 1960) and *Myzus persicae* (Devonshire, 1977). In our study the Est I and II are expressed in all categories of populations whereas Est III, IV and V were highly expressed only in insecticide-exposed populations of *S. dorsalis*. These enhanced expressions of the three bands of GEs indicate their relation with insecticide tolerance in *S. dorsalis.* Similarly, in *E. flavescens* Est I, II and III were highly expressed only in insecticide-exposed populations. So, it appears that continuous exposure of different insecticides has induced these two sucking pests to become more tolerant to the insecticides.

Host based variation in detoxifying enzymes activity:

Helopeltis theivora

Detoxifying enzymes activity

Detoxifying enzymes activity data from different host-based (specific) populations of *H. theivora* were subjected to two-way ANOVA followed by Student's *t* test at *p*=0.05 (**Table 4.22)**. General esterases activity was significantly high in specimen reared on *M. micrantha* and *P. guajava* than on *C. sinensis*. But the activity of the same enzyme between first two alternative hosts was non-significant. However, there was no significant difference in GSTs activity in *H. theivora* when fed on different host plants.

Table 4.22 General esterases (GEs), Cytochrome P450 monooxygenases (CYPs) and glutathione S-transferases (GSTs) activity (Values are means ± SE) in *H. theivora* reared on three different host plants.

Populations	No. tested (n)	General Esterases (GEs) (µmol α napthol min^{-1} $mg\ protein^{-1}$)		Cytochrome P450 monooxygenases (CYPs) (n mol min^{-1} $mg\ protein^{-1}$ as equivalent to Cytochrome P450)		Glutathione S-transferase (GSTs) (µmol GSH conjugated $min^{-1}mg\ protein^{-1}$)	
		Male	Female	Male	Female	Male	Female
C. sinensis (Tea)	60	$0.14\pm0.04^{a*}$	0.15 ± 0.03^{a}	0.96 ± 0.17^{a}	0.99 ± 0.13^{a}	0.31 ± 0.07^{a}	0.36 ± 0.08^{a}
M. micrantha (Mikania)	60	0.21 ± 0.04^{b}	0.22 ± 0.04^{b}	1.18 ± 0.12^{b}	1.36 ± 0.14^{b}	0.32 ± 0.05^{a}	0.37 ± 0.05^{a}
P. guajava (Guava)	60	0.23 ± 0.05^{b}	0.24 ± 0.04^{b}	1.27 ± 0.13^{c}	1.33 ± 0.18^{b}	0.32 ± 0.05^{a}	0.36 ± 0.05^{a}

*Within columns, means followed by the same letter do not differ significantly (P<0.05).

Densitometric analysis of electro-phoregram of General Esterases:

Non-denaturing PAGE analysis of general esterases revealed four (Est I-IV) isozyme bands in male *H. theivora* (Figure 4.7). The Rf values of the Est I-IV were 0.17, 0. 24, 0.41 and 0.55 respectively. The expression levels were higher when reared on two alternative hosts (*M. micrantha* and *P. guajava*) than on *C. sinensis*, in which the expression level was very low. In female *H. theivora*, only Est I and III had expressed and Est II and IV isozyme bandss were not expressed at all. Est I showed high expression on two alternative hosts in both sexes whereas on *C. sinensis* expression was low. Esterase II also showed differential expression in the sexes on two alternative hosts and on C. *sinensis*. Interestingly in female individual reared on two alternative hosts, but for *C. sinensis*, Est II did not express. This was evident from the densitometric analysis of esterase bands (Figure 4.7 & 4.8). Esterase III showed almost equal level of expression both in male and female *H. theivora* reared on two alternative hosts, but the same isozymes was of low intensity when reared on *C. sinensis*. However, Est IV isozyme expressed only in male *H. theivora* on *C. sinensis* and other two alternative hosts as well. *H. theivora*, reared on *P. guajava* showed higher expression of esterase isozymes in both sexes than that on *M. micrantha* and *C. sinensis* hosts. This was also reflected in quantitative estimation of the activity (Table 4.22). Female individuals reared on all three hosts lacked expression of Est IV isozymes (Figure 4.7).

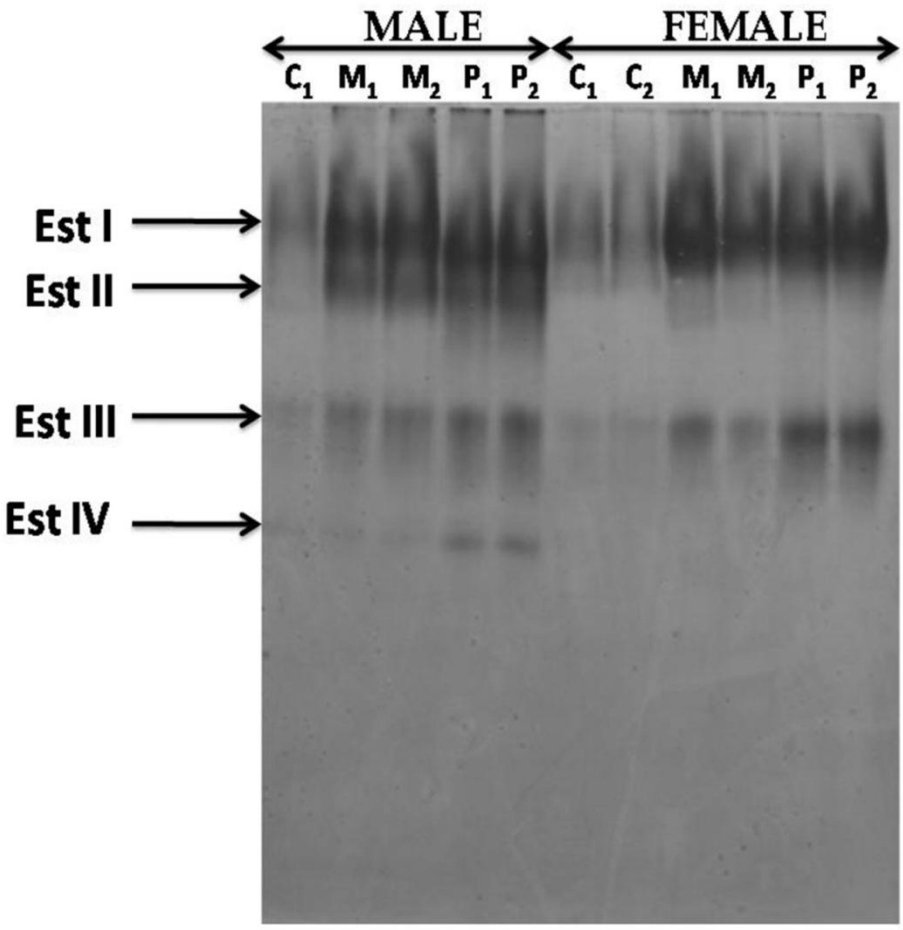

Figure 4.7 An electrophoregram of general esterases of *H. theivora* specimens reared on three alternative hosts i.e. *C. sinensis* (C_1, C_2), *M. micrantha* (M_1, M_2) and *P. guajava* (P_1, P_2).

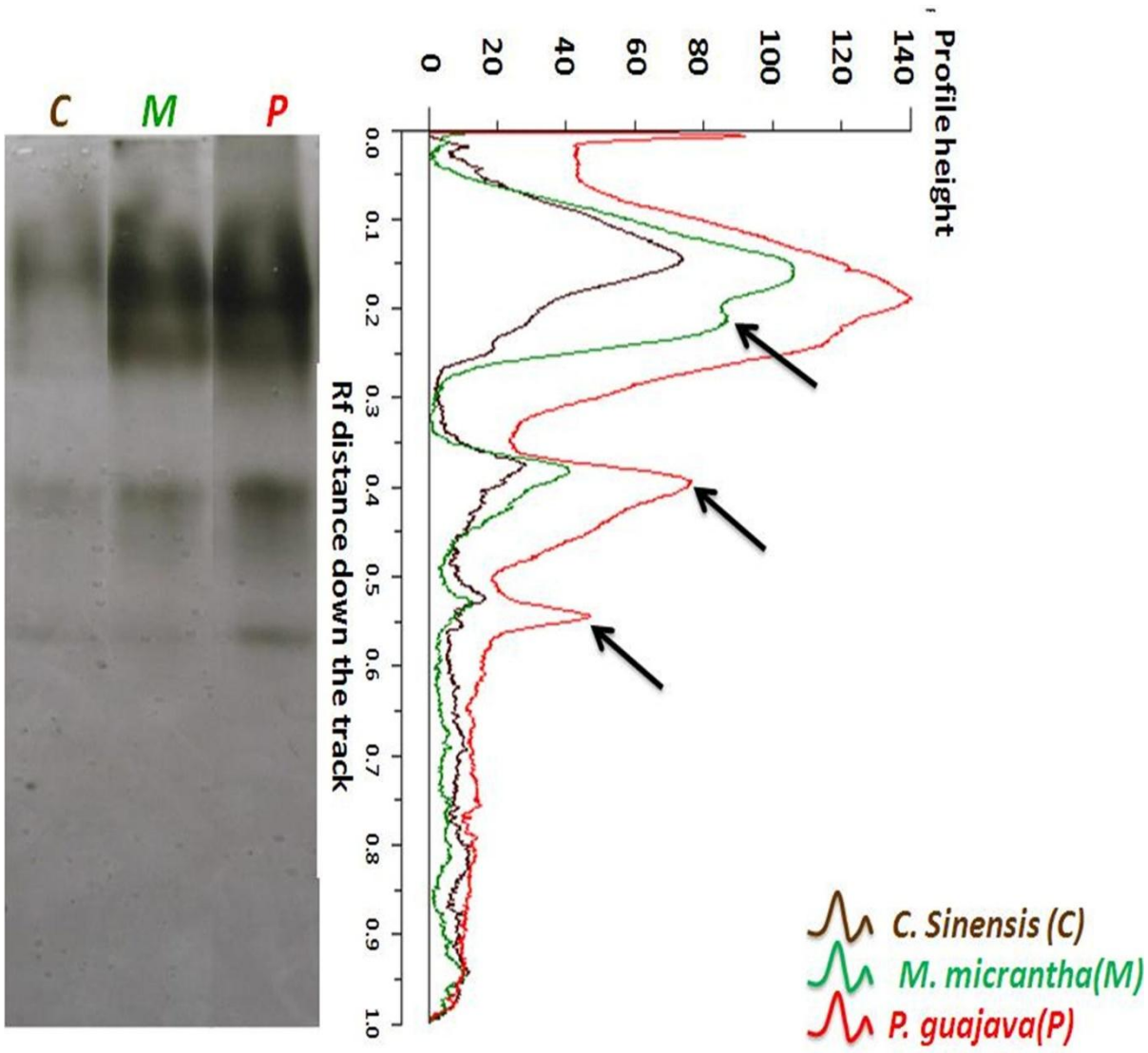

Figure 4.8 Densitometric analysis of phoregram of general esterase of male *H. theivora* reared on *Camellia sinensis* (*C*), *Mikania micrantha* (*M*) and *Psidium guajava* (*P*). Arrow (→) indicates higher expression of general esterases in *M. micrantha* and *P. guajava.*

