



Tea is a perennial monoculture crop, which provides suitable microclimate as well as continuous supply of food to number of arthropods. Each tea population within an agro-climatic area has its distinctive pest problem. The classical work of Watt and Mann (1903) offers the basic knowledge of tea pests occurring in Asia. Cranham (1966) and subsequently Muraleedharan (1983) reviewed the pest problems of this crop, on a global basis. Our knowledge of tea pests in North-East India is mainly based on the contribution of Cotes (1895), Watt and Mann (1903), Hainsworth (1992), Das (1965), Banerjee (1964, 1966a, 1966b, 1967, 1969, 1970, 1971, 1976 and 1977) Gurusubramanian *et al.*, (2005).

Origin, taxonomic character and distribution of *Helopeltis* spp

Plant bugs of Genus *Helopeltis* are serious pest of various cultivated plants in the old world tropics. The damaging effect of these insects on tea plants in India was documented over a century ago in reports by Peal (1973) and Wood-mason (1884). It was in these early accounts that the common name “tea-bug” and “tea-mosquito” were established, along with various names referring to feeding injury such as “tea-blight”, “mosquito-blight” and “spot-blight” etc. Since the late 1800s over 100 species of plants have been reported as host for *Helopeltis* spp. including a number of major cash crops such as black pepper (*Piper nigrum*), cashew (*Anacardium occidentale*), cinchona (*Cinchona* spp.), cocoa (*Theobroma cocoa*) and Tea (*Camellia sinensis*). In tea it was first recorded in Java in the year 1847 (Rao, 1970). In India the pest was noticed in the year 1968 in Cachar (Watt and Mann, 1903)

Helopeltis belongs to the subfamily Bryocorinae, tribe Dicyhini and subtribe Monaloniina, which is distinguished from other tribes of Bryocorinae by the elongate,

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cylindrical body form, the structure of the pretarsus, the reduced numbers of meso and metafemoral trichomae, the metathoracic sent efferent system lacking developed ostiole and evaporative area on metaepisternum and eggs with respiratory horns (Schuh, 1995). *Helopeltis* is readily distinguished from other members of the tribe by the large, spine like process on the scutellum and by characteristic of the male and female genitalia, particularly of the genital chamber of the female.

Helopeltis theivora (Hemiptera: Miridae), commonly called tea mosquito bug (TMB) is distinguished from congeners by a combination of characters i.e. base of antennal segment-I narrowly pale, distal third of antennal segment II and much of segment III of male with long, erect setae; head usually with broad, pale stripe laterally; pronotum usually with anterior half of collar and at least anterolateral margins of disc pale or sometime more broadly pale, leaving only posterior margin of disc infuscated, abdominal sterna I-IV uniformly pale laterally (Stonedahl, 1991).

The genus *Helopeltis* has a palaeotropical distribution extending from West Africa to New Guinea and Northern Australia. The genus *Helopeltis* was reported from Java (Rao, 1970 and Roepke, 1916), Sri Lanka (Mann, 1907; Ballard, 1921), Indonesia (De Jong, 1934), Africa (Harris, 1937 and Boulard, 1967), Zambia (Javaid, 1985), Malaysia, (Miller, 1939), Kanya (Nganga, 1977) and India (Watt and Mann, 1903). Schuh (1995) reported *H. theivora* from Java, Sumatra, Thailand, China and Hainan Is. beside Srilanka India and Malaya. ◦

Das (1965) reported that *H. theivora* was a major pest of tea in the Dooars, Darjeeling and Cacher. The tea mosquito bug *Helopeltis theivora* was the first pest to threaten the south India tea industry (Rao, 1970).

Pesticide consumption in tea in India and the Dooars.

During the last several decades, the control of pests, diseases and weeds in tea fields is predominantly by the use of synthetic chemicals. Though broad spectrum pesticides offer powerful incentives in the form of excellent control, increased yield and high economic returns, they have serious drawbacks such as development of resistance to pesticides, resurgence of pests, outbreak of secondary pests, harmful effects on human health and environment and presence of undesirable residues. The average use pattern of chemical pesticides was estimated to be 11.5 kg/lit/ha in the Assam Valley and Cachar, 16.75 kg/lit/ha in Dooars and Terai and 7.35 kg/l/ha in Darjeeling (Barbora and Biswas 1996). In a recent survey, an average of 14.16 l/kg of pesticides was used per hectare per year of which synthetic pesticides constituted 85% and the rest 15% were of organic and inorganic origin in the Dooars area. Within the synthetic insecticides, organophosphate compounds (64% - 5 rounds per year) were most preferred followed by organochlorine (26% - 2 rounds/year) and synthetic pyrethroids (9% - 7 rounds per year) (Sannigrahi and Talukdar, 2005). In North East India, Tocklai Experimental Station of the Tea Research Association (TRA), Jorhat, is the premier institute to test and certify the plant protection chemicals for use in tea plantations. Earlier, TRA recommended different classes of pesticides as endosulfan, quinalphos, phosphomidon, phosalone, acephate, dimethoate, chlorpyrifos, monocrotophos, oxydemeton methyl, lambda cyhalothrin, beta cyfluthrin, etofenprox, cartap-hydrochloride, alphamethrin, cypermethrin, deltamethrin, profenfos, thiomethoxam, imidacloprid, dicofol, ethion, propargite, fenazaquin, sulfur and neem

formulations for controlling tea pests (Anonymous, 2001). Organophosphate, organochlorine, carbamate, synthetic pyrethroid insecticide have been in use on tea in northeast India for the past 100 years. Much of the efficacy and sustainability of these groups of insecticides in tea pest management depend on the levels susceptibility of the major target pests.

During early forties, DDT dusting was a routine practice to combat the problem of Tea mosquito bug (*H. theivora*). As such by 1954 occurrence of this pest was rather low and the damage could hardly be regarded as causing loss in crop. After four decades the pest (TMB) has reappeared (during 1994) in an alarming way (Sudhakaran, 2000). In recent Years the management of tea mosquito bug has become the central problem for the planters (Rahman *et al.*, 2006a), due to persistent infestation round the year, the consumption of insecticides reaching up to 8.20-16.94 l/ha and costs shooting up by Rs.2500 – Rs.6000/ ha. Of late dimension of *H. theivora* infestation has alarmingly increased resulting in double consumption of toxic chemical pesticides (Gurusubramanian *et al.*, 2005).

Assessment of crop loss and damage potential of *H. theivora* in tea:

It is evident from the work of Watt and Mann (1903) that the loss in production due to *H. theivora* was as high as 22500 kgs made tea in the District of Cacher, Assam, in the year 1967. Similarly Das (1965) reported the crop loss of 750000 kgs of made tea in eleven gardens in the Dooars in the year 1958. Prasad (1992) and Barbora and Singh (1994) reported that usually the crop loss due to *H. theivora* infestation was 25% to 50%. Average crop loss due to *H. theivora* was 150 kg made tea per ha in Bangladesh (Ahmed, 1996). Earlier studies indicated that crop loss due to *H. theivora*

could be total if the attack was very severe (Rao and Murthy, 1976). It is a fact that tea mosquito bug has been the major challenge in recent years in N.E. India. Out of 4.36 lakh hectares of total plantation 80% of tea plantations have been suffering from *H. theivora* infestation. Peak season of *H. theivora* infestation (May-July) and rainy period (June-July) coincides with second flush having more quality and quantity. (Gurusubramanian *et al.*, 2005). In South Indian tea Muraleedharan and Selvasundaram, (2002) found ETL value for *H. theivora* in as 5% infestation, which was almost similar to that recommended for *H. schoutedeni* affecting tea in Central Africa during main cropping season (Rattan, 1987). But ETLs change with stage of crop growth, with costs of pesticides and labour, with weather conditions, with market prices, etc., so, ETLs vary from region to region and even from field to field.

