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Use Pattern of Insecticides in Tea Estates of the Dooars in North Bengal, India

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ABSTRACT

A survey was conducted on pesticide use patterns in the tea plantations of Dooars during the period 1998 to 2004. The study revealed that on an average 7.499 l/kg of insecticides was used per hectare per year of which the organo-chlorine, organo-phosphate and Carbamate (Non-pyrethroid) accounted 73.5% and pyrethroid represent 36.6% during the survey period. Among the Different Sub District in Dooars, lowest consumption was noted in Damdim Sub district (5.799 kg/l per hectare) followed by Chulsa (6.433 kg/l per hectare), Binnaguri (7.399 kg/l per hectare), Nagrakata (7.655 kg/l per hectare), Dalgong (7. 920 kg/l per hectare), and finally highest consumption was noted in Kalchini (9.793kg/l per hectare).The requirement of Synthetic pyrethorid gradually increased with every passing year in all Sub district in Dooars. Endosulfan, Monocrotophos, Deltamethrin and Cypermethrin were extensively used in all the regions of Dooars.

Key words: Insecticides, used pattern Dooars Tea Estates

INTRODUCTION

The tea industry is one of the oldest organized industries in India and Indian teas are appreciated world over as health drink for their unique flavour, aroma, and medicinal properties. India produces four speciality teas – Darjeeling, Dooars, Assam and Nilgris, which are partially exported world over. Tea is grown in 13 of which states and Assam, West Bengal, Tamil Nadu and Kerala are the major producers. Total absorption of tea in 2004 is estimated to be 900 million kg, up by 30 million kg from last year. Approximately 78% of tea is harvested from North East India, and North Bengal produces 23% of total Indian production(Priyakumar,2005). According to the statistics of Tea Board – India, there are 308 big and 1232 small tea gardens in North Bengal. Total area under tea is 107479 hectares and in 2003, total production of made tea was 200 million kg. (Anon., 2003 a,b,c). More than one thousand species of arthropod pests are known to attack tea all over the world, though only about 300 species of insects are recorded from India in that 167 species from North-east India (Das, 1965) resulting 11 to 55% loss in yield. In North East India, tea plant is colonized by a complex of insect species including the tea mosquito bug, red, pink, and purple mites, thrips, termites, red slug caterpillar, looper caterpillar, green leaf hopper and so on. Among the tea growing regions of North east India, pest activity has always been reported to be high in Dooars (Borbora & Biswas 1996 and Sannigrahi & Talukdar 2003). The climatic conditions in these region, monoculture of tea over vast stretches of land contribute largely to high pest incidence. With incerase in the quantity of pesticide being applied every passing year, the problem virtually has aggravated

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and the cost of pest control is increasing spirally (Sannigrahi & Talukdar 2003). To understand the problem of high pest activity and the consequent use pattern of insecticides in the region, these survey was conducted in Dooars spanning over 7 years from1998 to 2004.

MATERIALS & METHODS

The information on insecticide use pattern in Dooars was collected from tea estate of each subdistrict namely Damdim (25 tea estates), Chulsa (19 tea estates), Nagrakata (14 tea estates), Binnaguri (23 tea estates), Dalgong (19 tea estates) and Kalchini (19 tea estates) respectively. Pesticide consumptions data of the tea estate were collected for a period of seven years (1998-2004) from the pesticide stock book maintained by each tea garden.

RESULT AND DISCUSSION

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, Quinolphos, Phosphomidon, Phosalone, Acephate, Dimethoate, Chlorpyrifos, Monocrotophos, Oxydemeton methyl, Lamdacyhalothrin, Betacyfluthrin, Ethofenprox, Cartap hydrochloride, Alphamethrin, Cypermethrin, Deltamethrin, Profenophos, Thiomethoxam, Imidacloprid and neem formulations for controlling tea pests.

In the Dooars, the control of insect pests is predominantly done by Endosulfan, Qunalphos, Acephate, Chlopyriphos, Momocrotophos, Cypermethrin and Deltamethrin for last several decades, but subsequently Oxydematon methyl, Lamda-cyhalothrin, Ethofenprox, Alphamethrin, Fenpropathrin, Profenophos, Thiomethoxam and Imidaclopyrid have been introduced tea protection since 1999.