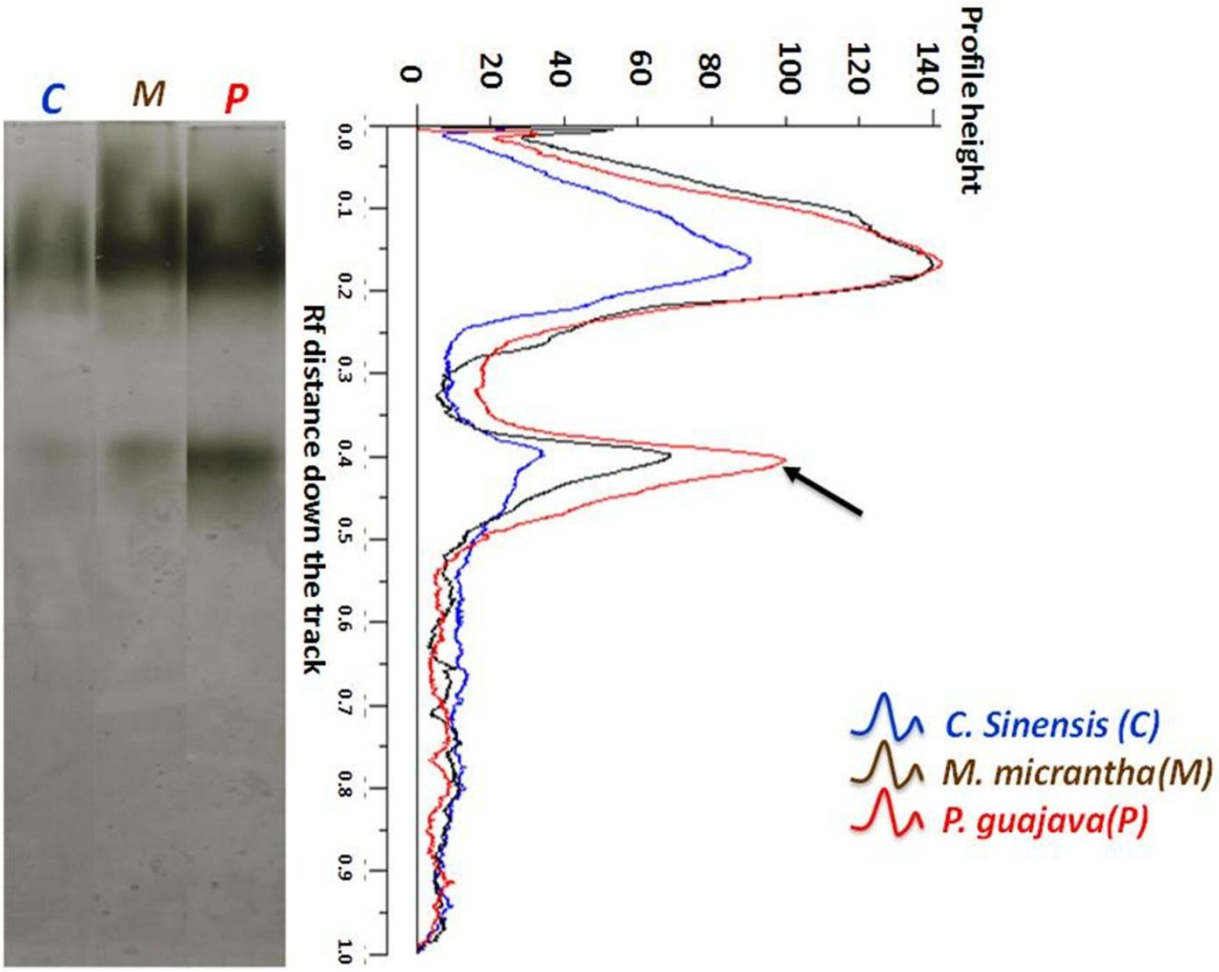

Figure 4.9 Densitometric analysis of phoregram of general esterase of female *H. theivora* reared on *Camellia sinensis* (*C*), *Mikania micrantha* (M) and *Psidium guajava* (*P*). Arrow (→) indicates higher expression of general esterase in *P. guajava* and *M. micrantha* than *C. sinensis*.

Empoasca flavescens

Detoxifying enzymes activity

In *E. flavescens*, general esterases showed 6.8 fold enhancements in activity when reared on *R. communis* in comparison to *C. sinensis*-reared specimens (Table 4.23). Similarly, a moderate enhancement in activity of general esterases was noticed in *S. tuberosum*-reared specimens (3.0 fold). Cytochrome P 450 monooxygenase also showed 3.6 and 4.7 fold higher activity when reared on *S. tuberosum* and *R. communis* in comparison to *C. sinensis* reared *E. flavescens*. Glutathione S-transferases showed 1.2 and 1.4 fold higher activity when reared on *S. tuberosum* and *R. communis* in comparison to *C. sinensis* reared *E. flavescens* (Table 4.23).

Table 4.23 General esterases (GEs), Cytochrome P450 monooxygenases (CYPs) and glutathione S-transferases (GSTs) activity (Values are means ± SE) in *E. flavescens* reared on three different host plants.

Populations	No. tested (n)	General Esterases (GEs) (µmol α napthol min^{-1} mg $protein^{-1}$)	Cytochrome P450 monooxygenases (CYPs) (n mol min^{-1} mg $protein^{-1}$ as equivalent to Cytochrome P450)	Glutathione S-transferases (GSTs) (µmol GSH conjugated min^{-1} mg $protein^{-1}$)
C. sinensis	85	0.18±0.02[az]	0.21±0.02[a]	1.35±0.03[a]
S. tuberosum	80	0.55±0.04[b]	0.76±0.06[b]	1.62±0.08[b]
R. communis	65	1.22±0.07[c]	0.98±0.11c	1.94±0.04c

[z] Within columns, means followed by the same letter do not differ significantly (P=0.05) in Tukey's multiple comparison test (HSDa)

Densitometric analysis of electro-phoregram of General Esterases

Densitometric analysis of general esterases (GEs) showed six esterase isozyme bands i.e. Est I-Est VI with Rf values 0.06, 0.25, 0.36, 0.39, 0.45 and 0.53. The pixel density and accordingly the profile height varied in different host-reared (specific) *E. flavescens* specimens. Est I-III showed distinct profile height among three host reared specimens (Figure 4.10)

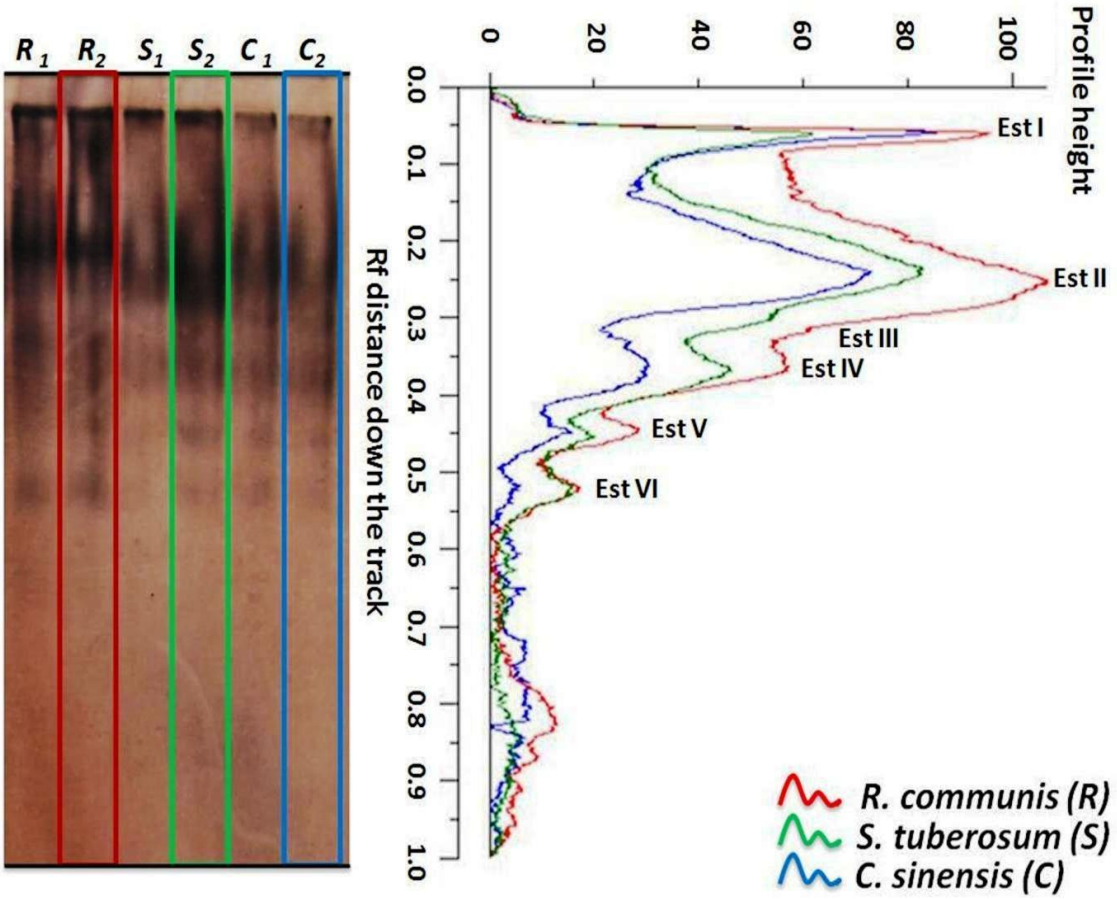

Figure 4.10 Densitometric analysis of phoregram of general esterase of *E. flavescens* reared on *C. sinensis* (C_1, C_2), *S. tuberosum* (S_1, S_2) and *R. communis* (R_1, R_2).

Scirtothrips dorsalis

Detoxifying enzymes activity

In *S. dorsalis*, general esterases showed 2.7 fold enhancements in activity when reared on *R. communis* in comparison to *C. sinensis*-reared specimens (Table 4.24). Similarly, an enhancement in activity of general esterases was also noticed in *C. annuum*-reared specimens (2.4 fold). Cytochrome P 450 monooxygenase also showed 1.6 and 2.0 folds higher activity when reared on *C. annuum* and *R. communis* in comparison to *C. sinensis* reared *S. dorsalis*. Glutathione S-transferases showed 2.0 and 2.3 folds higher activity when reared on *C. annuum* and *R. communis* in comparison to *C. sinensis*-reared *S. dorsalis* (Table 4.24).