Life cycle and oviposition pattern of tea mosquito bug, *Helopeltis theivora* on tea.

Extensive studies have been conducted on biology of *H. theivora* (Anonymous, 1974 and 1976; Sudhakar, 1975; Ambika and Abraham 1979; Das, 1965; Sathiamma, 1984). Das (1965) had given an account of the life history of this pest in North East India. The Duration of life cycle varied during different seasons of the year depending on climatic conditions. The life cycle was completed in about a fortnight during June and July but during cold weather the developmental period extended for 5-8 weeks (Das, 1984). The life cycle of the *H. theivora*, its fecundity, longevity and other parameters throughout the year along with detailed description of the different stages of *H. theivora* are also reported by Gope and Handique (1991) from North East India. Bio-Ecological Studies on the *H. theivora* in South India have been reported by Muraleedharan *et al.*, (2000). *Helopeltis* spp., in general, has overlapping

generations. In the tea growing regions of North-East India, population density of *H. theivora* reaches the peak in June to September and gradually declined from November, as the weather cools down. Few adults are found on the tea plants during winter months but their numbers increase again in March as nymphs emerge from over wintering eggs (Mann, 1902; Das, 1984). In North East India, life table of *H. theivora* have been worked out by Kalita *et al.*, (1996). In this study the population increased with the intrinsic rate (r_m) of 0.152 whereas the finite rate of increase (λ) was 1.164 per female per day. The net reproductive rate (R_0) was 28.58 and the period required for completion of a generation was 22.05 days. The rate of population increase was 2.9 times per week and the population comprised approximately of 93.10 per cent of immature stages on reaching a stable age distribution. The life cycle of the bug, and its fecundity, longevity and other parameters throughout the year have been studied by Gope and Handique (1991) in North East India.

Preference of oviposition site of *H. theivora* is dependent on the host plant. On cocoa *H. theivora* preferred the pods, but would occasionally oviposit on young shoots (Miller, 1941; Tan, 1974). On tea the young shoots were mostly preferred with occasional laying in petioles and midribs of leaves (Mann, 1902; Das, 1984). *Helopeltis antonii* laid eggs primarily on the young cashew shoots, inflorescence stalks and sometimes on the petioles and ventral midrib of leaves (Devasahayam and Nair, 1986). It was reported that the preferred oviposition site was close to feeding lesions, which helped to minimize the injury by further feeding (Muhamad and Way, 1995). The number of egg laid by the female varied. Normally, only two to three eggs

were laid but exceptionally 8-12 could also be laid (Sweeny, 1965). Smee (1928), however, reported a female laying as high as 28 eggs in one day.

Mass rearing of *H. theivora*.

Mass rearing of any insect pest is essential for detail studies of their management. Smith (1973) had reported that about 70% nymphs of *H. clavifer* reached adult stage when reared on separate cocoa pods. However, Entwistle (1972) indicated that most of the mirids are difficult to breed and maintain in the laboratory. Sundararaju and John (1992), Satapathy (1993) and Angaiah (1995) have standardized a method for mass rearing of *H. antonii* under laboratory condition on cashew shoots. Sudhakaran (2000) has standardized a tube method of mass rearing technique for *H. theivora* on tea shoots under laboratory condition. According to his findings the survival rate of both adults and nymphs and hatching success was > 90% to 96%.

Seasonal abundance of *H. theivora* in tea ecosystem:

Detailed information on seasonal prevalence of *H. theivora* in the Dooars area is not precisely available but in North East India *H. theivora* occurs on tea almost throughout the year. However serious attack develops in May, June and July, often extending up to September when the number of rainy days is large. With steady rains and dull weather conditions, its activities increase when compared to smaller rainy days with bright sunshine. 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 (Das, 1957).

Ahmed *et al.*, (1992) developed a population model for *H. theivora* in Bangladesh tea based on the data. The model simulated the population dynamics of the pest

throughout the season. The output of the model indicated that the population density of eggs, nymphs and adult was low during the early part of the season which increased rapidly to a peak in the September and then declined slightly in October and quickly in November to become very low from December to March. Detailed information on the seasonal prevalence of *H. theivora* in South India was reported by Muraleedharan (1992 a and b). In South India incidence of *H. theivora* was high during July to December and low during January to June. Two peaks in population were observed in a year, which coincided with the flushing seasons.

Abiotic factors influencing *H. theivora* population

Insect pest being poikilothermic, the climatic factors influence the life cycle and populations of insect pest considerably. Devasahaym (1985) reported that population development of *H. theivora* was negatively correlated with minimum temperature, maximum temperature, and relative humidity. Muraleedharan *et al.*, (2000) indicated that *H. theivora* population showed a negative relationship with maximum temperature and maximum relative humidity but significant positive relationship with minimum relative humidity. There are some evidence indicating that tea mosquito bug populations fluctuates in response to more localized and less regular climatic events, tending not to do well under conditions of heavy rains, high winds or low relative humidity (Lever, 1949; Miller, 1941; Betrem, 1950; Pillai *et al.*, 1976a). Lever (1949) noted that *H. theivora* on Malaysian tea was the most severe during the period of dull, calm, misty weather, the insect being less active under conditions of heavy rains, wind and or bright sunshine. Das (1965) and smith *et al.*, (1985) reported the photonegative nature of tea mosquito bug in tea ecosystem. The available

literature supports that the *Helopeltis* are active in early morning late afternoon, shaded area, cloudy, dull day and in evening time. The recent experiment conducted by Dhar *et al.*, (2001) distribution of tea mosquito bug in different regions of light intensity in tea bushes has been documented from North East India. They found that maximum concentration (26.5%) of adult was recorded where the light intensity ranged between 801 - 1100 lux followed by 1101-1400 lux (21.5%) and 5001 - 10000 lux (10.60%). The population gradually decreased with the increase in light intensity. Local conditions also have a great influence on its incidence. Tea growing areas bordered by jungles, particularly on the South and South-West, remain more or less permanently infested and from these areas, the attack usually spreads into others (Das, 1957)

Alternate host of *H. theivora*:

A number of plants, especially in the Jungle, *Melastoma malabathricum*, L. (commonly known as wild rhododendron); *Maesa ramentacea*, A.D.C.; *Eurya acuminata* D.O.; *Acalypha* sp and *Jasminum sandens* Vahl. have recorded as common alternative hosts for *H. theivora* (Das, 1965). According to Das (1965) *Mikania micrantha*, a creeper, which grows abundantly in and around tea gardens found to be another host plant in which the insect breeds. Barbora and Singh (1994) identified *Oxalis acetosella*, *Psidium guajava* *Gardenja jesminoid*, *Morus alba*, *Enthocephalus cadamba*, *Eugenia jambolans*, *Premna latifolia* and *Ehretia acuminata* have been identified as alternate hosts of *H. theivora* in North East India. Sudhakar (1975) observed that the life cycle of the species was completed within 22-35 days on Guava covering an incubation period of 9.2 days and nymphal period of

19.5 days. Sudhakaran and Selvasundaram (2000) reported life history of *H. theivora* on *Maesa indica* of 24.6 days duration. Somchowdhury *et al.*, (1993) reported overwintering of *H. theivora* observed in *Mikania* sp, *Similax* sp., *Polygonum chinens* and *Phlogocanthus pubinervious* in the Jiti Tea estate in Dooars, West Bengal.