The over all use pattern for seven years (1998 2004) showed that the consumption of insecticides in the Dooars varied between 5.799 kg/l per hectare and 9.793 kg/l per ha and the average annual requirement of insecticide during the period was 7.499 $\pm$ 0.56 kg/l per hectare (Table:1).

Table: 1 Average consumption of Insecticides (kg or lt per hectare) in different subdistricts of the Dooars (1998-2004).

Subdistrict	Total insecticide used	Non-Synthetic pyrethorid	Synthetic pyrethorid
Damdim	5.799 $\pm$ 0.49	4.609 $\pm$ 0.206	1.7194 $\pm$ 0.274
Chulsa	6.433 $\pm$ 0.166	4.73 $\pm$ 0.237	1.696 $\pm$ 0.262
Nagrakata	7.655 $\pm$ 0.166	5.324 $\pm$ 0.965	2.331 $\pm$ 1.197
Binnaguri	7.399 $\pm$ 0.549	5.407 $\pm$ 0.926	1.992 $\pm$ 0.404
Dalgong	7. 920 $\pm$ 0.066	5.435 $\pm$ 0.257	2.484 $\pm$ 0.305
Kalchini	9.793 $\pm$ 0.48	7.59 $\pm$ 0.357	2.196 $\pm$ 0.475
Mean consumption	7.499 $\pm$ 0.56	5.515 $\pm$ 0.439	2.749 $\pm$ 0.620

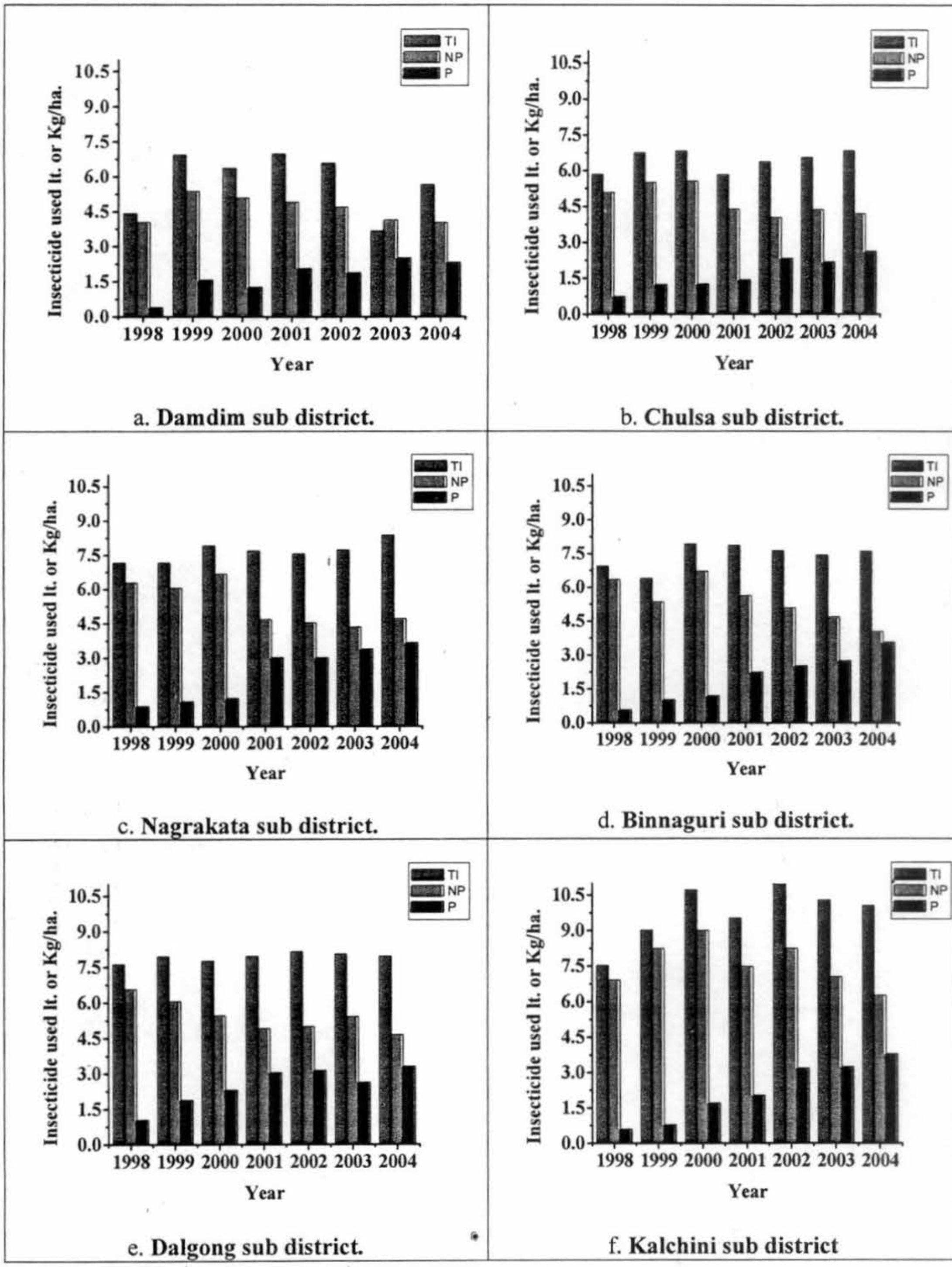
An analysis of the insecticide use pattern revealed the non-pyrethroids such as organo-chlorine, organo-phosphate and Carbamate accounted for 73.5% and pyrethroids represented 36.6%