Table 4.24 General esterases (GEs), Cytochrome P450 monooxygenases (CYPs) and glutathione S-transferases (GSTs) activity (Values are means ± SE) in *S. dorsalis* reared on three different host plants

Populations	No. tested (n)	General Esterase (GEs) (µmol α napthol min^{-1} mg $protein^{-1}$)	Cytochrome P450 monooxygenases (CYPs) (n mol min^{-1} mg $protein^{-1}$ as equivalent to Cytochrome P450)	Glutathione S-transferases (GSTs) (µmol GSH conjugated min^{-1} mg $protein^{-1}$)
C. sinensis	80	0.53±0.07[az]	0.83±0.06[a]	0.87±0.09[a]
C. annuum	80	1.26±0.11[b]	1.36±0.07[b]	1.75±0.06[b]
R. communis	60	1.44±0.12[b]	1.68±0.08[b]	1.96±0.09[b]

[z] Within columns, means followed by the same letter do not differ significantly (P=0.05) in Tukey's multiple comparison test (HSDa)

Densitometric analysis of electro-phoregram of General Esterases

Densitometric analysis of general esterases (GEs) showed five isozyme bands of esterase i.e. Est I- V with Rf values 0.17, 0.22, 0.27, 0.35, 0.45 and 0.52. The pixel density and accordingly the profile height varied in different host-reared (specific) *S. dorsalis* specimens. Est I-III showed distinct profile height for the specimen reared on three different hosts (Figure 4.11)

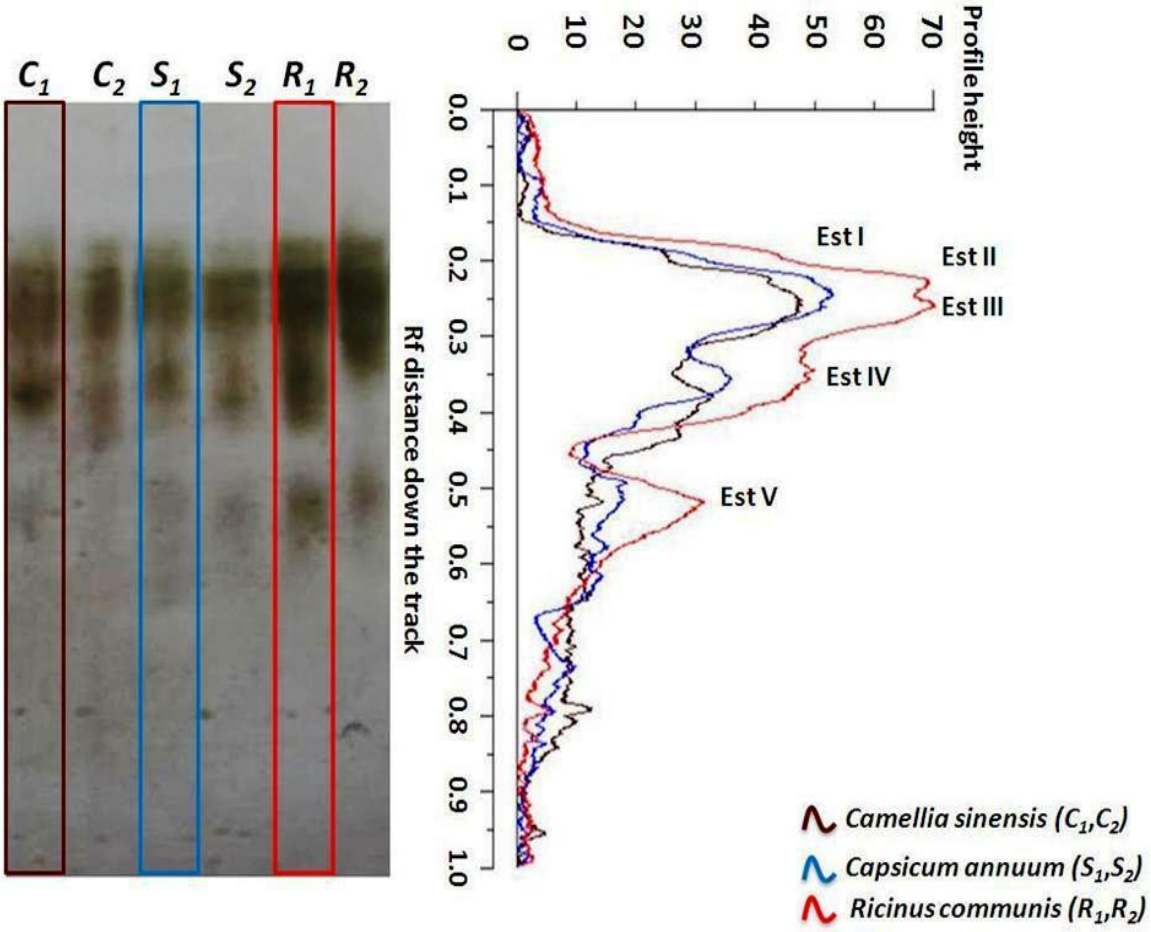

Figure 4.11 Densitometric analysis of phoregram of general esterase of *S. dorsalis* reared on *C. sinensis* (C_1, C_2), *C. annuum* (S_1, S_2) and *R. communis* (R_1, R_2).

The general esterases showed a significant increase in activity in *M. micrantha* and *P. guajava*-reared specimens than that of *C. sinensis*-reared *H. theivora*. However, the activity of the enzyme in the insects were non-significantly different when feed on the former two alternative hosts. Esterase is involved in allelochemicals metabolism (Li *et al.*, 2007). The metabolic enzyme may be present in multiple forms and is inducible by plant allelochemicals causing change in insect's susceptibility to insecticides (Yang *et al.*, 2001; Riley and Tan, 2003). So the enhanced activity of esterases in *H. theivora* may be due to induction by the allelochemicals present in both the non-tea host plants. GST is a broad-spectrum detoxifying enzyme, and has detoxifying effects on many plant allelochemicals (Yu, 2008). The activity of GST is also inhibited by a number of allelochemicals (Lee, 1991; Yu and Abo-Elghar, 2000). In the present study, esterases and cytochrome P450 monooxygenases of *H. theivora* appeared to play significant role in detoxification of allelochemicals while GSTs might not to be much involved in detoxification (Siti Noor, 2006). Monooxygenase showed enhanced activity in *H. theivora* female on two alternative hosts than on *C. sinensis* but the difference in activity in the two former host plants was not much different, therefore, indicating similar influence of the latter hosts on defense enzyme(CYP) of the bug. Monooxygenase enzymes are more versatile and have wide acceptance of variety of chemical (Terriere, 1984) including wide range of plant allelochemicals (Li *et al.*, 2007). They can be induced by plant allelochemicals (Krieger *et al.*, 1971; Terriere, 1984; Schuler, 1996; Zeng *et al.*, 2007a) and maintain an elevated level of activity (Brattsten *et al.*, 1977; Brattsten, 1983; Yu *et al.*, 1979; Yu, 1983). The induction of the enzyme systems depends on the frequency with which a host plant is fed upon (Li *et al.* 2000, 2002, 2003; Zeng *et al.* 2007a). Males puncture and suck host leaf less frequently than femalesas evident from the choice test. This may be the reason for the less induction of monooxygenase

system in male population of *H. theivora*. The same explanation possibly shall hold good for the difference in GST activity recorded between the sexes on the three host plants.

The densitometric study documents the differential activity of general esterases in between the sexes. Absence of Est II and IV isozyme bands in femalesmay be due to sex-specific allelochemicals-suppression process as the same are induced in male. This type sex specific suppression and induction was evident in *Drosophila melanogaster* (Thornton, 2009) when exposed to atrazine, an herbicide. So, similar phenomenon may be operative in *H. theivora* in the present study. The versatility in the adaptation of an insect to their environment depends on the phenomenon of induction (Terriere, 1984) through a rapid process (Brattsten, 1979a,b) in which a chemical stimulus of a host enhances the activity of the detoxification system leading to the production of additional enzymes. Polyphagous insects can selectively express a broad range of enzymes that assist in the detoxification of numerous xenobiotics including secondary plant metabolites (Brattsten *et al.*, 1984). The enhancement in detoxifying enzymes in different host plants studied for *H. theivora* may be an ideal example. The denser and heavier bands in PAGE for the specimens reared on two alternative hosts (other than tea) corroborates with the enhanced quantity of the general esterases as estimated in them. Adaptations to plant allelochemicals through induction of detoxifying enzymes confer an adaptive plasticity to insects which enable them to optimize their fitness in presence of varying level of toxins (Despres *et al.*, 2007). Furthermore, a mutation leading to an increased enzymatic affinity is retained by selection if it occurs in an inducible gene that overproduces the enzyme, than in a constitutive gene because it readily confers higher resistance to its bearer in presence of the toxin (Le, 2006). Therefore, induction of detoxifying genes by plant allelochemicals is considered as the first step toward further specialization (Després *et al.*, 2007).

Insects capacity to metabolize plant allelochemicals also cross protects them against synthetic insecticides (Kennedy, 1984; Li *et al.*, 2000) and therefore, significantly enhances insect tolerance to insecticides (Zeng *et al.*, 2007a) causing difficulty in its insecticidal control and management. So, the present findings give inkling that induction of defense enzymes due to feeding on alternative hosts is possibly rendering *H. theivora* more tolerant to xenobiotics, thus posing a pest management problem in Eastern Himalayan tea plantations. By and large its seems that, the other two sucking pests, *E. flavescens* and *S. dorsalis* also enjoyprotection by enhancing their titer and profile of defence enzymes, mainly GEs and CYPs, through feedingon the alternate hos plants other than tea.

Genetic variability in sucking insects:

Helopeltis theivora

Genetic variability of twenty populations of *Helopeltis theivora* was studied from collected among different tea plantations from the study area (Table 4.25). They differ in their management practice and location (Table 4.25). For RAPD-PCR amplification, eight random primer (four from Kit 'A' and four from Kit 'D') were used and 64 loci were amplified (Figure 4.12 - 4.15). The RAPD bands were scored as present (1) or absent (0) in each pattern. Allele frequencies were estimated based on the square root of the frequency of the null (recessive) genotype. All calculations were carried out using the population genetic analysis software, PopGene 1.32 (Yeh *et al.*, 2000). The UPGMA (Unweighted Pair Group Method Analysis) dendrogram of the three populations was constructed based on Nei's (1978) genetic distances using Tools for population genetics analysis (TFPGA) software (Miller, 1997). Percentage of polymorphic loci, gene diversity and Shannon's information index (Lewontin, 1972) are recorded (Table 4.26)**.** The genetic relatedness and genetic distances among twenty different populations of *H. theivora* are summarized (Table 4.27). UPGMA dendrogram based on the Nei's (1978) genetic distance among different *H. theivora* populations from Terai, the Dooars and Darjeeling hill, formed nine different population group (Figure 4.16). *H. theivora* population collected from tea plantations with similar mode of management practice formed a single group (Group 1 and Group 10).

