# 1. Introduction

## 1.1. Early history of tea cultivation

The first attempt of tea cultivation in Darjeeling dates back to January 1834 when Lord Willam Bentlnck proposed, to the Council of the East India Company, setting up of a tea committee to investigate and make recommendations on suitability of tea cultivation in India. The tea committee decided to send their secretary G.J. Gordon to China in order to acquire tea seeds and some tea workmen familiar to tea cultivation and manufacture. From this original consignment of China seed around 42,000 young plants could be raised which were allocated to three main areas, 20,000 to the hill districts in the Kumaon in North India, 2,000 to the hills of South India and the remaining 20,000 to the then North-East (N.E.) frontier (Weatherstone, 1992). Out of this initial trial, seed tried in Darjeeling grew well. As per the available records one Dr. Campbell, a civil surgeon, planted tea seeds in his garden at Beechwood, Darjeeling 2100 m above mean sea level (amsi) as an experiment with reasonable success. Subsequently the government, in 1847, selected the area to raise tea nurseries. With the plants raised in the government nurseries, the first commercial tea gardens in Darjeeling hill area were Tukvar, Steinthal and Aloobari tea estates in 1852 (Pathak, 2004).

## 1.2. Present scenario of tea cultivation and its arthropod pest problem

Tea plantation of North Bengal is spread over three regions – the Darjeeling hills, its Terai region, and the plains of the Dooars. North Bengal produced some 10,853 Th kgs of tea in March 2006. According to the statistics of Tea Board of India, there are 308 big and 1232 small tea gardens in North Bengal. Total area under tea is 5,19, 700 hectares and in 2005, total production of tea was 927.98 million kilograms.

At present there are 86 running gardens producing 'Darjeeling Tea' on a total land of 19,000 hectares. The cool and moist climate, the soil, the rainfall and the sloping terrain all combine to give Darjeeling tea its unique "Muscatel" flavour which is regarded as the "Champagne of Teas". The total annual production of such tea is in the range of about 10 to 12 million kilograms. Tea grown in the Darjeeling foothills, Terai and the Dooars plains are mostly high yielding clones.

Each tea growing region has its own distinctive pest fauna, though many species have been recorded from more than one country. About 300 species of arthropod pests are known to attack tea in India (Banerjee, 1993; Muraleedharan *et al.*, 2001). During last three decades several changes have taken place in the agronomic practices, which have also magnified our pest problems. Every part of the tea plant is subject to attack of pests. A steady loss of 10% due to overall pest attack is a generally accepted figure though it could be 40% in devastating attacks by defoliators (Banerjee, 1993). In addition to direct crop loss, pest damage can adversely affect the quality of processed tea. General observation and planters' experience indicate that looper (*Buzura suppressaria*), red slug (*Eterusia magnifica*), tea mosquito bug (*Helopeltis theivora*) and red spider mite (*Oligonychus coffeae*) are the most common tea pests of Darjeeling foothills, Terai, the Dooars areas with their incidence also in the plantations of North-East (N.E.) India. Planters of Terai and the Dooars are facing serious problems in combating the outbreaks of these folivores and sucking pests. *Oligonychus coffeae* breeds throughout the year and subsists on mature sustenance leaves. *Helopeltis theivora* causes extensive damage by attacking the tender leaves and the growing shoot. The defoliators like *Buzura suppressaria* and *Eterusia magnifica* have their share and defoliate tea in Terai, the Dooars and North-Eastern plantations (Muraleedharan, 1993). In the past, all tea phytophages were not active simultaneously and a well-marked seasonal

appearance for each was evident with the seasonal cycle of the growth and productivity of the plants (Banerjee, 1977). Now a day, the planter's experiences indicate that most of the pest species remain active throughout year with overlapping seasonal cycle of the growth and productivity of the tea plants. The appearance of the pests needs timely management mostly by use of synthetic pesticides. Since 1962, increasing use of pesticides in protection of tea plantations has been the common and popular practice.