during the survey period. Attention is drawn to the fact that the requirement of synthetic pyrethorid gradually increased with every passing year in all sub districts of the Dooars . For example in the year 1998, 10.82% of the total insecticide used was Synthetic pyrethorid but in 2004 it was computed to the extent of 40.91% in over all Dooars tea plantation senerio. Among the different sub districts, lowest consumption was noted in Damdim sub district (5.799 $\pm$ 0.49 kg/l per hectare) followed by Chulsa (6.433 $\pm$ 0.166 kg/l per hectare), Binnaguri (7.399 $\pm$ 0.549 kg/l per hectare), Nagrakata (7.655 $\pm$ 0.166 kg/l per hectare), Dalgong (7. 920 $\pm$ 0.066 kg/l per hectare), and finally highest consumption was noted in Kalchini (9.793 $\pm$ 0.48kg/l per hectare).

Data analysis revealed the trends of insecticide consumption in tea plantations of the sub districts over a period of seven years (Fig. 2a – f). In general, consumption pattern of major chemical groups of insecticides has been found to be similar in all the regions. By and large Endosulfan and Monocrotophos are extensively used in all the regions of the Dooars. It is note worthy that the use of chlorpyriphos has drastrically come down from year 2000,instead many tea estates have started to used neo-nicotinoid group of insecticides (Thiomethoxam and Imidaclopyrid) from the year 2002. Among the synthetic pyrethroid group, deltamethrin, and cypermethrin dominate, followed by Fenpropathrin, Alphamethrin, Lamda-cyhalothrin and Ethofenprox. The introduction of Lamda-cyhalothrin is comparatively very recent (2002). There has been a gradual increase in yearly consumption of total insecticides in the period under review in all the sub districts. Attention is drawn to the fact that there has been a definite trend to maximize the yearly use of synthetic pyrethroids in all sub districts but with a narrow choice for the spectrum of the insecticides. Earlier study (1990- 1994) reported that the average use pattern of insecticides was estimated to be 2.05 kg/lit/ha in the assam Valley, 2.05 kg/lit/ha 3.76 in Cachar, 7.05 kg/lit/ha in Dooars and Terai and 2.50 kg/l/ha in North Bank (Barbora & 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. Among which, acaricides accounted for 25% (3.60 l/ha) and insecticides 60% (8.46 l/ha). Of these 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).

The consumption pattern of insecticides gives an indication that the insect pest are more dominant and problematic in the Dooars tea. The preference of synthetic pyrethroids has probably increased because the conventional non-pyrethorid insecticides have become less effective against the target pests of Dooars. Moreover, the question, why do the estates/areas in Kalchini require 25% to 50% more chemicals than Chulsa and Damdim need to be addressed. Probably this is due to severity of pests or their higher pesticide tolerance or lack of proper planning and supervision during spraying. The situation can be analysed and verified by conducting a few critical experiments in different agroclimatic zones of Kalchini sub district, paying special attention to choice of chemicals, dosage, time and method of application.