Table 4.25 Different *H. theivora* populations, their sampling sites (tea plantations) with geographical coordinates (latitude and longitude) and management practice.

Populations	Location	Longitude	Latitude	Management Practice
HT 01	Darjeeling hill	26.908 N	88.241 E	Organic
HT 02	Darjeeling hill	26.875 N	88.177 E	Organic
HT 03	Darjeeling hill (Low altitude)	26.842 N	88.188 E	Conventional
HT 04	Terai Plains	26.803 N	88.260 E	Conventional
HT 05	Terai Plains	26.821 N	88.176 E	Conventional
HT 06	Terai Plains	26.679 N	88.243 E	Conventional
HT 07	Terai Plains	26.700 N	88.262 E	Conventional
HT 08	Terai Plains	26.663 N	88.112 E	Conventional
HT 09	Terai Plains	26.165 N	87.941 E	Conventional
HT 10	Dooars Plains	26.309 N	90.937 E	Organic
HT 11	Terai Plains	26.540 N	88.135 E	Conventional
HT 12	Terai Plains	26.445 N	88.195 E	Conventional
HT 13	Dooars Plains	26.911 N	88.319 E	Conventional
HT 14	Dooars Plains	26.859 N	88.684 E	Conventional
HT 15	Dooars Plains	26.866 N	88.803 E	Conventional
HT 16	Dooars Plains	26.931 N	89.117 E	Conventional
HT 17	Dooars Plains	26.753 N	89.533 E	Conventional
HT 18	Dooars Plains	26.736 N	89.156 E	Conventional
HT 19	Dooars Plains	26.834 N	89.995 E	Conventional
HT 20	Dooars Plains	26.591 N	89.964 E	Conventional

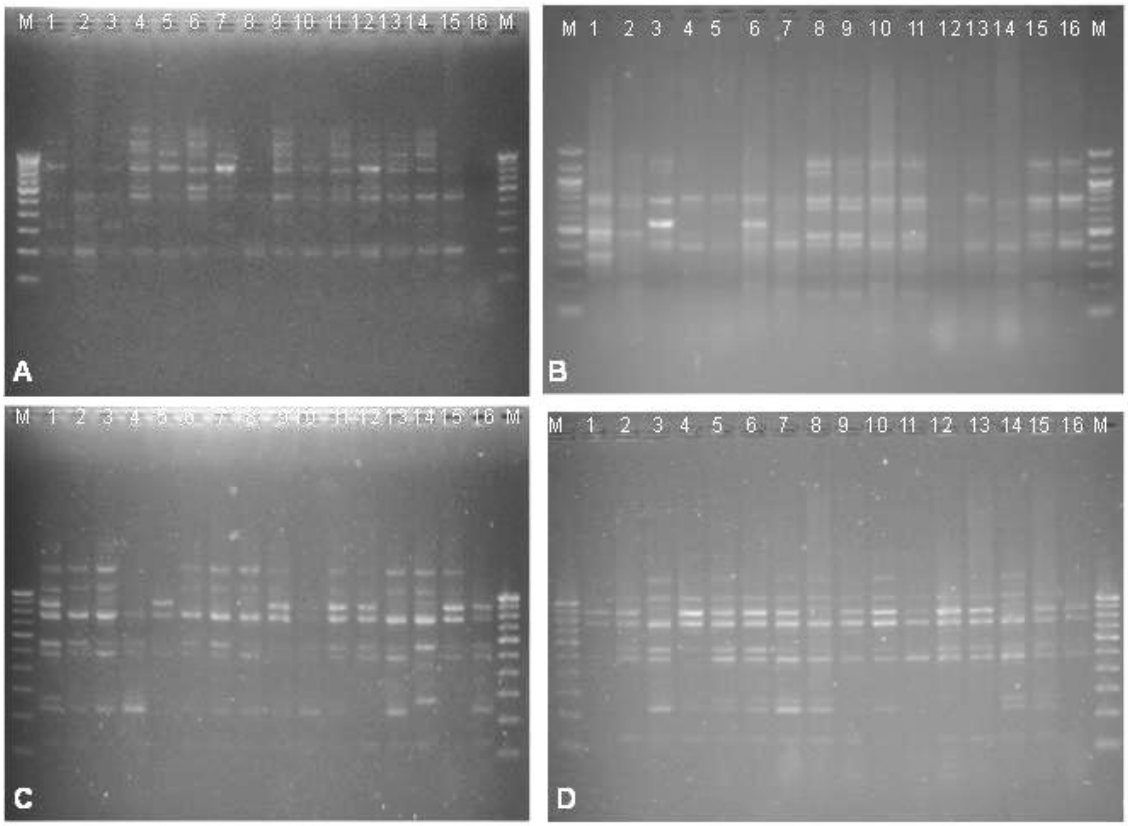

Figure 4.12 RAPD banding pattern of different populations of *H. theivora* using different OPA primers. M = Marker, 1-2 = Organic plantations, 3-8= Conventional plantations of Terai, 9-16= conventional plantations of the Dooars. A= OPA 02, B= OPA 08, C= OPA 09 and D= OPA 11.

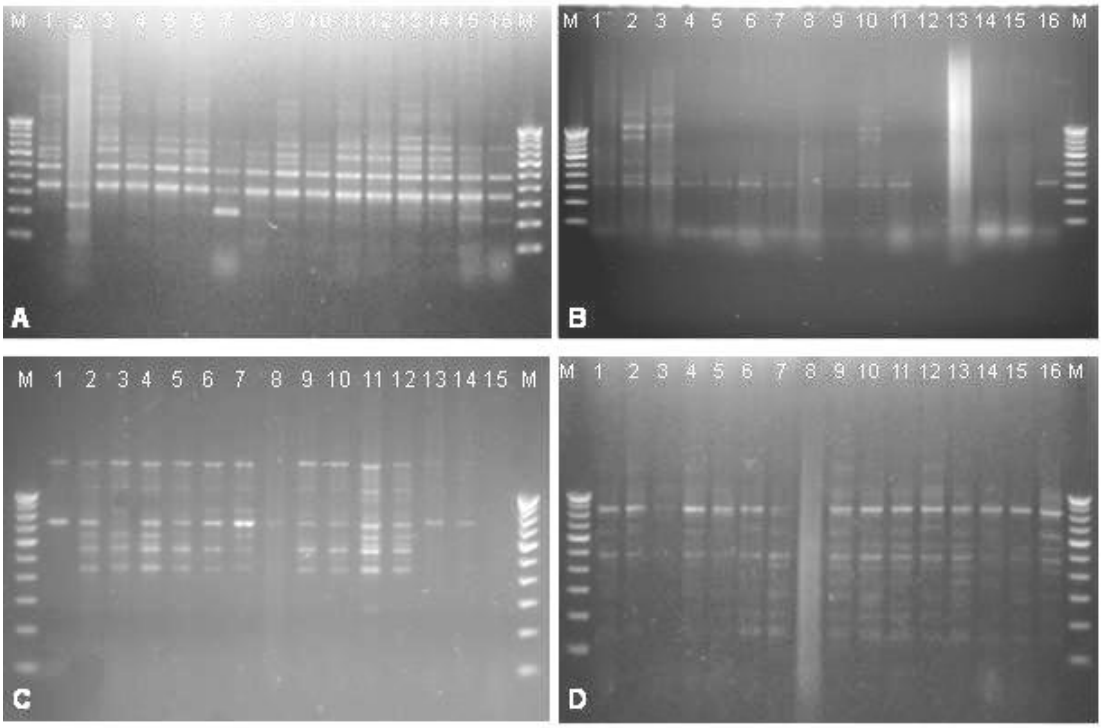

Figure 4.13 RAPD banding pattern of different populations of *H. theivora* using different OPD primers. M = Marker, 1-2 = Organic plantations, 3-8= Conventional plantations of Terai, 9-16= conventional plantations of the Dooars. A= OPD 05, B= OPD 08, C= OPD 12 and D= OPD 20.

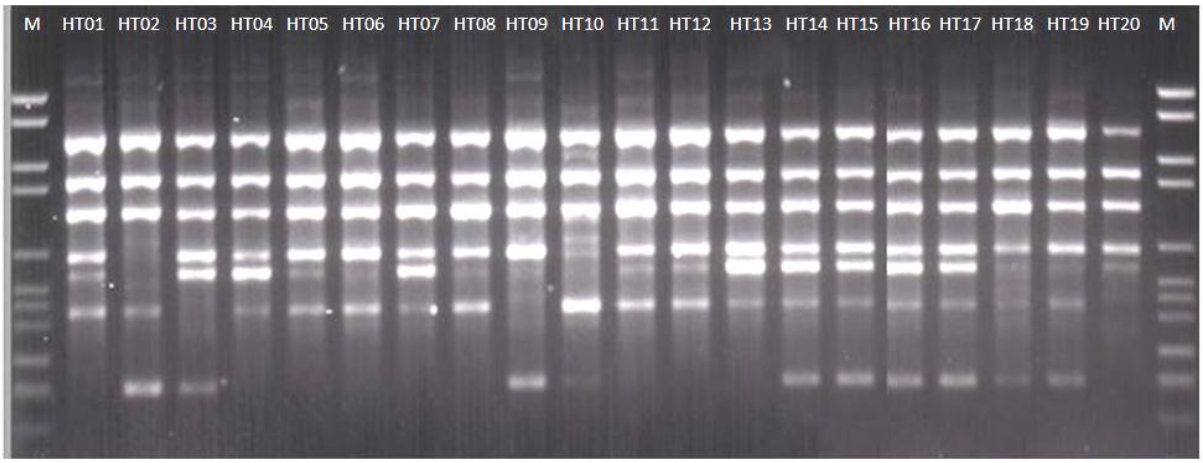

Figure 4.14 RAPD banding pattern of twenty different populations of *H. theivora* (HT01-HT20) using different OPD 08 primers. M = Marker.