Feeding behaviour and Nature of damage of *H. theivora*

The nymphs and adults of *H. theivora* suck the sap of the young leaves, buds and tender stems; while doing so, it injects toxic saliva which causes the breakdown of tissues surrounding the puncture, which becomes dark brown shrunken spots after 24 hours. The badly affected leaves became deformed and even curl-up. In severe attack, bushes virtually cease to form shoots and the affected area may not flush for weeks together (Ahmed, 1996). The rate of feeding and selection of site for sucking by nymph and adults of the tea mosquito bug, *H. theivora* were studied in the laboratory by Kalita *et al.*, (1996) who noted that an adult female produced lesions in the form of "fluid-soaked" spots on the upper surface of leaves over an area of 412.43 sq. mm per day. Likewise the first instar nymphs caused leaf damage of 43.81 sq. mm per day producing 114.70 spots. The 2nd leaf was the most preferred site by the third and fourth instars and adults. On the other hand, the first and second instars preferred the 1st leaf. A recent study (Rahaman *et al.*, 2007) indicated that rate of feeding was high in female (56-339^o feeding spots) than males (9-182 feeding spots). Feeding site preference in both male and female was second leaf followed by third leaf, first leaf and the bud being the least. Chinery and hybrid tea jats were found invariably more susceptibility to *H. theivora* than 'Assam' varieties (Angstead and Ballare, 1992; Rau, 1940). However in Assam, all tea clones were found infested and among them TV-1

was the most susceptible (Das, 1984). The clones TV11, TV17, TV21, TV25 and TV26 had been reported tolerant (Somchowdhury *et al.*, 1993). In an extensive study, Rahaman *et al.*, (2007) found that fifteen different tea cultivars namely TV1, TV2, TV9, TV17, TV18, TV20, TV22, TV25, TV26, P126, TS426, T491, T653, T652, and Teenali clone were susceptible to *H. theivora* attack. The mechanism of feeding by *H. theivora* had been studied by Cohen-Stuart (1992) who showed that the proboscis (stylets) of insect penetrated the vascular bundle resulting in the collapse of tissues. The collapse of parenchyma was seen 90 min after feeding. Sarker and Mukhopadhyay (2006 a and b) reported the presence of three common hydrolytic enzymes, amylase, protease and lipase from salivary gland and midgut homogenates of *H. theivora*.

Morphological observation of *H. theivora*:

Heavy rain fall adversely affected its population. Mann, (1907) strongly correlated the colour variation in *H. theivora* with season in which males from summer / autumn (July – October) were on an average much darker than those from winter / spring brood (November – June) with the converse being true for females. Furthermore, this observation on colour variation was corroborated by Stonedahl, (1991) who reported that populations collected from Vietnam, South India and Assam showed variation in colour pattern of head and pronotum. In recent study on male and female within a single season three colour variations were identified in both the sexes of *H. theivora* of Jorhat tea plantation area of Southern south Assam, India (Bora and Gurusubramanian, 2007).

Plant based insecticide against *Helopeltis theivora* in tea

Muraleedharan and Radhakrishnan (1989), Kakoty *et al.*, (1993), Borthakur *et al.*, (1993) and Sannigrahi *et al.*, (1995) mentioned about the use of a fairly large number of neem based formulations for pest management in tea plantation. The survey on use pattern of pesticides in tea estates of N.E. India conducted by Barbora and Biswas (1996) noted that many tea estates started to use neem products in place of hard insecticides from the year 1994 which represented 43% of total insecticide consumption in Dooars. Bioneem, Nimgreem, Neemgold, Azadirachtin 0.3%, Neemazal-F 5%, Pestoneem 0.3%, Neembicidin, Econeem 0.3% and Fortune Aza 0.15% were used as common neem formulations in N.E. India. (Anonymous, 1993).

Beside neem based formulations, several workers (Bhattacharya, 1994; Rahaman *et al.*, 2006 a and b; Sarmah *et al.*, 1999; Gogoi *et al.*, 2003; Bisen and Kumar, 1997 and Ghosh Hajra, 2001 and 2002) reported that certain wild and weed plants having pesticidal properties against sucking and chewing pest in tea. Among them, 5 and 10 % aqueous extracts of *Annona squamosa*, *Lantana camara*, *Adhatoda vasica*, *Clerodendron inerme*, *Pongamia pinnata*, *polygonum orientale*, *Polygonum hydropiper*, *Equisetum arvensis*, *Eupatorium glandulosum*, *Urtica dioica*, *Artemesia vulgaris*, and seeds (5 and 10 % aqueous extract) of *Azadirachta indica* *Melia azaderch* were found having insecticidal efficacy against sucking and chewing pests in tea: Antifeedant and repellent properties of *Clerodendron inerme* and *Polygonum orientale* extract against tea mosquito bug have identified by Deka *et al.*, (1998) and Rahman *et al.*, (2005) and Sarmah *et al.*, (2006a, 2006b and 2007).

Use of synthetic chemical insecticides in control of *H. theivora*

The importance of chemical method of pest control in plantation crops and in particular had in tea always been emphasized. During the early forties, DDT dusting was a routine practice to combat the problem of tea mosquito bug (*H. theivora*). In North East India, Tocklai Experimental Station of the Tea Research Association (TRA), Jorhat, is the premier institute to test and certify the plant protection chemicals for use in tea plantation. Earlier, TRA recommended different pesticides (Chlordane 10% dust; 50% DDT W.P.; Endrex 20 E. C.; Gammexane 50% W.P; 5 % BHC dust, Lindane 20% E.C Aldrin, Dieldrin, and Endrin) for controlling tea pest (Glover,1955, Mukerjea, 1962). A comparative trial with these pesticides in the Dooars tea plantation which was moderately infested with *H. theivora* was tried by Mukerjea, (1962). The Thiodan 35 E. C. was found to be a good as standard insecticide like DDT and Dieldrin. The Thiodan (endosulfan) were applied with a micronette power sprayer at 1.25 litres per hectare. After that endosulfan was introduced in tea of North East India. By the virtue of the Tocklai Circular No. V/75/129/of 11.2.70 use of DDT was restricted to for pest management in tea (Anonymous, 1970). During seventies TRA approved only use of eight different types of chemical insecticides viz. endosulfan, monocrotophos, phosalone, shalimar Tar oil, dimethoate, fenitrothion, chlorpyrifos and quinalphos for tea pest management (Banrejee, 1973). The synthetic pyrethroid was first introduced in tea industry in North East India in the year 1982 to 1983 in the form of permethrin, cypermethrin, deltamethrin and fenvalerate (Satyanarayana, 1982 and 1983). From

the year 2001, TRA recommended several pesticides [endosulfan, quinalphos, phosphomidon, phosalone, acephate, dimethoate, chlorpyrifos, monocrotophos, oxydemeton methyl, lambda-cyhalothrin, beta-cyfluthrin, etofenprox, cartop hydrochloride, alphamethrin, cypermethrin, deltamethrin, profenfos, thiomethoxam, imidacloprid, and neem formulations] for controlling insect pest in tea (Anonymous, 2001). Dilutions for conventional insecticides used to control tea mosquito bug population in N.E. India were recommended by Barbora and Singh (1994). These were monocrotophos 36 SL, endosulfan 35 EC, quinalphos 20 AF, acephate 75 WP and chlorpyrifos 20 EC at 1:200 and 1:400 with low and high volume sprayers respectively; methomyl 12.5 EC at 1:250 (High volume sprayers); phosphamidon 80 WSC at 1:500 (Low volume) and 1:1000 (High volume); all synthetic pyrethroids at 1:2000 (high volume). A few promising cocktails such as methomyl 12.5 + DDVP (2 l + 329 ml/ha), alphamethrin 10 EC + DDVP (200 ml + 329 ml/ha), monocil 36 + DDVP (695 ml + 329 ml/ha), ripcord + DDVP (400 ml + 329 ml/ ha) and endosulfan 35 EC (1:600) + neem gold (1:150) or Godrej ahook (1:100) were also suggested for control of sucking pests. Kakoty (1994) mentioned proper selection of pesticide, dilution, discharge of measured quantity of spray fluid, dispersion of spray fluid on the proper target, time of spraying, use of properly cleaned sprayers, quality of water used in the preparation of spray fluid, maintenance of nozzles and supervision at the time of preparation and spraying of the fluid for greater the effectiveness of spraying. Somchowdhury *et al.*, (1993) suggested the schedule of integrated control against tea mosquito bug. According to his view alphacypermethrin and the controlled release formulation of DDVP or DDVP in tank mixture with pesticides like methomil,