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 natural enemies of pests, human health and environment and above all the presence of undesirable residues. The effective control of pests in tea is essential to ensure the marketability



a. **Damdim sub district.**

b. **Chulsa sub district.**

c. **Nagrakata sub district.**

d. **Binnaguri sub district.**

e. **Dalgong sub district.**

f. **Kalchini sub district**

Fig 2(a – f): Subdistrict wise insecticide use pattern in the Dooars tea plantation.

"**P**" denotes Synthetic pyrethorid; "**NP**" represents Non-Synthetic pyrethorid and "**TI**" indicates Total insecticide.

of this crop. Excessive use of Pesticides in the Dooars has led to more problem of maximum residual limits, contamination of food products and underground water. Non-prescribed use of chemical pesticides, wrong advice, force marketing of pesticides to planters by vested interests, non observance of prescribed waiting period, use of sub-standard pesticides, effluents from pesticide manufacturing and repacking units, continued use of persistent pesticides for public health programmes; lack of awareness and lack of adequate educational programme for planters/consumers (Anonymous, 2003) appear to be responsible for creation of the current grim situation.In the recent years, it has become a major concern to the tea industry as the importing countries are imposing stringent restrictions for acceptability of the made tea due to pesticide residues. Changes in pest management strategy have to be contemplated to ensure environmental and human safety. Development of insect pest susceptibility change against a few insecticides is now a reality (Sarker and Mukhopadhyay 2003, 2006 a,b) along with an increase in pesticide cost and availability. Public concerns over pesticide use have resulted in government action such as a mandated 50% cut in European countries' pesticide use (Matteson, 1995).Public anguish has compelled the EPA, USDA and FDA to take initiatives to implement IPM in the U.S. (U.S. Congress OTA, 1995); FIFRA and FQPA requirements for pesticide use in the U.S. (EPA, 1997; Klassen, 1998), and consequently led to CIB label claim and PFA clearance for usage of chemicals in tea in India (Gurusubramanian *et al.*, 2005). So it is high time that tea planters should consider i) the impact of pesticides on non-target organisms, human health, wild life habitat and environment and ii) adoption of IPM strategies to reduce the pesticide load and to produce residue free tea. Such special drives would increase the exports and the popularity of tea in domestic front as a true health drink.

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Approaches for the Management of Tea Mosquito Bug, *Helopeltis theivora* Waterhouse (Miridae : Heteroptera)

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Abstract

Helopeltis (*Helopeltis theivora*) has gained the status as one of the major pests of tea in recent years and its management has also become the central problem for the planters. The effectiveness of different approaches as cultural operations, regulation of shade status, use of botanicals, natural enemies abundance and their conservation and selection and judicious use of chemicals have been integrated to manage the havoc of tea mosquito bug infestation.

Cultural operations like Black Plucking (BP) and Level of Skiff (LOS) along with chemical spraying significantly decreased the infestation level of Helopeltis (9 – 50 fold) and increased the crop yield (2-3 times) in comparison with spraying only chemicals without cultural operations. Moderate shade status (60 %) coupled with cultural operations (BP and LOS) protected the crop from *Helopeltis* with lesser rounds of spray. Unshaded plots suffered more *Helopeltis* attack and crop loss.

Clerodendron infortunatum, a weed plant abundant in and around tea growing areas, was found to possess antifeedant property (50.94 – 80.76 % - laboratory) as well as insecticidal activity (18.54 – 75.85 % - Field) against *Helopeltis* which showed promise as a plant based insecticide. Lady bird beetles and spiders and braconids and ichneumonids were the predominant predators and parasites in tea ecosystem. Among 42 species of identified spiders, *Oxyopes, Plexippus, Phidippus* and *Marpissa* were the dominant genera.

In a separate study on field persistency, toxicity of some of the insecticides lasted for about 11-14 days which showed the need for 10-15 days interval between two subsequent rounds. Most of the commonly used insecticides were found to have lesser ovicidal action against the eggs of *Helopeltis*. During rainy season, a rain-free gap period of 1-6 hours is needed after spraying of chemicals to retain their toxicity. Desired *Helopeltis* control was not achieved when acaricides were mixed with insecticides showing the chemical incompatible nature of Fenazaquin and Propargite with Deltamethrin, Alphamethrin, ë-cyhalothrin, â-cyfluthrin, Endosulfan and Cypermethrin and Sulfur exhibited chemical incompatibility with Deltamethrin and neem. Mixing of incompatible chemicals not only decreased the insecticide toxicity but also shifted the level of relative toxicity. Variation in relative toxicity to Deltamethrin was observed between male and female populations of the pest in Jorhat and to different insecticides among the populations of *Helopeltis* collected from different sub-districts of Dooars.