As the four major pest species have a wide occurrence attacking different tea cultivars in the plantations of the Darjeeling foothills and their adjoining plains of Terai and the Dooars, an assay of the major hydrolases (digestive enzymes) and oxidoreductases of the four common tea pests in question has been undertaken. The findings would likely furnish an understanding of the trophic strategies of these pests in exploiting and utilizing the tea plant. These pests have been exposed to conventional pesticide sprays such as organophosphates and synthetic pyrethroids for long, so there is possibility of development of different levels of pesticide resistance. This often makes the pests to a greater or less degree unmanageable by synthetic pesticides. Attempts have, therefore, been made to study the variation based on certain biochemical parameters of the four pests. Profiles of three main isozymes related to resistance and their quantities were studied from the local populations. As very little work has been done on the resistance / tolerance status of these pests, the contemplated study on three detoxifying enzymes is expected to reveal for the first time the biochemical nature and level of pesticide resistance / tolerance in the population of the four concerned pests occurring in this region.

## 1.3. Location and Physiognomy of the study area

Tea, the most ancient non-alcoholic beverage, is still a popular drink in the world. Tea, *Camellia sinensis* (L.) O. Kuntze, cultivation in India has a long history which is found to spread over approximately 5.19 lakh hectares. The Districts of Darjeeling and Jalpaiguri of West Bengal and a large part of Assam of N.E. India (**Fig. 1**) has gained importance both for quality tea and high yield. The tea growing regions of Darjeeling district is located between 26°31' and 27°13' north latitude and between 87°59' and 88°53' east longitude. The Northern part of the district has the distribution of eastern Himalayan range while the southern part consists of a stretch of alluvial plain at the base of the hills and is known as the Terai. The Terai is situated 91 meter above sea level with an average rainfall of 350 cm and an average temperature of maximum $30^0$C and minimum $12^0$C. The soil is moderately acidic, rich in organic material and is suitable for tea plantation. Besides heavy rainfall, Terai and its adjacent Dooars regions are well watered by a number of major rivers and a host of rivulets. The major rivers from east to west of Terai and the Dooars are: Siltorsha, Torsha, Jaldhaka, Teesta, Mahananda, Balason and Mechi.