monocrotophos and endosulfan etc. provided excellent control of the pest. Rahaman *et al.*, (2007) reported highest ovicidal action of dimethoate against *H. theivora* eggs. Profenofos, lambda-cyhalothrin, and oxydemeton-methyl gave 20-28 per cent egg mortality followed by deltamethrin and thiomethoxam (20%), cypermethrin, beta-cyfluthrin, etofenprox. The latter were less effective as ovicides in Assam condition and they also indicated that field persistency of different classes of pesticides range from 10 to 23 days were imidacloprid, thiomethoxam and beta-cyfluthrin which persisted for long time.

Relative toxicity of insecticides used in tea against *Helopeltis theivora*

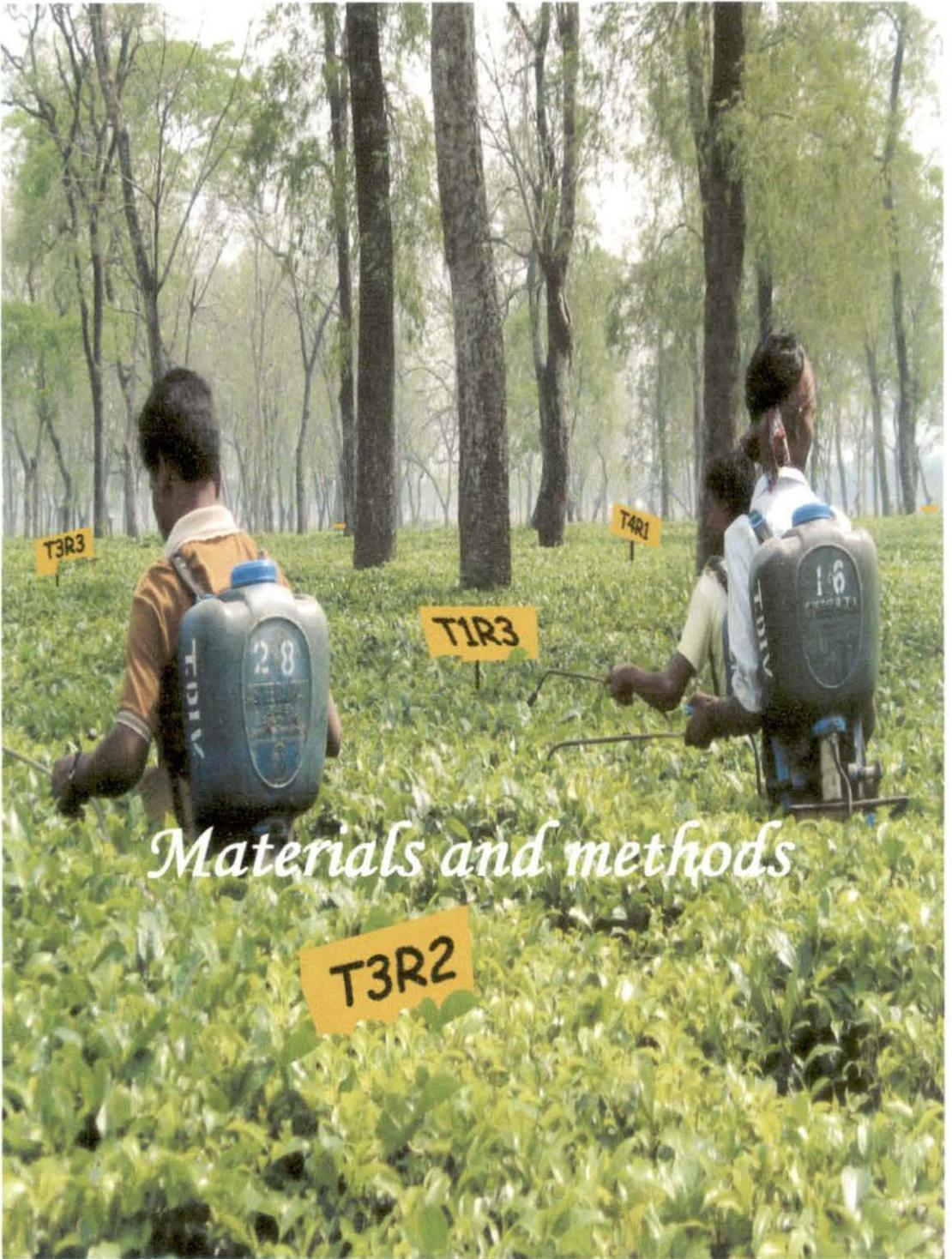
Widespread use of insecticides has lead to resistance development in many insects. Prudent insecticide use must be taken seriously as resistance problems in mirids may arise (Dzolkhifli *et al.*, 1986). Liew *et al.*, (1992) and Ho, (1994) have shown degrees of tolerance between populations of the cocoa mirid. Muhamad and Omar (1997), and Muhamad and Dzolkhifli (1996) have also reported resistance in cocoa Mirid, *H. theivora* to gamma-HCH (99% a.i.), deltamethrin (99.5% a.i.) and cypermethrin (50% and 90% a.i.). Recent report indicates that variation in relative toxicity between male and female population *H. theivora* of Jorhat, Assam (Bora and Gurusubramanian, 2007) and Darjeeling population (Bora *et al.*, 2007). The change in susceptibility was found in the male and female populations of *H. theivora* to dimethoate, endosulfan, cypermethrin, imidacloprid and deltamethrin in Assam population (Rahman *et al.*, 2005). A comparison of expected effective dose of five insecticides based on their LC₅₀ values with recommended dose revealed a pronounced shift in the level of susceptibility of *H. theivora* to all the chosen

insecticides. The usually recommended dose of synthetic pyrethroids (deltamethrin and cypermethrin), neonicotinoid (imidacloprid), organophosphate (dimethoate) and organochlorine (endosulfan), however, was practically ineffective against this pest (Bora *et al.*, 2007).

In addition, qualitative and quantitative changes were recorded in the enzyme pattern of the mosquito bug especially in general esterase, glutathione S-transferase and Acetylcholinesterase (Sarker and Mukhopadhyay, 2003 and 2006 a and b). Fat body content of pest has been found to contribute to the resistance through the possess of storage, detoxification and insulation (Brown, 1960; Garfield, 1990; Yu *et al.*, 2003; Enayatl, *et al.*, 2005; Abdallah *et al.*, 2005 and Srivastava, 2004).

Management strategies of *H. theivora* using chemical pesticides

A general recommendation for management of tea pests is available for N.E. India (Das, 1965; Choudhury *et al.*, 1995; Samanta, 1995; Dhar, 1999; Rahman *et al.*, 2006 and 2007) and South Indian tea planters (Muraleedharan and Chen 1997; Muraleedharan and Selvasundaram, 2002). But very little suggestions are available to tackle the pest population at local levels, specially the Dooars area in the North Bengal.