Key words : *Helopeltis theivora*, Black plucking, Level of skiff, shade status, *Clerodendron infortunatum*, Relative toxicity, Chemical incompatibility of pesticides.

Introduction

Tea Mosquito Bug, *Helopeltis theivora* Waterhouse (Miridae: Heteroptera), has been the most destructive pest of tea for last few decades and the endeavour to control this pest continues. Now we have been facing two constraints with respect to pest management, i.e., regulatory measures imposed by the Government of India (CIB and PFA) and current practices of adoption of plant protection measures. After recent detection of pesticide residues in food products, the regulatory agencies under the Prevention of Food Adulteration Act (PFA) the ministry of health imposed stringent rules and regulations for the application of chemicals used in tea. Recently CIB approved 30 pesticides out of which only 11 were insecticides. Current trend of over-reliance on the use of synthetic pesticides in tea crop protection programs around the North-east India has resulted in disturbances to the environment, pest resurgence, variation in susceptibility, residue problems in made tea, impedance for natural regulatory agents and lethal and sub-lethal effects on non-target organisms, including humans. These side effects have raised public concern about the routine use and safety of pesticides. In the recent years, it has become a major concern to the tea industry as the importing countries are imposing stringent restrictions for acceptability of the made tea due to pesticide residues. However, the concerns outlined above dictate movement from pesticide-based pest management systems to more truly integrated insect pest management approaches, creating opportunities for increased inclusion of ecofriendly based pest management tools. Pest control materials are selected and applied in a manner that minimizes risks to human health, beneficial to non-target organisms, and the environment. Further more emphasis is being given towards the alternative measures complemented with the current practices of chemical control.

With this likely future in mind, reducing dependence on chemical pesticides in favour of ecosystem manipulations is a good strategy for planters. Hence the current study focused with the objectives of i) reduction in infestation pattern of *Helopeltis* through cultural operations like Black Plucking (BP) and Level of Skiff (LOS) ii) quantify the relationship between shade status and infestation iii) tapping the sources of a weed plant, *Clerodendron infortunatum* as plant based insecticide iv) species composition of biocontrol agents and their conservation v) selection and usage pattern of insecticides and development of a package and vi) future alternative control strategies.

Materials and Methods

Effect of plucking and skiffing along with pesticide application on infestation pattern of H.theivora

Heavily infested section (Section no. 9) at Kotalgoorie T.E was selected to study the effect of different cultural practices as plucking (Black plucking and Normal plucking) and skiffing (LOS – Level of Skiff) on infestation pattern of *H.theivora* along with chemical control. Seven plots each containing 100 bushes were chosen and between the plots four rows were kept as guard rows. Randomised Block Design was followed and pre and post treatment operations were recorded at weekly intervals in terms of percent infestation and crop yield for a period of one season(March 2003- Mid December2004).

Effect of shade status on Helopeltis population

In order to put more emphasis on cultural practices and quantify the effect of shade status for control of *Helopeltis*, a field trial was initiated at Borbhetta T.E. during May 2004. The experiment was laid out in three sites as **Site I** - full sun shine in unshaded condition (1780 μ mole / m2 /sec); **Site II** - 89% shade (187-228 μ mole / m2 /sec) under *A. odoratissima*; **Site III:** an average shade status of 60% (775 --339 μ mole / m2/ sec) under *A. odoratissima*. Pre and post treatment observations on infestation pattern and crop yield were taken at weekly interval for a period of four months.

Bioassays with *Clerodendron infortunatum*

Leaves and Succulent stems of *Clerodendron infortunatum* were and dried under shade for 20 --30 days and powdered by using mixer grinder. After grinding the plant powder was sieved through 20-mesh sieve and required amount of plant powder was soaked in water as per the chosen concentrations (2,5,10 %) for a day. Insecticidal, ovicidal and antifeedant activities under laboratory (Isman *et al.*, 1990) and field trial, phytotoxic, tainting and organoleptic tests were assayed along with untreated control.