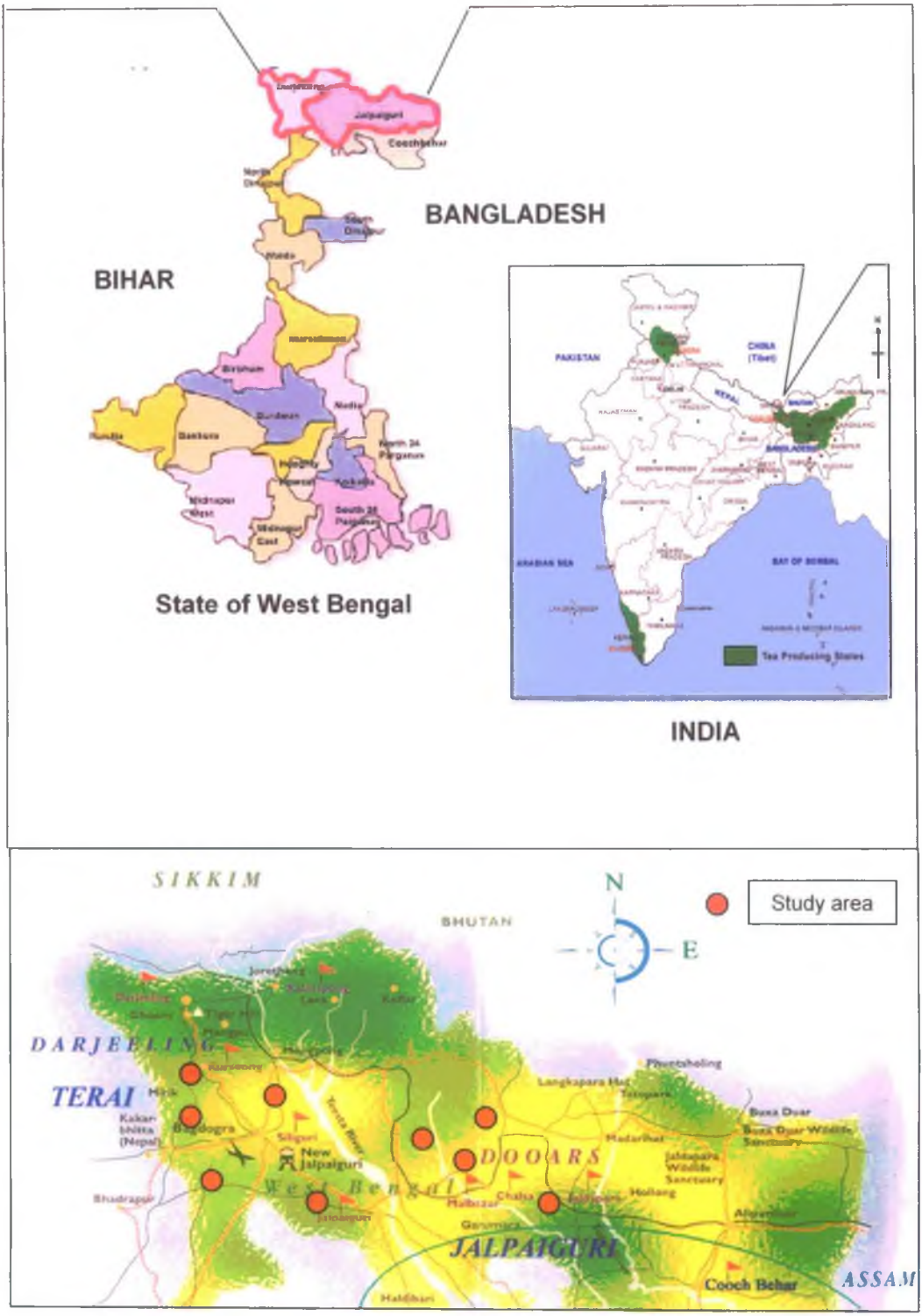


**Fig. 1. Tea growing areas of Darjeeling and Jalpaiguri districts of West Bengal (Not to scale)**

## 1.4. Major arthropod pests of tea from Darjeeling foothill and its adjoining plain

Tea, as a perennial monoculture crop provides an inexhaustible resource for colonization by several guilds of insects and mites (**Fig. 2**) many of which easily attain the pest status in such a stable ecosystem. Each tea-growing region has its own distinctive pest fauna though they may be found in other areas as well. More than a thousand species of arthropods are known to attack tea all over the world though only about 300 insects and mites are recorded from India (Muraleedharan *et al.*, 2001). Out of them Lepidoptera form the major component (31.53%) followed by Hemiptera (26.29%) (Chen and Chen, 1989).


**Fig. 2. A Tea bush showing distribution of major pests (four) attacking leaves**

Insects may consume every anatomical part of the plant but show specialization with regard to the feeding sites they occupy (Schoonhoven *et al.*, 1998). Every part of the tea plant has a specific guild of pests. They may be grouped as per their feeding activity on stems, roots and foliage. Some sucking insects such as thrips, jassids and aphids cause extensive damage to the plant by making the shoot unproductive. The tea mosquito bug (*Helopeltis theivora*) is a serious pest both in South and N.E. India. *H.theivora* primarily feeds on leaves and new flushes and to a lesser extent on tender stem of tea plants. The feeding damage by this pest appears as a discoloured

necrotic area or a lesion around the point of entry of the stylets into the plant tissue. The lesion can be elongate and becomes darker with age as the tissue around the stylet puncture dies, presumably in response to the enzymatic action of the insects salivary secretions (Stonedahl, 1991). In the salivary gland of *H.antonii*, hydrolytic enzymes (protease, lipase), oxido-reductase enzymes (catechol oxidase, catalase and peroxidase) were detected. The salivary enzymes were implicated for the cause of phytoxaemia on various host plants as well as detoxification of defensive chemicals (Sundararaju and Sundara Babu, 1996). Among the leaf attackers, mites are most widespread. Distribution of foiivores, such as flushworms, leaf rollers, bunch caterpillars are more in the lower elevations of Darjeeling. In the Terai and the Dooars regions of Darjeeling foothills and plains, there are often outbreaks of defoliating pests such as *Buzura (Biston) suppressaria* Guenee (Looper caterpillar) and *Eterusia magnifica* Butler (Red Slug caterpillar) causing extensive damage of the foliage. Hence, the need for introduction of Integrated Pest Management in tea (Barbora *et al.*, 1994) in N.E. India.

The damage potential and recurrence of many pests are well known from tea plantations for long and many region specific new pests are now being recorded, but due to inappropriate measures taken for their control, the pest problem has further intensified. Although about Rs.15 crores is being spent in N.E. India alone for the control of pests and diseases, it has been found that about 6 to 14% of tea is lost due to insects, mites and weeds (Banerjee, 1976). Normally 10% of the total crop is lost annually due to pests but this could rise to 40% in devastating attack by lepidopteran defoliators (Banerjee, 1993). Sivapalan (1999) also reported that various assessments on crop loss by respective tea pest had been done from time to time in the different tea growing countries. This loss ranges from 5 to 10% to as high

as over 50%. But, these differences are also dependent on the prevailing climate, genetic variation / uniformity (seed /clone), age of tea, soil type and the prevailing fertility status etc. as such, it is difficult to estimate the crop loss accurately caused by a particular species.