Materials and methods

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History of occurrence of *H. theivora* and insecticide used pattern in the Dooars tea plantation

The information on history of occurrence of *H. theivora* and pesticide used pattern in the Dooars was collected from most of the tea estates (mentioned in parenthesis) of each subdistrict covering about 75% of the area namely Damdim (25), Chulsa (19), Nagrakata (14), Binnaguri (23), Dalgong (19) and Kalchini (19) respectively. Data on occurrence of *H. theivora* was collected from each tea garden from the year 1980 - 2004 as per visit report of advisory officers, scientists and extension pesticides specialist. Pesticide consumption data of the tea estates was collected from the pesticide stock book for a period of seven years (1998-2004) maintained by the respective tea estates.

Seasonal abundance and influence of abiotic factors

The study was conducted during 2004 and 2005 in experimental plot at Nagrakata (Jalpiguri district, West Bengal). Four heavily infested TV1 section was selected to study among them two un-pruned (section 1 and 6) and two pruned (section 4 and 11) section, which was not treated with insecticides. The plot consist 100 bushes. Monthly twice observations were recorded. The relative population abundance of *H. theivora* was assessed by percentage of infestation which was calculated by collecting 100 shoot (medium plucking i.e. all 2 + bud; 3 + bud; single and double banjhis) at random from each replicates and counting the number of infested shoot and un-infested shoots then by using the following formulae:

$$\text{Per cent infestation} = \frac{\text{Infested shoots/replicate}}{\text{Total No. of shoots/replicate}} \times 100$$

Data on abiotic factors viz., morning and afternoon humidity (%), maximum and minimum temperature ($^{\circ}\text{C}$), mean daily sunshine (hours) and rainfall (mm) were collected from the meteorological observatory. The influence of weather parameters on seasonal abundance was determined by using multiple regression analysis and simple regression analysis (Gomez and Gomez, 1984).

Morphological variations

Colour variation in pronotal area of males and females in the Dooars population was observed by collecting the adults from the field and killing them using cyanide jar. The observation was done under an advanced research microscope. One hundred males and one hundred females specimens were subjected to observation of the pronotal colour variation.

Culture and maintenance of *H. theivora*

Adults and nymphs of *H. theivora* were collected from tea fields. The field collected nymphs and adults were reared and maintained in the insect cage and glass chimneys in the laboratory by providing the shoots of TV 1 at a temperature of $28 \pm 4^{\circ}\text{C}$ and 80-90% RH. The mass culture of insects was maintained throughout the study period to carry out the experiments.

Rearing techniques and biology:

For mass rearing in laboratory, transparent glass chimney (5 liter capacity) was used with its mouth covered by muslin cloth. Two to three healthy tea shoots (three leaves and a bud) were kept in glass tube (2cm long x 2 cm wide) containing water and wrapped with cotton. Glass tubes along with the tea shoots were placed in Petri dish

(4" diameter) towed with blotting paper. The whole arrangement was covered by the aforesaid glass chimney. Nymphs and adults collected from field were introduced into the glass chimney by opening the mouth.

In a container, 10-12 nymphs were allowed to feed on tender shoots. Shoots were renewed daily and the glass chimney replaced everyday with new glass chimney. While replacing the vial, the nymphs on the shoot were carefully removed by single hair brush and introduced on a new shoot. This continued till the emergence of adults. Adults emerging on a particular day were paired and reared in separate glass chimney.

After mating, females were allowed to oviposit in the shoots and the egg laden shoots were removed. By using a strong magnifying glass, these hairs can be seen sticking out from the hiding place of the eggs. Twigs containing eggs were inserted to a 5 ml glass tube containing water. The egg-laden shoots were kept in an upright position in such a way that the stem portions, which contain egg, do not touch the water. Fresh tender shoots will be provided in the tube till the emergence of the nymphs. As a prophylactic measure water in the glass vials will be mixed with carbendazim 0.1% to prevent fungal growth on the shoots. After a few hours of hatching, newly emerged first instar nymphs were transferred to Petri dish (4" diameter) individually by providing tender host shoots moistened with wet cotton around petiole daily.

Data regarding pre-oviposition, oviposition, post oviposition, fecundity, nymphal duration and number of nymphs attaining adult stage, sex ratio and adult longevity were recorded. Fresh tender twigs inserted in glass vial shall be replaced daily till the death of adults. Twenty such sets will be kept for observation. The data of biological

parameters were subjected to analysis with the help of computer programmer "GrahPad InStat".

To determine life cycle pattern of *H. theivora* in different seasons of the Dooars agro-climate condition the experiment was conducted in the laboratory at room temperature, in different month of the two consecutive years.

The comparative study on biology of *H. theivora* on *Mikania micrantha* and Tea (TV1) in the laboratory condition was conducted in September at 25 ± 2 °C temperature and 85 ± 5 % RH. Fisher's t- test was used for estimating difference in biological characteristic.

Feeding behaviour of *H. theivora*

For studying feeding behaviour of *H. theivora* in laboratory, immediately after hatching one nymph was released to feed on five shoots kept inside a reagent bottle and was covered with a glass chimney whose upper end was closed with muslin cloth. The shoots were changed daily and the number of spot, area of spots, total puncture marks of different instars and adults were recorded. The diameter of the spot was measured with stage ocular micrometer under binocular microscope. Total experiment was conducted on TV1 tea clone during September to October, 2005.

For determination of tea clones and alternate host preference twenty-eight different tea clones or varieties available in the Dooars area were chosen. A five shoot or twig (three leaves and a bud) of the respective cultivar was kept in a reagent bottle filled with water and placed inside the rearing cage (15x30x15 cm). For each treatment five numbers of adult *H. theivora* were introduced separately. The *H. theivora* used in the experiment were starved for eight hours only; further starvation was not done to avoid

emaciation and death out of starvation. Observations were taken for 24 hours and feeding spots were counted.

Bioassays employing whole plants or plant parts of thirty eight botanicals (weed to tree) commonly available in the Dooars region were subjected to screening of alternate hosts for *Helopeltis theivora* feeding by no-choice type and binary choice methods recommended by Schoonhoven *et al.*, (1998). In no-choice type of experiment *H. theivora* was subjected to feed only respective plants but in binary choice methods the insect have offered a choice between tea plant and other alternatives. Observations were taken for 24 hours and feeding spots were counted.

Oviposition preference:

The adult *H. theivora* were collected from the commercial tea fields of the Dooars (Kalchini subdistrict) and maintained on TV1 tea leaves and kept under laboratory conditions at the temperature of $28 \pm 4^\circ \text{C}$, 70-80% RH and a 16:8 LD photoperiod for one day. Then they are divided in to three groups. Among them two are treated with sub-lethal concentrations of endosulfan (800 ppm) and deltamethrin (0.5 ppm) by using glass atomizer (tropical application). The bugs survive after treatments were used for respective experiments. Ten females and a similar amount of males in each batch were introduced in transparent glass chimney (5 liter capacity) which contained two to three healthy TV1 tea shoots (four to five leaves and a bud) kept in glass tube (2cm long X 2 cm wide) containing water and wrapped with cotton. As *H. theivora* oviposits its eggs in the tender green part of the host plants and only the two unequal chorionic processes of each egg are visible, the number of eggs in the plants was counted using a stereo microscope. These experiments were conducted five times.