Species composition of biocontrol agents

A survey was attempted to explore the incidence of predators and parasites available in tea crop especially in North Bengal area from December 2002-2003. For this study 12 gardens (Dooars - Satali, Birpara, Lakhipara, Grossmore, Songachi, Manabari; Terai – Mohorgong, Gulma, Hasqua; and Darjeeling – Makaibari, Castleton, Pusimbing , Namring) were selected. Samplings were made at random in the sections of the chosen gardens for 10 minutes duration on a fortnightly basis by using De-vac suction sampler (USA). Potent predators and parasites were identified and reported.

Selection and usage pattern of insecticides

Ovicidal activity, persistence of residual toxicity (Sarup *et al.*, 1969), tea foliage adsorption time, chemical compatibility, variation in relative toxicity (Dooars and Jorhat populations) to different insecticides were performed and proposed a package for *Helopeltis*.

Data were statistically analysed by using ANOVA method to derive F - value, CD @ 5 % level and CV (%) (Zar, 1974).

Results and Discussion

Effect of plucking and skiffing on infestation pattern of H.theivora

An experiment was conducted at Kotalgoorie T.E to prove the significance of cultural practices like LOS and Black Plucking(Removal of all infested leaves) as remedial measures to eliminate the eggs of *Helopeltis* and alleviate the problem of complete shut down in shoot production with two different schedules of new generation pesticides as Imidacloprid, Lamdacyhalothrin, Etofenprox (ILE) and Lamdacyhalothrin, Etofenprox and Betacyfluthrin (LEB). Before adopting the said cultural practices as well as the chemical treatments the percent infestation and yield were to the tune of 76.5 - 80 % and 1.45 – 2.5 kg respectively (Table 1). After following the respective cultural practices along with normal operations and spraying of new generation pesticides as per the treatments mentioned in Table 1, the percent infestation was decreased to the tune of 0.2 % in LOS, 1.22 – 1.30 % in BP and 9.82 10.2 % in normal plucking in comparison with untreated control (82%).

The yield was in increasing trend in LOS toward 27.8 – 29.0 kg followed by BP (21 – 26 kg) whereas declined in Normal Plucking (only chemical spray) to the extent of 10.0 -- 10.5 kg followed by 3.2 kg in untreated control. Increased productivity (2.7-2.9 times) and decreased infestation (50 fold) were achieved with LOS followed by Black plucking (yield- 2.1-2.6 times; infestation- 8.5-9.0 fold) with 3 rounds of chosen new generation pesticides in comparison with normal plucking. Regarding the pesticide schedule in terms of selection of pesticides no significant differences were noticed with respect to infestation level. From the above findings it was confirmed that to increase the productivity of *Helopeltis* infested sections removal of all infested shoots was mandatory i) to eliminate the eggs of *Helopeltis* in infested shoots, ii) to initiate the shoot production and iii) to check Helopeltis population in the next generation (Table 1).

The whole pluckable shoots dried up due to feeding when the infestation level was severe whereas no shoot development occurred when the *Helopeltis* fed on. Badly damaged buds could not be plucked, which badly affected the next flush of shoots. This fact was proved through the experiment on the effect of plucking and skiffing. Further, splitting and callousing of tender stems resulted due to oviposition thereby blocking the nutrient channels affecting the physiology of tea plant in the later stage. In course of time this condition led to the formation of gall and stunted growth.

Effect of Shade status on Helopeltis population

In unshaded condition (1780 µ mole / m2 /sec) plots *Helopeltis* infestation was decreased to the tune of five times (10.0 %) than pretreatment count (59.0 %) following black plucking (May) and three rounds of pesticide spraying (from June- September). Yield recorded was minimum and resurgence of the pest was quick in the unshaded plots.

Plots receiving 89% heavy shade (187-228 µ mole / m2 /sec) under *A. odoratissima* recovered from severe infestation (90.0 %) to below economic injury level (3.0 % - 30% decrease in infestation) after a black plucking operation followed by light skiffing and two rounds of pesticide application (during late June). Shoot production was higher compared to the unshaded plots and the pest was well under control up to the month of November.