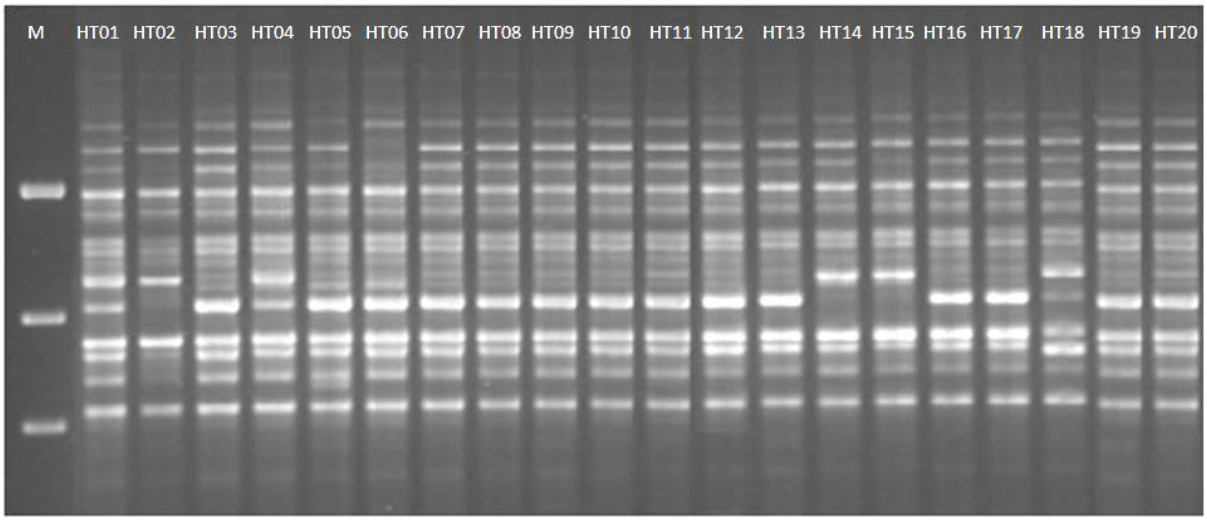

Figure 4.15 RAPD banding pattern of twenty different populations of *H. theivora* (HT01-HT20) using different OPD 20 primers. M = Marker.

Table 4.26 Primer name, sequence, and number fragments amplified and percentage of polymorphic loci in *Helopeltis theivora* populations.

Primer	Sequence (5'-3')	No. of Fragments amplified (% of Polymorphic loci)
OPA-02	TGCCGAGCTG	8 (75.0)
OPA-08	GTGACGTAGG	8 (87.5)
OPA-09	GGGTAACGCC	7 (80.0)
OPA-11	CAATCGCCGT	9 (83.3)
OPD-05	TGAGCGGACA	8 (62.5)
OPD-08	GTGTGCCCCA	9 (75.0)
OPD-12	CACCGTATCC	7 (83.3)
OPD-20	ACCCGGTCAC	8 (75.0)

Table 4.27 Genetic diversity and genetic identity of Different *H. theivora* populations.

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
01	****	0.9965#	0.9912	0.9893	0.9821	0.9909	0.9830	0.9782	0.9790	0.9914	0.9844	0.9746	0.9868	0.9858	0.9812	0.9740	0.9904	0.9773	0.9811	0.9873
02	0.0035	****	0.9956	0.9954	0.9832	0.9946	0.9916	0.9866	0.9885	0.9828	0.9948	0.9622	0.9847	0.9839	0.9797	0.9689	0.9839	0.9707	0.9774	0.9853
03	0.0089	0.0044	****	0.9983	0.9924	0.9985	0.9935	0.9884	0.9904	0.9852	0.9938	0.9747	0.9931	0.9939	0.9911	0.9781	0.9955	0.9838	0.9910	0.9966
04	0.0108	0.0046	0.0017	****	0.9915	0.9988	0.9987	0.9940	0.9930	0.9843	0.9952	0.9680	0.9837	0.9913	0.9891	0.9734	0.9916	0.9781	0.9827	0.9900
05	0.0181	0.0170	0.0077	0.0086	****	0.9969	0.9906	0.9918	0.9875	0.9784	0.9850	0.9725	0.9880	0.9922	0.9889	0.9773	0.9949	0.9818	0.9820	0.9946
06	0.0091	0.0054	0.0015	0.0012	0.0031	****	0.9952	0.9956	0.9930	0.9869	0.9940	0.9752	0.9904	0.9954	0.9901	0.9760	0.9954	0.9870	0.9861	0.9950
07	0.0171	0.0084	0.0065	0.0013	0.0094	0.0048	****	0.9971	0.9999	0.9647	0.9972	0.9484	0.9739	0.9810	0.9759	0.9542	0.9822	0.9619	0.9682	0.9817
08	0.0221	0.0135	0.0116	0.0061	0.0083	0.0045	0.0029	****	0.9923	0.9674	0.9932	0.9533	0.9761	0.9804	0.9785	0.9568	0.9824	0.9662	0.9675	0.9798
09	0.0213	0.0116	0.0097	0.0070	0.0126	0.0070	0.0001	0.0077	****	0.9580	0.9988	0.9348	0.9643	0.9714	0.9653	0.9423	0.9792	0.9547	0.9579	0.9751
10	0.0086	0.0174	0.0149	0.0158	0.0218	0.0132	0.0359	0.0331	0.0430	****	0.9703	0.9942	0.9943	0.9917	0.9927	0.9925	0.9940	0.9938	0.9919	0.9960
11	0.0157	0.0052	0.0063	0.0048	0.0152	0.0060	0.0028	0.0068	0.0012	0.0301	****	0.9451	0.9766	0.9759	0.9740	0.9499	0.9809	0.9629	0.9643	0.9817
12	0.0257	0.0385	0.0256	0.0326	0.0278	0.0251	0.0530	0.0479	0.0674	0.0058	0.0565	****	0.9945	0.9935	0.9919	0.9973	0.9890	0.9980	0.9959	0.9904
13	0.0132	0.0154	0.0069	0.0164	0.0120	0.0096	0.0264	0.0242	0.0363	0.0058	0.0237	0.0055	****	0.9955	0.9965	0.9928	0.9956	0.9914	0.9933	0.9977
14	0.0143	0.0162	0.0061	0.0087	0.0078	0.0046	0.0191	0.0198	0.0291	0.0083	0.0244	0.0066	0.0045	****	0.9990	0.9919	0.9985	0.9986	0.9983	0.9961
15	0.0190	0.0206	0.0089	0.0109	0.0111	0.0100	0.0244	0.0217	0.0353	0.0073	0.0263	0.0082	0.0035	0.0010	****	0.9960	0.9956	0.9934	0.9938	0.9961
16	0.0264	0.0316	0.0221	0.0269	0.0230	0.0242	0.0469	0.0442	0.0594	0.0075	0.0514	0.0027	0.0072	0.0081	0.0040	****	0.9921	0.9911	0.9936	0.9900
17	0.0097	0.0162	0.0045	0.0084	0.0051	0.0046	0.0179	0.0177	0.0210	0.0060	0.0193	0.0110	0.0044	0.0015	0.0044	0.0079	****	0.9946	0.9951	0.9988
18	0.0229	0.0297	0.0164	0.0222	0.0184	0.0131	0.0389	0.0343	0.0464	0.0062	0.0378	0.0020	0.0087	0.0014	0.0066	0.0089	0.0054	****	0.9984	0.9949
19	0.0191	0.0229	0.0091	0.0174	0.0182	0.0140	0.0323	0.0330	0.0430	0.0081	0.0363	0.0041	0.0067	0.0017	0.0062	0.0064	0.0049	0.0016	****	0.9981
20	0.0128	0.0148	0.0034	0.0101	0.0054	0.0050	0.0185	0.0205	0.0252	0.0040	0.0185	0.0097	0.0023	0.0039	0.0039	0.0100	0.0012	0.0052	0.0019	****

Nei,s genetic identity (above diagonal) and genetic distance (below diagonal).

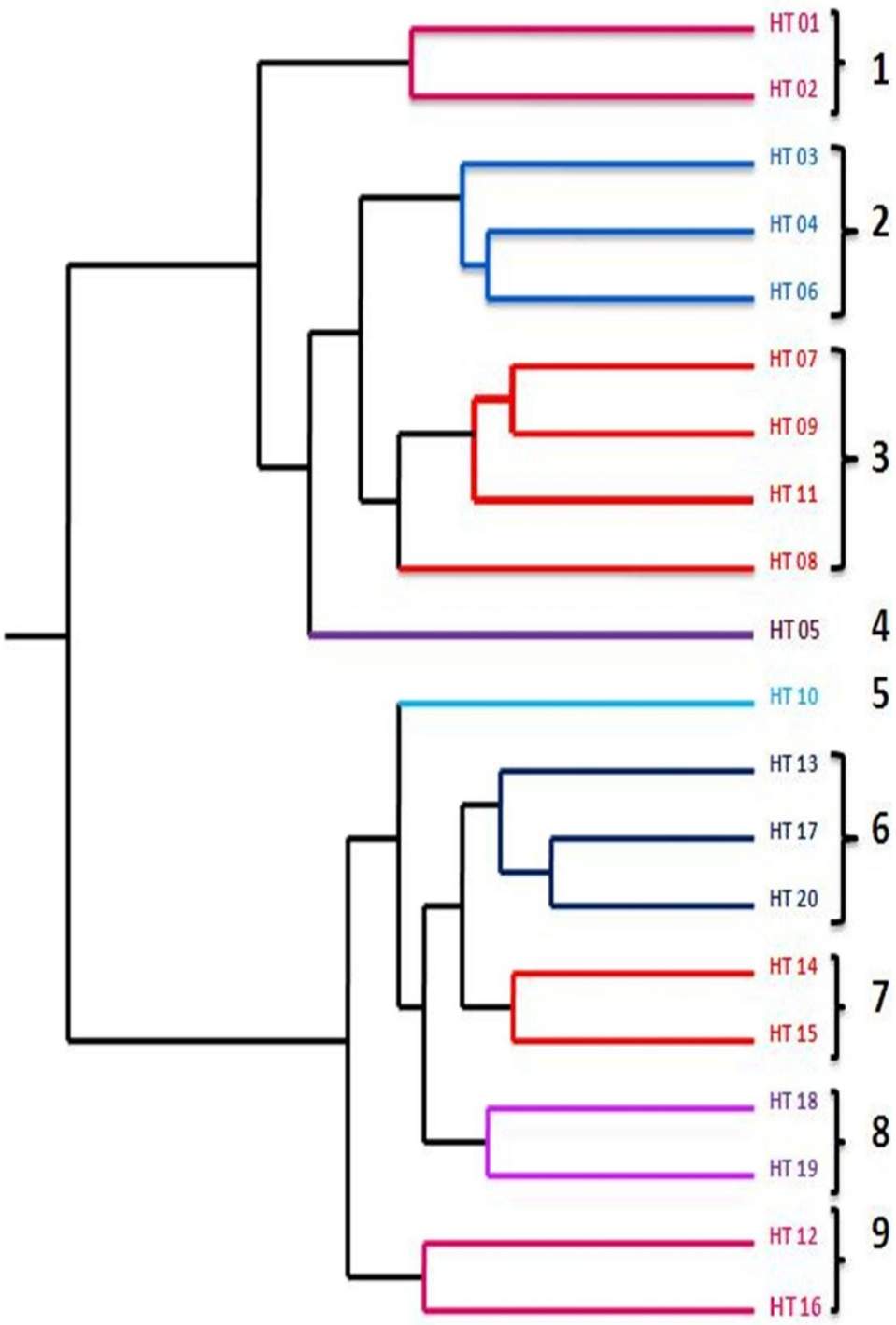

Figure 4.16 Dendrogram of different *H. theivora* populations based on Nei's (1978) genetic distance method i.e. UPGMA (Unweighted Pair Group Method Analysis) modified from NEIGHBOR procedure of PHYLIP Version 3.5.