Determination of relative toxicity of 12 insecticides against *H. theivora* in different subdistricts of the Dooars

The adults of *H. theivora* (about 500) were collected by hand from commercial tea fields of 6 different subdistricts in Jalpaiguri province (Damdim, Chulsa, Nagrakata, Binnaguri, Dalgong and Kalchini) of West Bengal during the last week of September 2004 to November 2006. Then the collected *H. theivora* adults were placed in rearing jars (20cm x 15 cm) for preconditioning under laboratory conditions at the temperature of 27 ± 2 °C, 70-80% RH and a 16:10 LD photoperiod for a period of seven days. Insecticides used in the studies were include imidacloprid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25EC, lamda-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37SL, endosulfan 35 EC, quinalphos 25 EC and oxydemeton methyl 25 EC. Graded concentrations of insecticides were prepared in distilled water by mixing commercial formulations of the insecticides. Toxicity assays were conducted as per the standard method, 'Leaf Dipped Method' recommended by FAO Method No. 10a (FAO, 1980), Martin Rathi and Gopalakrishnan (1995), Yaqoob and Arora (2005), Allahyari *et al.*, (2005), Bora *et al.*, (2007) and Gurusubramanian and Bora (2007). TV 1 clones three and a bud healthy shoots were collected from the experimental garden plots and brought to the laboratory. The leaves were washed thoroughly with distilled water and air-dried. Fifteen tea shoots for each treatment were dipped up-to five seconds in the pesticides solutions to ensure complete wetting and then they were kept in a glass

tube containing water and wrapped with cotton. The treated tea shoots were kept under ceiling fans for 15 minutes to evaporate the emulsion. The glass tubes containing tea shoots were placed in glass chimneys. Muslin cloth was tied with the help of rubber bands on top of the glass chimneys, and the tubes were kept at $28 \pm 4^{\circ}\text{C}$ in culture room.

Preliminary test- Dose selection is a very important element in each insecticide bioassay test. More than 5 preliminary tests were done to find proper doses. The process of dose selection was based on Robertson *et al.*, (1984) and Robertson and Preisler (1991).

Final test-

Preparation of insecticidal concentrations: Based on results of preliminary tests and availability of insects, the proprietary product of insecticides was used to prepare one per cent (1000 ppm) stock solution in distilled water from which further dilutions were prepared subsequently. Seven to eight concentrations resulting in mortalities of less than 10 to more than 90 percent and a zero concentration in each replicate were used as check. Ten insects were allocated to each concentration. Observations of adult mortality were recorded in all the five replications of each concentration 24 hours after the treatment. Moribund insects were counted as dead. Five to seven concentrations of each insecticide were tested to obtain a concentration – probit mortality curve. The mortality data was converted to percent mortality and subjected to probit analysis (Finney, 1971; Busvine, 1971).

Quantification of insecticidal susceptibility:

The degree of susceptibility of different insecticides was determined by working out LC_{50} values of *H. theivora* in each subdistrict. Data were log transformed and analyzed by means of the probit analysis method of maximum verisimilitude proposed by Finney 1971, to obtain LC_{50} values and a regression equation.

Resistance factors (RFs) were determined at LC_{50} relative to the corresponding lowest LC_{50} s of the respective insecticides due to the unavailability of a suitable reference susceptible strain normally used to calculate resistance factors (Chaturvedi, 2004).

The expected effective concentration of each insecticide was calculated by doubling the LC_{50} value to attain a LC_{100} value, and then effective field dosages of these insecticides were computed based on the following formula and compared with recommended dosages as per the standard method of Misra (1989).

Expected effective concentration (LC_{100}) (%) = 2 X LC_{50} %

Expected effective dose (g a.i./ ha) = ED /100 X EC X 20 fold

ED = % concentration / EC X 1000 X 400 liters of spray fluid/ha

Persistence of residual toxicity of insecticides against *H. theivora*

TV 1 clone was sprayed with imidacloprid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25EC, lamda-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37SL, endosulfan 35 EC, quinalphos 25 EC and oxydemeton methyl 25 EC at three different concentrations, *i.e.*, 0.05, 0.1 and 0.25 per cent for evaluating the persistence of residual toxicity against *H. theivora*. Each treatment contains 150 bushes with three replications. One plot was not treated

and used as untreated control. Two and a bud from five tea bushes were selected randomly after one hour of spray from each treated and untreated plots for "0" day observation and collected in marked paper bags separately. Bags with shoots were brought to the laboratory. Five tea shoots were kept in the glass tube containing water and wrapped with cotton. Glass tubes containing tea shoots were placed in the glass chimneys. The muslin cloth was tied with the help of rubber bands on top of the glass chimneys and the tubes were kept at $28 \pm 4^{\circ}\text{C}$ in a culture room. Ten numbers of field collected and preconditioned adults of *H. theivora* were released in each glass chimney containing tea shoots collected from the respective plots. Observations were recorded after 24 hours release of *H. theivora* adults. Moribund insects were counted as dead. Same procedure was repeated every day till insect mortality declined to the tune of ten per cent (4-28 days) in all the three observations (Sarup *et al.*, 1969; Rahman *et al.*, 2007).

The relative efficacy of each treatment was determined by a criterion developed by Saini (1959). According to this criterion the product (PT) of average residual toxicity (T) and the period in days (P) for which the toxicity persisted was determined. The average residual toxicity was calculated by first adding the values of corrected percent mortality caused by the insecticidal residues on the tea plant at various intervals and then divided by the total number of observations.

Sum of per cent mortality of tea mosquito bug on different days

$$T = \frac{\text{-----}}{\text{-----}}$$

No. of observations

The LT_{50} values for different concentration of insecticides were calculated by probit analysis (Busvine, 1971; Finney 1971) in all the three replications of the experiment.

The

t- test was employed for comparing the log LT_{50} values of different insecticides used in the present investigation (Singh *et al.*, 1998 a and b). For example, “t” value for testing the difference between the log LT_{50} values of endosulfan 0.05 % and 0.25 % was calculated as

$$\text{Sum SEM} = \sqrt{(\text{SEM of endosulfan } 0.25 \%)^2 + (\text{SEM of endosulfan } 0.05 \%)^2}$$
$$“t” = \frac{\log LT_{50} \text{ value of endosulfan } 0.25 \% - \log LT_{50} \text{ value of endosulfan } 0.05 \%}{\text{Sum SEM}}$$

The table value of “t” is 2.0369 and 2.7385 at 5 and 1 per cent level respectively.

Ovicidal activity of different classes of pesticides against eggs of *H. theivora*

Seventeen commercial grade synthetic pesticides and one botanical pesticide viz., endosulfan, chloropyriphos, monocrotophos, quinalphos, oxydemeton methyl, profenfos, dimethoate, acephate, deltamethrin, alphamethrin, cypermethrin, fenpropathrin, β - cyfluthrin, etofenprox, λ - cyhalothrin, imidacloprid, thiomethoxam, and azadirachtin at three concentrations viz., 0.05, 0.1 and 0.25 per cent were tested against eggs of *H. theivora*. Thirty gravid females of *H. theivora* were introduced on TV-1 freshly collected shoots for egg laying and kept for 12 hours inside the chimney. The next day the egg laden shoots were subjected to pretreatment count. After counting, the eggs were exposed to pesticide treatments by using glass atomizer. Tap water was sprayed on eggs kept as control. Observations were taken periodically after treatment along with control. Observations on percent hatching and

percent mortality of neonate nymphs were recorded collectively up to 28 days (Gope and Handique 1991). Those eggs that did not hatch after this period were regarded as non-viable. The cumulative mortality data was recorded on fourteenth day after the spraying. Data was analyzed statistically.

From the observed egg mortality corrected per cent mortality was calculated using Abbott's formula (1925).

$$\text{Percentage egg Mortality} = \frac{S - K}{100 - K} \times 100$$

Where S = % Mortality of the treated group.

K = % Mortality of the control group.

Comparison of life cycle traits of population *H. theivora* infesting organic and conventional tea plantations, with emphasis on endosulfan resistance.

Rearing of H. theivora.