Among the tea growing regions of North-East India, pest activity has always been reported to be high in the Dooars and Terai regions. The average use pattern of the synthetic pesticides was estimated in the year 1990 to 1994 to be 11.5 kg / l / hectare / year in Assam Valley and Cachar; 16.75 kg / l / hectare / year in the Dooars and Terai, and 7.35 kg / l / hectare / year in Darjeeling. Further the average annual consumption of insecticide and acaricide in the Dooars and Terai was 7.05 and 3.49 kg / l / hectare / year respectively (Barbora and Biswas, 1996). A recent survey, 1998-2000 suggested that the pesticide (insecticide + acaricide) consumption increase to 24.076 kg / l / hectare / year in Terai region and around 14.16 kg / l / hectare / year in the Dooars region and average pest management cost is about Rs 7,000-10,000 / year / hectare in these regions. The average pesticide consumption was highest in the Dalgaon (19.56 kg / l / hectare / year) and lowest in Binnaguri (11.27 kg / l / hectare / year) sub districts of tea located in the Dooars area of Jalpaiguri district. Survey also reveals that on an average, the synthetic acaricides accounted for nearly 25% and synthetic insecticides around 60% while the rest 15% constituted safer pesticides. Organophosphates accounted for nearly 64%. The use of synthetic pyrethroid was also found to be alarmingly high at 0.73 kg / l / hectare / year (approx. 9%). Conversion of the quantity of pesticide used into number of effective spraying rounds on the basis of standard recommended dilution showed that, on an average nearly 18 rounds of pesticide application were resorted to by the estates in the region (Sannigrahi and Talukder, 2003).

Routine application of chemical pesticides for the protection of tea crops has been a common and effective practice since the last 50 years but with growing usage of pesticides, the resistance of the arthropod tea pests to these chemicals has likely increased (Banerjee, 1968; Sarker and Mukhopadhyay, 2003, 2006a, b, c). Therefore extreme precautionary measures must be adopted before a pesticide is introduced to tea for pest control (Das, 1962), which will also save residue problem. In 1934, there were 10 insect species known to be resistant to pesticides whereas by 1980 the figure rose to 432 and by 1990 about 500 cases were reported (Schulten, 1990). High incidence of pests in tea has led to the indiscriminate use of pesticides leading to problems such as killing of non-target organisms including natural enemies of pests (Anonymous, 2003), human health hazards, enhanced environmental hazards and above all the problems of insecticide resistance. The need for conserving natural enemies of pest (Banerjee, 1967) and an integrated approach for controlling mite pests of tea has been emphasized (Banerjee, 1975). Further, the role of Integrated Pest Management has been stressed by Muraleedharan and Selvasundaram (2002) for the ever-growing pest problems in agricultural crops. It goes without saying that in the same spirit Integrated Pest Management (IPM) in tea requires planning to cover all the serious arthropod pests.

The major folivores, *B.suppressaria* (Looper caterpillar), *Et.magnifica* (Red slug caterpillar) and major sucking pests, *H.theivora* (Tea mosquito bug) and *O.coffeae* (Red spider mite) that are important tea pests of Darjeeling foothills, Terai and the Dooars areas and the State of Assam in North-East India have been considered in this study. A brief introduction to these pests, the damage symptoms and the synthetic pesticides usually applied for their control are provided herewith:

### 1.4.1. Looper caterpillar: *Buzura suppressaria* Guenee

**(Geometridae: Lepidoptera)**

**Description**

**Fig. 3. An advanced stage of looper caterpillar of tea**

Moth usually gray, finely speckled with black, forewing with yellow wavy bands, indistinct irregular yellow median lines and an ill defined median-postmedian maculate black and marginal series of yellow spots. Wing expanse 5-7cm, female wings larger than male. Moths have high degree of melanism ranging from different shades of brown to creamy white.