Genetic variability in Empoasca flavescens:

To study the genetic variability of *E. flavescens* eight random primer (OPA 02, 08,09,11 and OPD 05, 08, 12, 20) were used which amplified a total of 53 loci (Figure 4.17, Table 4.28). Percentage of polymorphic loci, gene diversity and Shannon's information index (Lewontin, 1972) was calculated by using population genetics software PopGene version 1.32 (Yeh *et al.*, 2000) (Table 4.29). The percent polymorphism was found to be higher (88.7 %) in populations from the Dooars, compared to the populations from Darjeeling hill (83.0 %). The genetic relatedness and genetic distance are summarized in Table 4.30. The genetic relatedness and the genetic distance between Terai and the Dooars population were found to be 0.9768 and 0.0235 respectively. UPGMA (Unweighted Pair Group Method analysis) dendrogram was constructed based on the Nei's (1978) genetic distance method, using Tools for population genetics analysis (TFPGA) software (Miller, 1997) (Figure 4.18).

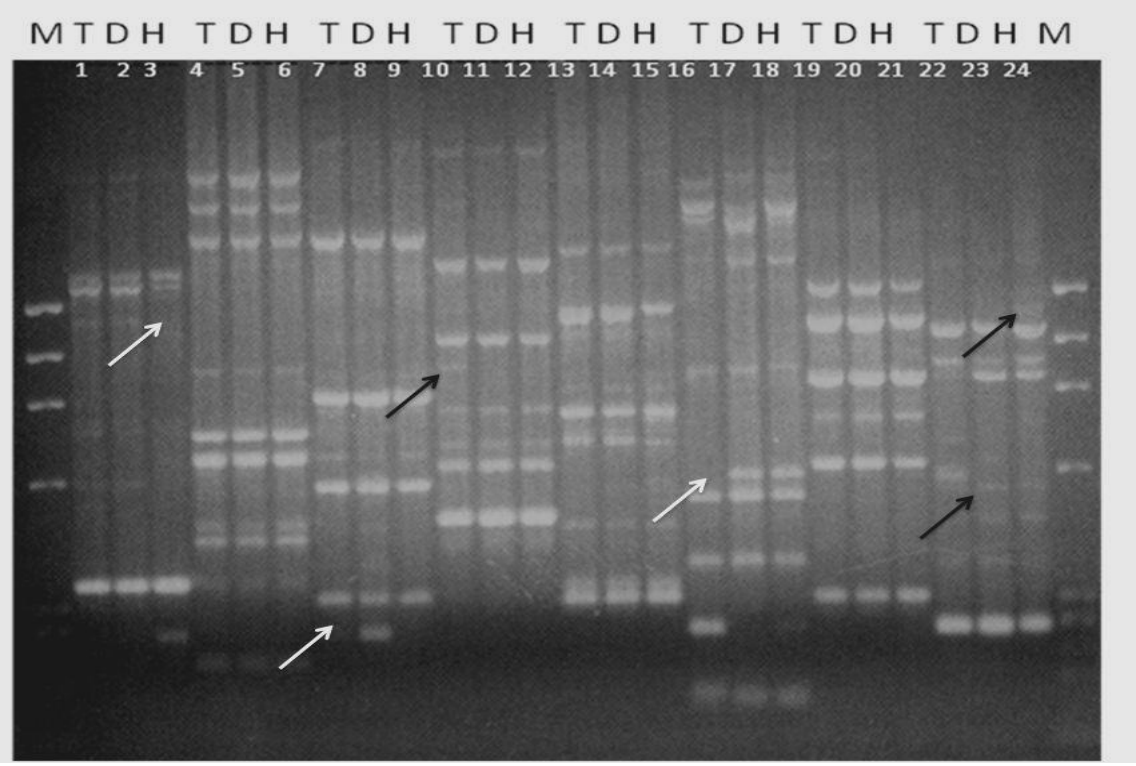

Figure 4.17 RAPD banding pattern of three *Empoasca flavescens* populations using different primers. M=Marker; T= Terai; D=Dooars; H=Darjeeling Hill, 1-3=OPA02; 4-6=OPA08; 7-9=OPA09; 10-12=OPA11; 13-15=OPD05; 16-18=OPD08; 19-21= OPD12 and 22-24=OPD20 primer. Arrow indicates presence (black) and absence (white) of bands.

Table 4.28 Primer name, sequence and number fragments amplified and percentage of polymorphic loci in *Empoasca flavescens* populations.

Primer	Sequence (5'-3')	No. of Fragments amplified (% of Polymorphic loci)
OPA-02	TGCCGAGCTG	3 (100)
OPA-08	GTGACGTAGG	8 (87.5)
OPA-09	GGGTAACGCC	5 (80.0)
OPA-11	CAATCGCCGT	6 (83.3)
OPD-05	TGAGCGGACA	8 (62.5)
OPD-08	GTGTGCCCCA	9 (100)
OPD-12	CACCGTATCC	6 (83.3)
OPD-20	ACCCGGTCAC	8 (75.0)

Table 4.29 Genetic diversity of three *Empoasca flavescens* populations based on RAPD markers.

Populations	n	na*	ne	h	I	P
Darjeeling hill	31	1.83±0.38	1.62±0.33	0.35±0.17	0.51±0.24	83.02
Terai	30	1.85±0.36	1.64±0.34	0.36±0.17	0.51±0.25	84.91
Dooars	30	1.89±0.32	1.65±0.31	0.36±0.15	0.53±0.21	88.68

*na = Observed number of alleles; ne = Effective number of alleles [Kimura and Crow (1964)]; h = Nei's (1973) gene diversity; I = Shannon's Information index [Lewontin (1972)]; P = Percentage of polymorphic loci;

Table 4.30 Genetic identity and Genetic distance of three *Empoasca flavescens* populations [Nei (1978) Genetics 89:583-590].

Populations	Darjeeling hill	Terai	Dooars
Darjeeling hill	****	0.9748	0.9674
Terai	0.0255[#]	****	0.9768
Dooars	0.0332	0.0235	****

[#]Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

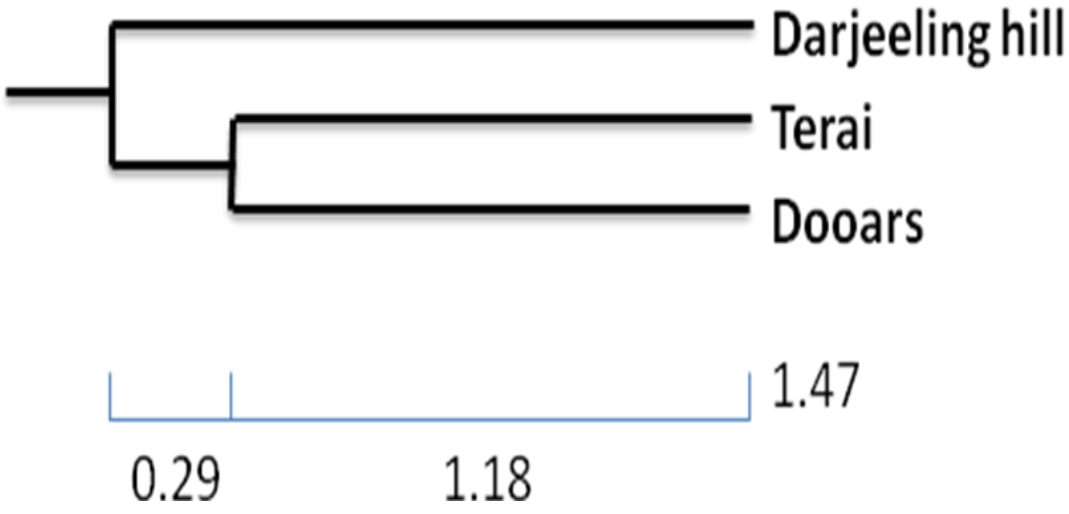

Figure 4.18 Dendrogram of different *E. flavescens* populations based on Nei's (1978) genetic distance method i.e. UPGMA (Unweighted Pair Group Method Analysis) modified from NEIGHBOR procedure of PHYLIP Version 3.5.

Genetic variability in Scirtothrips dorsalis:

To study the genetic variability of *S. dorsalis* nine random primer (OPA 02, 08, 09, 11 and OPD 05, 08, 09, 12, 20) were used which amplified a total of 92 loci (Figure 4.19, Table 4.31). Percentage of polymorphic loci, gene diversity and Shannon's information index (Lewontin, 1972) were calculated by using population genetics software PopGene version 1.32 (Yeh *et al.,* 2000) (Table 4.32). The percent polymorphism in the populations from Dooars, and from Darjeeling hill was observed to be 92.8 % and 88.8 %. The genetic relatedness and genetic distance are summarized in Table 4.33. The genetic relatedness and the genetic distance between Terai and the Dooars population were determined to be 0.9768 and 0.0235. These two populations have been found to form a single clade in UPGMA (Unweighted Pair Group Method analysis) dendrogram based on the Nei's (1978) genetic distance (Figure 4.18).