The laboratory culture of *H. theivora* was initiated by collecting about 600 nymphs from the tea plantations in and around the Dooars during last week of September 2005. Specimens brought to the laboratory were maintained at $26 \pm 2^\circ\text{C}$ in a BOD incubator. The nymphs were provided with tea leaves of TV1 (Tocklai variant) clone till they attain adult stage. After multiplying the culture in the laboratory for two successive generations, the stock was divided into two groups: A and B. Group A was maintained untreated as parental stock (CFF) and Group B was exposed to endosulfan (ERF). Due to the nonavailability of a suitable susceptible strain from North East India, nymphs of *H. theivora* were collected from Makibari Tea Estate, a pure bio-organic garden in Darjeeling slope where no synthetic chemical pesticides

have ever been used. These specimens (SOF) were used as baseline of susceptible form in the bioassay study. Moreover the LC_{50} of Makabari (Darjeeling strain) populations of *H. theivora* against synthetic organic insecticides have been found to be very low than other tea growing region of India (Bora *et al.*, 2007).

Bioassay and Laboratory Selection

The proprietary product of endosulfan (Thiodan 35 EC) was used to prepare one per cent stock solution in distilled water.

Subsequently graded concentrations of insecticides were made in distilled water from stock solution. Bioassay was performed by the film residue method mentioned earlier. Healthy TV 1 tea shoots (two and a bud) were collected and washed thoroughly with distilled water and air dried. Fifteen tea shoots for each treatment were sprayed with endosulfan separately at the respective dilutions by using glass atomizer and then air-dried. Three shoots were kept in a glass tube containing water, which in turn were placed in the glass chimneys. Glass chimneys were covered with pieces of muslin cloth and were kept in B.O.D. incubator ($26 \pm 2^{\circ}\text{C}$; 12: 12 L/D period). Thirty-preconditioned group B (ERF) *H. theivora* were released separately in each glass chimney containing treated tea shoots. Observations for adult mortality were recorded in all the five replications of each concentration 24 hours after the treatment. Moribund insects were counted as dead (Gurusubramanian and Bora 2007). Six concentrations of endosulfan were utilized for exposure in each generation. Besides, a set of control (with water only) was also maintained with each exposure to work out the corrected mortalities. The mortality data was recorded 24 hours after the treatment. The survivors obtained at higher concentrations ($\geq LC_{80}$) were shifted to

clean rearing glass vial and provided with fresh TV1 tea leaves. The progeny of the first surviving lot was termed the F1 generation. Such endosulfan treatments and the selections were conducted subsequently up to 5 generations. The parental and susceptible strains were maintained without exposure through generation to observe the biological parameters.

Quantification of insecticidal resistance

The degree of development of resistance through different generations was determined by working out LC_{50} values in each generation by the mortality data. These data were converted to percent mortality and subjected to probit analysis (Finney, 1971), then computing the resistance ratio. The resistance ratio for any generation was worked out by dividing LC_{50} for that generation with LC_{50} value of the parental generation.

Biological studies

The newly emerged adult of the three forms ERF, CFF and SOF were kept in separate glass jars (15 cm diameter) covered with muslin cloth and provided with TV1 tea shoots to serve as food to initiate the studies on biological and developmental traits such as oviposition, fecundity, nymphal duration and longevity.

Statistical analysis of the data

The mortality data was analyzed to determine LC_{50} value at 95 per cent confidence interval by SPSS software (10.0- version). The data on the various biological parameters of the three forms were subjected to Turkey's HSD test for analysis of variance.

Estimation of total body lipid content in *Helopeltis theivora*

Body fat content of *H. theivora* was extracted using glass Soxhlet apparatus (AOAC, 1990). Round bottomed flask was oven dried and kept in desiccators for cooling. The weight (W_1) of the round bottom flask was taken. A cellulose thimble (dry and fat free) was taken in which 1-2 g. of *H. theivora* as sample (Collected from different subdistricts of the Dooars) was placed and subjected to the Soxhlet distillation using petroleum ether (with boiling range 40 – 60^o C) for 5 h. The round bottom flask with the extract was dried for 1 h. at 100^o C to evaporated the solvent ether and moisture, then cooled in desiccator and weighed (W_2). Fat was calculated in percentage.

$$\text{Fat (\%)} = (W_2 - W_1 / \text{sample weight}) \times 100$$

Screening and utilization of phytochemical from different parts of common native botanical against *H. theivora* in the Dooars:

Screening test:

Preparation of crude Plant Extracts:

Leaves and succulent stems of different plants were collected locally from nearby areas in the Dooars region of North Bengal. Each plant material was dried under shade and powdered by using electric grinder and pass through a 20 mesh sieve and kept in a 1 kg capacity polypropylene bag. 300 gm of each powdered plant material were taken into a 2 litre capacity conical flask and 1000 ml of distilled water was added to it and shaken for 8 hours in a mechanical shaker and then kept it for 24 hours. The extract was separated using fine muslin cloth and then filtered. The filtrate was collected in a 2 litre capacity conical flask and volume was made up to 1000 ml.

This was considered as stock solution. Required concentrations 20.0% were prepared from the stock solution.

Insecticidal Activity - Direct Spray Method

Different chosen concentration of crude extract of different botanicals was sprayed by using glass atomizer over tea shoots and then dried under a fan for 15 minutes. The dried treated shoots were inserted in glass bottles (4' X 2') and kept in a glass chimney (18"x6") covered with muslin cloth. For each treatment 5 adults of *H. theivora* were introduced and replicated for three times. Mortality counts were taken after 24 and 72 hr.

Antifeedant choice Bioassay

Antifeedant activity of plant extract was assayed (Isman *et al.*, 1990) by using young tea shoots for *H. theivora* treated on each side with 10 ml of water extracts along with untreated control. First the shoots were sprayed with different 20% concentrations of the respective extract then air dried and exposed to *Helopeltis*. Based on the number of lesions in the form of "fluid-soaked" spots on the upper surface of leaves produced, the absolute deterrence coefficient was calculated using the formula according to Kielczewski *et al.*, (1979) and Erturk (2006) with slight modification:

Absolute coefficient of deterrence (Antifeedant activity) = $(C - T) : (C + T) \times 100$,
where T is number of puncture marks by bug in the experimental variant and C is number of puncture marks in the control variant.

Ovicidal Activity:

Thirty gravid females of *H. theivora* were introduced on TV 1 freshly collected shoots for egg laying and kept for 12 hours inside the chimney. The next day the egg laden shoots were subjected to pretreatment count.

After counting, the eggs are exposed to respective different treatments as mentioned above. Observations were taken periodically after treatment along with untreated control. Per cent reduction in hatchability was calculated by using the following formula:

$$\text{Per cent egg mortality} = 100 - \left[\frac{\text{No. unhatched eggs/treatment}}{\text{Total No. of eggs/treatment}} \times 100 \right]$$

Based on results of preliminary tests and availability of insecticidal plant product was used to subjected different Solvent extraction project.

Preparation of plant extracts in different solvents:

Native botanical possessing anti-insect potential recorded from Preliminary tests was collected from adjacent areas and were washed thrice with tap water and once with distilled water and then shade-dried for two weeks. It was successively extracted with petroleum ether (40 – 60^oC), methanol, acetone and water extracts by using Soxhlet apparatus (250g in 500 ml). The last trace of the solvent was removed under reduced pressure distillation and the crude extract was dried in vacuum desiccators and used for the experiments.

Different concentrations of the plant extract viz., 1, 2, 4 and 8 per cent solutions were prepared by adding respective solvents and used for the study. These different concentrations were prepared on the basis of quantity of plant extract in 100 ml solvents and the actual concentration of active ingredients was not taken into consideration.