1st and 2nd instar looper caterpillars are black with ring like thin white bands (*c.*7) at certain gaps, spread over the entire length of body. All larval stages have 3 pairs of thoracic legs, a pair of prolegs and a pair of claspers at the end of the body forcing the animal to make looping movement. The body colour of larva changes in advanced stages to green then to brown and finally taking the colour of twig or stem of the tea bush. There are five larval stages. It grows from the average length of 2.9 mm in the 1st instar to a length of 30.55 mm in the fifth instar (**Fig. 3**). Freshly laid eggs are bluish green, cylindrical 0.27 mm length, laid in clusters (300-500) covered with buff coloured hair preferably on shade tree, sometime on axil of the tea leaf.

**Damage symptoms**

1st and 2nd instar caterpillar nibble edge of young leaves of tea bush making tiny holes. 3rd instar chews the margin of a leaf making small cuts and nicks. 4th and 5th instars can eat up the whole of the leaf resulting in large-scale defoliation of a tea bush.

**Control measures**

Insecticides usually applied for control of loopers are: Quinalphos 25EC @ 500 ml/ ha; Chlorpyriphos 20EC @ 500 ml/ha; Cypermethrin 10EC @ 250 ml/ha; Fenvalerate 20EC @180 ml/ha; Deltamethrin 2.8EC @ 180 ml/ha.

### 1.4.2. Red slug caterpillar: *Eterusia magnifica* Butler

### (Zygaenidae: Lepidoptera)

**Description**

Brightly coloured moths with an average wingspan of 50.6 mm in male and 56.46 mm in female. Body length of male is slightly smaller than that of female. Head, thorax and two basal segments of abdomen black and remaining abdomen except black tip in males pale yellow. Male antennae unipectinate, female antenna filiform. Forewing purple brown with a greenish tinge, a basal spot, medium white bands broken up usually into five spots. A white spot at the end of the cell and an irregular row of submarginal white spot. Hind wing black at the base followed by a yellow band, wide on the inner margin and with a few subtropical white spot. The apical area of hind wing marked with brilliant blue.

**Fig. 4. A fifth instar of red siug caterpillar**

Eggs oval. 0.9 mm in length and 0.4 mm breadth, yellow but turning green before hatching. 1st instar brown with tubercles on the back bearing hairs, three pairs of thoracic legs and five pairs of prolegs, last pair acting as clasper. Prolegs end with disc like structure for attachment to the plant surface. 3rd instar larva develops a brown coloured ring like band on dorsal surface. A full-grown larva is brick red with well-developed tubercles on the back (**Fig. 4**), on disturbance a thick clear fluid

exudes from the tubercles. Larval body length ranges from 1.32 mm for the 1st instar to 20.54 mm for the 5th instar.

**Damage symptoms**

1st and 2nd instar larvae nibble the epidermal surface of the leaf. 3rd instar feeds on the side of the leaf blade. Advanced larval instars eat entire leaf blade and parts of stem including the bark. Larvae usually attack the mature leaves of a tea bush.

**Control measures**

Insecticides applied for control of red slug caterpillars are: Endosulfan 35EC @ 750 ml/ha; Quinalphos 25EC @ 500 ml/ha; Chlorpyriphos 20EC @ 500 ml/ha; Cypermethrin 25EC @ 120 ml/ha; Fenvalerate 20EC @180 ml/ha.

### 1.4.3. Tea mosquito bug: *Helopeltis theivora* Waterhouse

**(Miridae : Heteroptera : Hemiptera)**

**Description**

**Fig. 5. *Helopeltis* bug resting on a tea leaf**

The egg is white, cylindrical and slightly curved. Two unequal silvery filaments, the respiratory horns, arise laterally on either side of the operculum. Tea mosquito bug have five nymphal stages. The newly hatched nymph, light orange in colour, measured about 1.49 mm. Second instar nymph measured about 2.00 mm and their abdomen deep orange in colour. Body reddish green and measured about 3.00 mm length in third instar nymph, scutellar horn distinct. Wing pads became dark and body greenish yellow measure about 4.17 mm in fourth instar. Fifth nymphal instar measured about 5.00 mm and resembled adults with a reddish green thorax and

green abdomen. The newly emerged yellowish brown adult turned metallic black (**Fig. 5**) with in an hour. Head black, eyes oval, wings black and abdomen green.