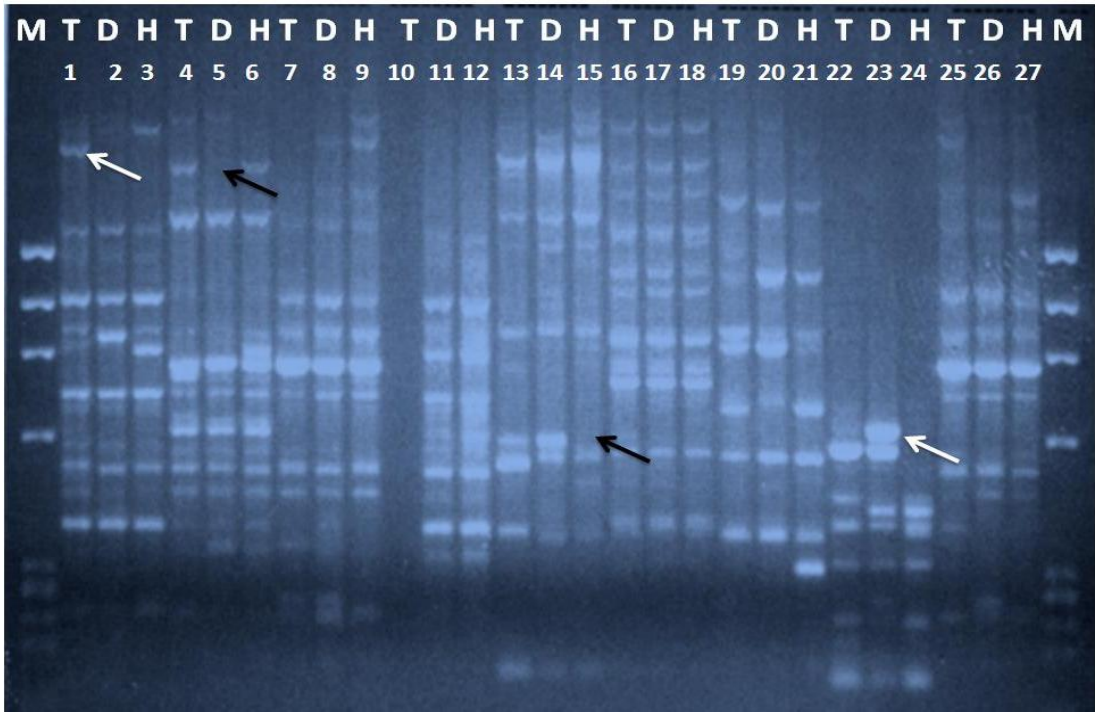

Figure 4.19 RAPD banding pattern of three *Scirtothrips dorsalis* populations using different primers. M=Marker; T= Terai; D=Dooars; H=Darjeeling Hill, 1-3=OPA02; 4-6=OPA08; 7-9=OPA09; 10-12=OPA11; 13-15=OPD05; 16-18=OPD08; 19-21= OPD09, 22-24=OPD12 and 25-27=OPD20 primer. Arrow indicates presence (white) and absence (black) of bands.

Table 4.31 Primer name, sequence and number fragments amplified and percentage of polymorphic loci in *Scirtothrips dorsalis* populations.

Primer	Sequence (5'-3')	No. of Fragments amplified (% of Polymorphic loci)
OPA-02	TGCCGAGCTG	11 (72.7)
OPA-08	GTGACGTAGG	14 (64.2)
OPA-09	GGGTAACGCC	11 (60.0)
OPA-11	CAATCGCCGT	10 (83.3)
OPD-05	TGAGCGGACA	10 (62.5)
OPD-08	GTGTGCCCCA	12 (50.0)
OPD-09	CTCTGGAGAC	8 (66.6)
OPD-12	CACCGTATCC	8 (83.3)
OPD-20	ACCCGGTCAC	8 (75.0)

Table 4.32 Genetic diversity of three *Scirtothrips dorsalis* populations based on RAPD markers.

Populations	n	na*	ne	h	I	P
Darjeeling hill	34	1.89±0.32	1.69±0.32	0.36±0.15	0.52±0.21	88.89
Terai	34	1.91±0.28	1.67±0.29	0.38±0.14	0.55±0.19	91.36
Dooars	34	1.93±0.26	1.74±0.28	0.41±0.13	0.58±0.18	92.89

*na = Observed number of alleles; ne = Effective number of alleles [Kimura and Crow (1964)]; h = Nei's (1973) gene diversity; I = Shannon's Information index [Lewontin (1972)]; P = Percentage of polymorphic loci;

Table 4.33 Genetic identity and Genetic distance of three *Scirtothrips dorsalis* populations [Nei (1978) Genetics 89:583-590].

Populations	Darjeeling hill	Terai	Dooars
Darjeeling hill	****	0.9575	0.9545
Terai	0.0434#	****	0.9647
Dooars	0.0465	0.0359	****

#Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Figure 4.20 Dendrogram of different *S. dorsalis* populations based on Nei's (1978) genetic distance method i.e. UPGMA (Unweighted Pair Group Method Analysis) modified from NEIGHBOR procedure of PHYLIP Version 3.5.

Random amplified polymorphic DNA technique (Williams *et al.*, 1990) is similar to DNA fingerprinting in that the technique generates characteristic spectrum of fragments from template of the total genomic DNA. Generation of RAPD fragments has been shown to be reproducible (Black *et al.*, 1992; Weeden *et al.*, 1992) and RAPD results correspond to those of RFLP's (Williams *et al.*, 1990; Thormann and Osborn, 1992).The RAPD technique has been used to identify genetic polymorphisms in aphids (Black *et al.*, 1992) to distinguish biotypes. In *Aedes* mosquitos using only three to five individuals per population, Kambhampati *et al.* (1992) demonstrated that RAPD markers generate polymorphisms numerous enough to differentiate species and populations. With larger population samples, the RAPD markers can be used to examine the genetic structure of the population and to assess the mechanism of maintenance of genetic variability (Skinner and Camacho, 1995).

Ecological research on insects provides invaluable information on population structure, gene flow and genetic diversity based on their interaction with environmental factors, either biotic or abiotic (Jain *et al.*, 2009). Most of the tea plantations in Terai and Dooars regions are conventional i.e., managed by regular spraying of different synthetic insecticides of different chemical functional groups. These plantations are managed by using similar synthetic insecticides. Increase in the activity of three detoxifying enzymes i.e., general esterases, glutathione S-transferases and cytochrome P450 mediated monooxygenases both quantitatively and qualitatively has also been recorded in these insects (Saha *et al.* 2012b). Therefore, expectedly, the selection pressures on insect pest populations from the two locations are similar. But the pest management practices in the Darjeeling hills are more organic and cultural i.e., managed by herbal and microbial insecticides rather than spraying synthetic insecticides. The results showed a less genetic relatedness and higher genetic distance in the specimens from

Darjeeling hills compared to that of Terai and the Dooars populations. Also, tea plantations on the hill slopes of Darjeeling are, on an average 3,000 ft high than the in Terai and the Dooars plantations. Apart from insecticide spraying there are geographical variations too.

The proportion of polymorphic loci is not a good measure of genetic variation (Nei, 1987). A more appropriate measure of genetic variation is average heterozygosity or gene diversity (h). Higher heterozygosity values indicate broader genetic diversity (Nei, 1987). Higher gene diversity and Shannon information index in the Dooars population indicates a high heterozygosity (Table 4.26, 4.28). In a similar study using insecticide resistant and susceptible populations of *Spodoptera litura,* Janarthan *et al.* (2003) have found consistently presence of a specific band in insectiside resistant population. In repeated amplifications using insectiside resistant and susceptible populations, they have confirmed the presence of polymorphism. Various parameters such as morphological characteristics, protein (detoxifying enzymes) and data on DNA polymorphism have been used to describe genetic variation and population structure of a number of pests (Loxdale *et al.*, 1996). In another study on pronotal colorations of the sucking pest, *Helopeltis theivora,* Roy *et al.* (2009) have also noted polymorphism between Darjeeling hill (organic) population and those of Terai-Dooars. RAPD has been used to study genetic variation between and among the insect pest populations (Puterke *et al.*, 1993; Lopez-Brana *et al.*, 1996). The UPGMA dendrogram of twenty different populations of *Helopeltis theivora* formed nine different clusters (group) indicating ample genetic variations among the populations collected from different tea plantations with different mode of pest management and geographic location.

In recent years, use of synthetic insecticides has become a major concern for the tea industry, as importing countries are imposing stringent restrictions on the acceptability of processed tea having insecticide residues. These conditions warrant a review of the levels and use patterns of insecticides, which would restrict application to only effective insecticides, on the one hand, and reduce insecticide load on tea and the environment, on the otherhand. So once the genetic variability established in the three sucking insect pests of tea namely, *H. theivora, E. flavescens* and *S. dorsalis* populations from different sub-Himalayan tea plantations, it would be easier to formulate population specific control measures and in developing integrated resistance management strategies that can help in the effective control of these major and emerging pest of tea.

Research Highlights

Research Highlights:

- *Helopeltis theivora* produced more number of feeding spots on *Camellia sinensis* than other two alternative hosts i.e. *Mikania micrantha and Psidium guajava*. The number of feeding spots was always higher on *C. sinensis* both in male and female *H. theivora* in combination with both the non- tea hosts.

- The diameter of feeding spot was also higher in *C. sinensis* than other two hosts in both sexes of *H. theivora.* Compared with *C. sinensis*, the number and diameter of feeding spots on *M. micrantha and P. guajava* leaves were few and smaller in diameter.

- In a non-choice test (forced feeding), being forced to feed, *H. theivora* fed all studied hosts more or less similarly with no significant difference in feeding puncture marks made on different host plants.

- Female *Helopeltis theivora* always produced significantly high number of feeding spots in *C. sinensis* than male implying that the former acquires more nutrition from leaf tissue. Reduced number of feeding spots in *M. micrantha* and *P. guajava* leaves in comparison to *C. sinensis* may be due to the insect's low preference due to poor availability of nutrients in the alternative hosts or 'presence of toxic allelochemicals'.

- Expenditure of more time in each sucking stance by female than male on all the three host plants further substantiated the female's 'requirement of a higher energy input' possibly to produce viable eggs and to establish sustainable second generation.

- Life history traits (fitness traits) of *H. theivora* varied significantly on three studied alternative hosts. The nymphal duration was significantly high on *P. guajava* (22.5±0.4 days) than on *C. sinensis* (20.0±0.3 days) and *M. micrantha* (21.9±0.4).

- The total nymphal duration in *C. sinensis* was significantly low than other two host plants. The development time of *H. theivora* is significantly shorter on *C. sinensis*

(13.3±0.16 days) than on the other two hosts, *M. micrantha* (14.2 ± 0.22 days) and *P. guajava* (14.7 ± 0.23 days).

- However, the fecundity (*C. sinensis*: 172.6 ± 4.5 no. egg / female, *M. micrantha*: 128.6 ± 4.4 no. egg / female, *P. guajava*: 118.7 ± 3.3 no. egg / female), oviposition period (*C. sinensis*: 24.1 ± 0.7 days, *M. micrantha*: 22.5 ± 0.6 days, *P. guajava*: 21.7 ± 0.8 days) and hatchability (*C. sinensis*: 80.9 ± 1.9 %, *M. micrantha*: 69.4 ± 1.6 %, *P. guajava*: 64.1 ± 1.7 %) are recorded to be significantly higher on *C. sinensis*. The age at reproductive maturity and egg incubation periods were lower on *C. sinensis* than on the other two host plants.

- Life history traits (fitness traits) of *Empoasca flavescens* varied significantly on three alternative host i.e. *C. sinensis, Solanum tuberosum* and *Ricinus communis* considered in the present work. The total development period (in days) was significantly high on *R. communis* (21.4±0.38) than on *C. sinensis* (18.2±0.35) and *S. tuberosum* (19.2±0.32). The total nymphal period (in days) on *C. sinensis* was significantly low than other two alternative hosts. The nymph development period (in days) of *E. flavescens* is significantly shorter on *C. sinensis* (11.7±0.38) than on the other two hosts, *S. tuberosum* (12.8 ± 0.26) and *R. communis* (13.6 ± 0.35). However, the fecundity, hatchability percent and oviposition period were significantly higher on *C. sinensis* than on the other two host plants.