Treatment:

The insecticidal efficacy was evaluated by 'Leaf Dip Method' (Martin Rathi and, Gopalakrishnan, 2005). The fresh TV1 tealeaves were dipped in the different concentrations of plant extracts (1, 2, 4 and 8 %) separately for 15 minutes. For control, the leaves were dipped in the respective solvents. After 15 minutes the leaves were taken out and shade-dried for 20 minutes and supplied to the pest. Laboratory reared nymphs or adults were released in each glass vials containing treated tea leaves and the glass vials were covered by muslin cloth. Same number of *H. theivora* nymphs or adults released into the vials having respective solvent treated leaves and served as control. Three replications were made for each concentration and control respectively. The insects were allowed to feed the respective solvent treated leaves as well as solvent extracts of experimental botanical treated leaves for a period of 3 days continuously. The observation was taken at 24 h interval and dead insect were removed daily. Moribund insect were also considered as dead. Statistical analysis of the experimental data was performed using probit analysis (Finney 1971). Ovicidal and antifeedant activity of different solvent extract was also determined as mentioned earlier.

Field experiment:

Different solvent plant extracts in different dilution and combination were tested under field condition by following randomized block design (RBD) with three replicates at Nagrakata experimental plot against *H. theivora*. Each plot in the experiment was separated by two buffer rows of non-experimental tea. 100 bushes per replication were considered for each treatment. Before treatment pre-treatment

data has been recorded and sprayed with hand operated high volume hand operated Knapsak sprayer @ 400 litres/ha. The control plot will be treated with water spray and cloth screen will be used for avoiding drifting of spraying material from plot to plot. After treatment observations were made at weekly interval for three to four weeks. The performance of each treatment against *Helopeltis* was assessed by recording the number of infested and uninfested shoots in each replication of the treatment during morning hours (9.00 – 10.00 hrs) and carried in muslin cloth bags (8" x 12") to the laboratory. Immediately after reaching the laboratory, infested and uninfested leaf count was done and the percent infestation and percent reduction were calculated by using the following formulae:

$$\text{Per cent infestation} = \frac{\text{Infested shoots/replicate}}{\text{Total No. of shoots/replicate}} \times 100$$

$$\text{Per cent reduction} = \frac{\text{Pre-treatment per cent infestation} - \text{Post-treatment per cent infestation}}{\text{Pre-treatment per cent infestation}} \times 100$$

The per cent reduction then subjected to angular or arcsine transformation and were statistically analysed by using ANOVA method to derive F – value, CD @ 5 % level and CV (%).

Phytotoxic Effect

Observations were taken up to 28 days after first spraying to assess the virtual phytotoxicity symptoms like injury on leaf tips, injury on leaf surfaces, leaf wilting,

necrosis, vein clearing, epinasty and hyponasty. The following scale was used to assess the phytotoxicity symptoms:

<u>Percentage</u>	<u>Grade</u>
0-10	1
11-20	2
21-30	3
31-40	4
41-50	5
51-60	6
61-70	7
71-80	8
81-90	9
91-100	10

Tainting and organoleptic test:

A field experiment was conducted to study whether different spraying materials have imparted any taint to black tea. Shoots were harvested on 7th and 14th day after spraying and processed separately in a mini CTC machine. The samples were forwarded to tea taster for assessment of taint as positive or negative and organoleptic test. Leaf, infusions and liquor strength were considered for organoleptic test and score was given as 1 being poor and 10 being very good.

Neem based pest management

Collection and Preparation of different concentrations of Azadirachtin content:

Samples of different azadirachtin contents (300, 1500, 3000, 10,000 and 50,000 ppm) were obtained from Entomology Research Institute, Loyola College, Chennai which were analyzed for azadirachtin content through HPLC. From the respective azadirachtin content sample five dilutions were prepared (1:200, 1:300, 1:500, 1:1000 and 1:1500) and tested against *H. theivora*,

Field Evaluation of Azadirachtin content and their bioactivity against H. theivora

A field trial was conducted in (Sep - Oct 2005) at Nagrakata experimental station to evaluate the efficacy of different azadirachtin contents (300, 1500, 3000, 10,000 and 50,000 ppm) at different dilutions (1:200, 1:300, 1:500, 1:1000 and 1:1500) against *H. theivora* along with untreated control. TV1 and TV9 (100 x 65 cm space) mixed plots were chosen for the current study by following Randomized Block Design with three replications. Each plot in the experiment was separated by two buffer rows of non-experimental tea. 100 bushes per replication were considered for each treatment of different dilutions of azadirachtin contents. Plots with heavy infestation of *H. theivora* were chosen for this study. After selection of the plots, pretreatment count was taken in the respective plots and two rounds of foliar spray were given at 15 days interval with hand operated Knapsak sprayer @ 400 litres/ha. Post treatment observations were taken for four weeks after treatment.

Bioefficacy of Neem formulations combined with reduced dosages of insecticides against *H. theivora* in tea:

Randomized block design field trials were laid out at Nagrakata experimental station to study the bioefficacy of Neemazal F 5 EC alone and in combination with monocrotophos and endosulfan at different dilutions against *H.theivora*. The control plot was treated with water spray. The treatments were applied at fortnightly interval with high volume Aspee knapsack sprayer. Pre and post observations were made at weekly interval for four weeks.

Effect of synergists on the toxicity of insecticide:

H. theivora were collected from the plots of tea estate of Kalchini subdistrict in the Dooars, North Bengal. Field collected insects were preconditioned for seven days in the laboratory (temperature of $27 \pm 2^\circ \text{C}$, 70-80% RH and a 16:10 LD photoperiod). A stock solution of technical grade piperonyl butoxide (PB; 90% w/v supplied by Aldrich Chemical Company, Inc.) was mixed with each tested insecticides namely endosulfan (Thiodon 35 EC, Hoechst), quinalphos (Ekalux 25 EC, Sandoz), deltamethrin (Decis 2.8 EC, Alkali) and imidacloprid (Confidor 17.5 SL Bayer India Ltd.) insecticides at the ratio of 1:5. Blends were tested against *H. theivora* using the leaf dip method recommended by FAO Method No. 10a (FAO, 1980). Graded concentrations of insecticide and synergists mixtures were prepared in distilled water. Five to seven concentrations of each insecticide with synergists mixture were tested to obtain concentration – probit mortality curve. The mortality data were converted to percent mortality and subjected to probit analysis to obtain LC_{50} values (Finney 1971): Synergistic ratio was calculated by the formula (Hsu *et al.*, 2004):

$$\text{Synergistic ratio} = \frac{\text{LC}_{50} \text{ of insecticide alone}}{\text{LC}_{50} \text{ of insecticide plus synergist}}$$

Effects of Carrier Water on Insecticide Efficacy against *H. theivora*:

The goal of pesticide is to optimize its effects on the targeted pest by applying the proper rate at the proper time with suitable equipment. The quality of the carrier water can be another important factor that should be considered to optimize pest control. pH was easily measured with an electronic pH meter. By mixing KOH (caustic potash) and HCL with distilled water we prepared alkaline (pH 9-10) and

acidic (pH 4-5) water respectively. A field trial was conducted in (Sep - Oct 2005) at Nagrakata experimental station to evaluate the efficacy of three different groups of insecticides viz. endosulfan (Organochlorine), monocrotophos (organophosphate) and fenpropathrin (synthetic pyrethroid) at TRA recommended dilutions mixed with three different categories of carrier water sources i.e. alkaline (pH 9-10), acidic (pH 4-5) and Neutral (pH 6.5-7.5) against *H. theivora*. Two batches of these foliar sprays were given after mixing the respective carrier water with insecticides at interval of 4hr and 24hr with hand operated Knapsak sprayer. TV1 and TV9 (100 x 65 cm space) plot was chosen for the current study by following Randomized Block Design with three replications. Each plot in the experiment was separated by two buffer rows of non-experimental tea. 100 bushes per replication were considered for each treatment. Plots with heavy infestation of *H. theivora* were chosen for this study. After selection of the plots, pretreatment count was taken in the respective plots. Post treatment observations were taken for two weeks after treatment.