**Damage symptoms**

Feeding on the leaf results in the formation of water-soaked spot, which becomes distinct within minutes after the commencement of feeding. Subsequently these spots become circular and pale green and then gradually turned dark brown within an hour. These circular areas later become dark brown sunken spots. Gradually, they dry up and hole appear in their place. Feeding near the petiole and on the midrib cause an elongated lesion resulting in the curling of leaves. Severely infested leaves become deformed and curled, which lead to retardation of shoot growth.

**Control measures**

The insecticides applied for control of mosquito bugs are: Quinalphos 25EC @750 ml/ha; Chlorpyriphos 20EC @ 750 ml/ha; Fenthion 80EC @ 200 ml/ha; Endosulfan 35EC @ 1000 ml/ha.

### 1.4.4. The Red spider mite: *Oligonychus coffeae* (Nietner)

**(Tetranychidae : Acarina)**

**Description**

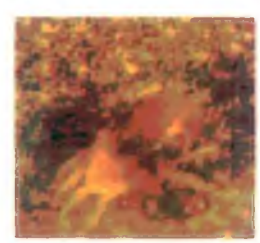

**Fig. 6. Adult Red spider mite**

Eggs red, spherical with 0.1 mm diameter and with filament. Immature stages include roundish six-legged larva, 0.15mm in length, protonymph with 4 pairs of leg, oval shape, 0.2 mm long, anterior part pale red and abdomen deep reddish brown; deutonymph similar to pronymph but larger about 0.25 mm in length, female deutonymph slightly larger than male and resembles adult. Anterior part of adult female elliptical and crimson, while posterior part purplish brown (**Fig. 6**). Female measures, 0.35 mm to 0.45 mm in length. Males smaller with tapering abdomen.

**Damage symptoms**

Increase in red spider mite population is mainly related to the dry season and rise in temperature during spring and early summer in North-East India. The mites attack the mature leaves on their dorsal side starting from the midrib and vein areas and gradually spreading on the whole of the leaf. The feeding impacts leave the leaf coppery-red coloured. Mites leave their cast skin and empty eggs as white spakes on the leaf surface. Heavy infestation leads to dropping of the leaf. Several factors like drought, unprunned tea, sunny days, unsmooth leaf surface texture and dust accumulation allow easy proliferation of the mite population. Further the increase in mite population may be influenced by organic manuring, poor drainage and may be influenced by the weeds acting as alternate host in the plantation areas.

**Control measures**

Chemical used for control of red spider mites are: Ethion 50EC @ 750 ml/ha; Quinalphos 25EC @ 750 ml/ha; lime sulphur or wettable sulphur (1 kg / ha), Dicofol 18.5EC @ 1000 ml/ha; Fenpropathrin 10EC @ 500 ml/ha.

## What prompted to undertake the present study?

In view of the growing pest problem in tea and increased resurgence of pests it was felt necessary to undertake the present study on "certain hydrolases and oxidoreductases of the major arthropod pests of tea from Darjeeling foothill and its adjoining plain". In the lower elevation of Darjeeling foothills and adjoining tea plantations lepidopteran larvae and sap-feeding bugs have been the subject of the study largely as far as their crop damage activities are concerned. In severe attacks by lepidopteran caterpillars, entire leaf as well as woody parts of the bush may be eaten, while young shoots and leaves dry up under attack of sap feeders. The digestive enzymes commonly found in the salivary and midgut of these pests are

therefore of interest in understanding their feeding relation with host (tea) as well as in devising methods of non-conventional pest management. Herbivore insects also possess an assemblage of enzymes that constitute their defense against chemical toxicants. These defense enzymes work by oxidation, reduction, hydrolysis or conjugation of molecules. The oxidoreductase enzymes are of great value because of their involvement not only in defensive but also in processing the secondary metabolites of the host plant. Many such enzymes involved in detoxification pathways act on a broad array of substrates found in plant allelochemicals and chemical pesticides. To characterize the array of digestive, oxidoreductase and detoxifying enzymes of major arthropod pests of tea, the present investigation is contemplated. This knowledge-base may be utilized in designing control programmes of these tea pests in future through use of enzyme inhibitors, host plant resistance programmes and by detection of pesticide resistance status of tea pests from this region. Moreover, no literature is available on biochemical identity of the tea–pest species from foothills and plains of Darjeeling and also on their pesticide resistance status as "strains", or "biotype". To fulfill these gaps and to usher in newer approaches of pest management, the following objectives of study have been laid down.