- The fecundity (*C. sinensis*: 20.3 ± 2.6 no. egg / female, *S. tuberosum*: 16.3 ± 2.8 no. egg / female, *R. communis*: 17.3 ± 3.2 no. egg / female), oviposition period (*C. sinensis*: 9.5 ± 1.12 days, *S. tuberosum*: 8.7 ± 1.22 days, *R. communis*: 8.4 ± 1.18 days) and hatchability (*C. sinensis*: 82.4 ± 4.4 %, *S. tuberosum*: 73.2 ± 5.3 %, *R. communis*: 68.6 ± 6.2 %) were recorded to be significantly higher on *C. sinensis*. The age at reproductive maturity and

egg incubation periods were lower on *C. sinensis* than the other two host plants (*C. sinensis*: 3.3 ± 0.35 days, *S. tuberosum*: 3.6 ± 0.35 days, *R. communis*: 3.8 ± 0.35 days).

- Similarly, in *Scirtothrips dorsalis,* the fecundity, egg incubation period, larval development period (LDP), total development period (TDP), pupal development period (PDP) varied greatly on three hosts i.e. *C. sinensis, R. communis, and Capsicum annuum*. In *C. sinensis* (11.4±0.36) significantly high fecundity was observed than on *C. annuum* (9.7±0.24) and *R. communis* (8.6±0.32). In *C. sinensis,* egg incubation period was 6.2±0.23 which was significantly less than other two hosts, *C. annuum* (7.1±0.34) *and R. communis* (7.5±0.52). The larval development period was 4.3±0.64 days on *C. sinensis*, 4.9±0.46 days on *C. annuum* and on 5.6 ±0.36 days on *R communis*.The total development period (TDP) was significantly less in *C. sinensis* (13.6±0.52) than other two alternative hosts i.e. *C. annuum* (15.5±0.36) *and R. communis* (16.7±0.14). On *C. sinensis* pupal development period was 3.1±0.42 days which is significantly less than on other two non tea hosts, *C. annuum* (3.5±0.34 days) and *R. communis* (3.6±0.22 days).
- The oviposition period (*C. sinensis*: 3.1 ± 0.52 days, *C. annuum*: 2.6 ± 0.36 days, *R. communis*: 2.5 ± 0.34 days) and hatchability (*C. sinensis*: 92.6 ± 1.14 %, *C. annuum*: 82.5 ± 1.16 %, *R. communis*: 74.6 ± 1.22 %) were noted to be significantly higher on *C. sinensis*. The age at reproductive maturity and egg incubation periods were lower on *C. sinensis* than the other two host plants (*C. sinensis*: 1.4 ± 0.29 days, *C. annuum*: 1.6 ± 0.21 days, *R. communis*: 1.9 ± 0.32 days).
- Activities of general esterases (GEs), Gluathione S-transferases (GSTs) and cytochrome P450 monooxygenases (CYPs) in *H. theivora*, *E. flavescens* and *S. dorsalis* populations collected from different Organic and Conventional tea plantations of the study area

showed significant variations in quantitative estimation. Populations sampled from Conventional plantations showed higher level of activity of all the detoxifying enzymes than the organic plantations.

- Alternate host reared populations also showed higher level of activity of all the detoxifying enzymes that tea-reared populations in quatitative estimation after rearing for five generation in respective hosts.
- The general esterase activity both in specimen sampled from conventional plantations and alternate host-reared populations varied significantly qualitatively.
- The populations of *H. theivora, E. flavescens* and *S. dorsalis* sampled from different tea plantations of Darjeeling foothill, Terai and the Dooars regions varied genetically forming different banding paptterns as revealed in the RAPD-PCR study,.
- The populations of *H. theivora, E. flavescens* and *S. dorsalis* sampled from different tea plantations of Darjeeling foothill, Terai and the Dooars regions formed different clades in UPGAM dendrogram based on Nei's (1978) genetic distance method.

List of Published /accepted paper related to sucking insect pests of tea by *Dhiraj Saha*

Scientific Paper Published in Peer reviewed Journal/ Proceedings:

1. **Saha, D.** and Mukhopadhyay, A. 2012. Insecticide resistance mechanisms in three sucking insect pests of tea with reference to North East India - an appraisal. *International Journal of Tropical Insect Science*. **33**(1): 46-70. (***Cambridge University Press, London,* U.K.**).

2. Mukhopadhyay, A., Roy, S., Das, S. and **Saha, D.** 2012. Some stress factors in tea plantation of Sub-Himalayan Terrain of North Bengal vis-à-vis the changing pest scenario -an overview. *Two and a Bud*. (In press).

3. **Saha, D.** and Roy, S. 2012. Evaluation of Insecticide susceptibility and activity of three principal detoxifying enzymes in female *Helopeltis theivora* Waterhouse (Miridae: Heteroptera) from Sub-Himalayan tea plantations of North Bengal, India. In: "***Proceedings of 2nd International Symposium on Biopesticide and Ecotoxicological Network (2nd ISBioPEN)".*** Department of Zoology, Kasetsart University, Bangkok, Thailand. pp. 387-400.

4. **Saha, D.**, Mukhopadhyay, A. and Bahadur, M. 2012. Variation in the activity of three detoxifying enzymes in major sucking pest of tea, *Helopeltis theivora* Waterhouse (Heteroptera: Miridae) from sub-Himalayan tea plantations of West Bengal, India. *Proceedings of Zoological Society*. DOI: 10.1007/s12595-012-0039-y. Published online 2nd August, 2012. (***Springer-Verlag Publication, Netherlands***).

5. **Saha, D.**, Mukhopadhyay, A. and Bahadur, M. 2012. Genetic diversity of *Empoasca flavescens* Fabricius (Homoptera: Cicadellidae), an emerging pest of tea from sub-Himalayan plantations of West Bengal, India. *Proceedings of Zoological Society* (July-Dec 2012) 65(2): 126-131. (***Springer-Verlag Publication, Netherlands***)

6. **Saha, D.**, Mukhopadhyay, A. and Bahadur, M. 2012. Effect of host plants on fitness traits and detoxifying enzymes activity of major sucking insect pest of tea, Helopeltis theivora (Heteroptera: Miridae). *Phytoparasitica.* **40:** 433-444. DOI: 10.1007/s12600-012-0244-2. Published online 19th July, 2012. (***Springer-Verlag Publication, Netherlands***).

7. **Saha, D.**, Roy S and Mukhopadhyay, A. 2012. Insecticide susceptibility and activity of major detoxifying enzymes in female *Helopeltis theivora* Waterhouse (Heteroptera: Miridae) from Sub-Himalayan tea plantations of North Bengal, India. *International Journal of Tropical Insect Science*. **32**(2): 85-93. (***Cambridge University Press, London, U.K.***)

8. **Saha, D.**, Roy S and Mukhopadhyay, A. 2012. Seasonal incidence and enzyme-based susceptibility to synthetic insecticides in two upcoming sucking insect pests of tea. *Phytoparasitica* **40**: 105-115.(***Springer-Verlag Publication, Netherlands***).

9. **Saha, D.**, Mukhopadhyay, A. and Bahadur, M. 2012. Variation in detoxifying enzymes activity in tea greenfly, *Empoasca flavescens* Fabricius (Homoptera: Jassidae) from sub Himalayan organic and conventional tea plantations. In: "***Proceedings of National Symposium on Biodiversity Status and Conservation Strategies with special Reference to North East India*****"**, Varatharajan, R. (Ed.). Centre for Advanced Studies, Department of Life sciences, Manipur University, Manipur, India. pp. 257-266. ***ISBN 978-81-923343-1-8.***

10. **Saha, D.**, Mukhopadhyay, A. and Bahadur, M. 2010. Variation in Detoxifying Enzymes of Assam Thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) from Organically and Insecticide Managed Tea Plantations. *NBU Journal of Animal Sciences* **4**: 45-52. ***ISSN 0975-1424.***

11. **Saha, D.** and Mukhopadhyay, A. 2008.Variation in Esterase activity of *Empoasca flavescens* Fabricius (Cicadellidae: Homoptera) in different tea Estates of Terai. *NBU Journal of Animal Sciences* **2**(2): 39-41. ***ISSN 0975-1424***.

Paper Presentations at National and International Symposium/ Conference related to sucking insect pests of tea by Dhiraj Saha:

1. Silver Jubilee Symposium (National) on ***"Dimensions of Research Applications in Animal Sciences" a***t Department of Zoology, University of North Bengal, Siliguri during December 2 – 3, 2008.
2. National Symposium on ***"Pesticide stress on Target, Non-Target Organisms and Human Health"*** at Department of Zoology, University of North Bengal, Siliguri during February 11 – 12, 2010.
3. National Conference on ***"Evaluation of Biodiversity of Eastern Himalaya and Adjoining Plains"*** at Department of Zoology, University of North Bengal, Siliguri during December 1 – 4, 2010.
4. National Symposium on ***"Biodiversity Status and Conservation Strategies with special Reference to North East India"*** at Department of Life Sciences, Manipur University, Manipur during March 17 – 18, 2011.
5. 3RD Congress on insect science ***"Pest Management for Food Security and Environment Health"*** at Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab during April 18 – 20, 2011.
6. National Symposium on ***"Biodiversity and Food Security: Challenges and Devising Strategies"*** at Indian Institutes of Pulses Research (IIPR), Kanpur during December 10 – 11, 2011.
7. National Symposium on ***"Anthropogenic Toxicants, Green Chemistry, Biodiversity and Sustainable Development: An Interdisciplinary Approach"*** at Department of Zoology, P.D. Women's College, Jalpaiguri, West Bengal, India during March 13 – 14, 2012.
8. National Symposium on ***"Research in Animal Sciences: Evaluation and Development"*** at Department of Zoology, University of North Bengal, Siliguri during March 19 – 20, 2012.
9. National Symposium on ***"Biodiversity and Sustainability vis-à-vis EconomicDevelopment in the Northern parts of West Bengal"*** at Raiganj Surendranath Mahavidyalaya, Raiganj, Uttar Dinajpur, West Bengal during August 26 – 27, 2012.
10. International Symposium on ***"Biopesticide and Ecotoxicological Netwirk (ISBioPEN)"*** at Department of Zoology, Kasetsart University, Bangkok, Thailand during September 24-26, 2012.
11. 4TH International Insect Science Congress (**IISC**) on ***"Exploring the Incredible Insect World"*** at University of Agricultural Sciences at Bangalore, India during February 14-17, 2013.
12. National Symposium on ***"Man, Animal and Environment Interactionin the Perspective of Modern Research"*** at Department of Zoology, University of North Bengal, Siliguri during March 8 – 9, 2013.

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