Plots receiving moderate shade status of 60 % (775 –839 µ mole / m2/sec) under *A. odoratissima* suffered least from attack (35.40% during May). One round of pesticide was applied in June. Maintaining regular plucking rounds, highest crop production compared to either of the Sites I and II was recorded in the plots up to the month of November and did not require further pesticide application as there was no resurgence of the pest (Table 2).

From the above experiment it was observed that a shade status of 60% was the best in terms of crop yield and protection from the pest attack with lesser rounds of spray. Heavily shaded (89%) condition suffered from severe attack and needed better management practices for recovery; unshaded condition was equally detrimental as that in the case of red spider mite in so far as *Helopeltis* attack and crop loss was concerned.

Bioactivity of *C.infortunatum*

No ovicidal activity was registered against the eggs of *H.theivora* at 2% concentration of water extract of *C.infortunatum*. The least percent [12.5 – 16.6 %] of eggs of *H.theivora* was unhatched at 5 and 10 % concentrations (Table 3). Prolongation of incubation period was observed in *H.theivora* to the extent of 4-5 days in comparison with untreated control. Further, the extracts of *C.infortunatum* were found to be effective in killing the neonate nymphs of hatched *H.theivora* to the tune of 20 – 30 % that successfully came out of treated eggs. Generally, chemical substances present in the plant extracts may block the micropyle region of the egg thereby preventing the gaseous exchange that will ultimately kill the embryo in the egg itself. Raja *et al.*,([2003) screened 9 plants with various solvent extracts against *Spodoptera litura* in relation to ovicidal and ovipositional deterrent activity and varied responses were noticed irrespective of the concentrations and the solvents used for extraction. In this study no promising impact was there on reduction in egg hatchability.

Antifeedant activity of *C.infortunatum* with water extracts against *H.theivora* revealed that in all the treatments the average number of feeding spots were invariably less (2-4 fold) as compared to that of Control (Table 4). The percent reduction in feeding spots over control varied from 61.52 to 71.14 %. Antifeedant property of *C.infortunatum* in relation to percent reduction in number of feeding spots over control increased with the increase in concentrations and were found to be effective significantly (Table 4). The antifeedant effect of different solvent extracts of *C.infortunatum* on the adults of *H.theivora* was similar to the ones as observed by Gogoi *et al* (2003), Breuer and Devkota (1990), Dilawari *et al.*. (1994) and Raja *et al.*, (2003) against *H.theivora, Thaumetopoea pityocampa, Plutella xylostella* and *Spodoptera litura.*

Irrespective of the concentrations, the insecticidal activity of *C.infortunatum* varied. Results of insecticidal action of water extract of *C.infortunatum* under laboratory condition are documented in Fig.1. Higher concentration of water extract (10%) of *C.infortunatum* was found to be potent in killing the adults of *H. theivora* (60.0%) than lower concentration counterpart (32.2% kill at 5% concentration) (Fig.1).

Field evaluation of the efficacy of water extract of *C. infortunatum* on *H. theivora* presented in Table 5 revealed that the toxicity of different concentrations of water extract of *C. infortunatum* under field conditions and all the treatments significantly reduced the population of *H.theivora* when compared with untreated check. Higher concentrations (10%) of water extract of *C. infortunatum* recorded the significant percent reduction of *H.theivora* to the tune of 57.09 % than the lower (2 and 5%) concentrations (2 % – 45.31 %; 5 % - 44.50 %). The insecticidal activity (44.50-57.09 % reduction) of *C. infortunatum* was at par with neem as standard (45.60 %).

The ovicidal activity, antifeedant property, and insecticidal action of *C. infortunatum* against *H. theivora* in the present study were of a high order. However, crude extracts of *C.infortunatum* were observed to suppress the population among *Oryctes rhinocerus* (Chandrika and Nair, 2000), Sweet potato weevil (Palaniswami, 2000), *S. litura*, *Earias vitella* and *P. xylostella* (Anon, 2003) and major rice pests (Gaby Stoll, 1986) which also had antifeedant and repellant effects against cereal pests (Grainage and Ahmed, 1988). It is, therefore, possible that some sort of olfactory, gustatory or contact response of adults of *H. theivora* to *C. infortunatum* extract has led to suppression in their egg hatchability, feeding and population size under field conditions.

No phytotoxic symptoms in terms of injury on leaf tips and leaf surfaces, leaf wilting, necrosis, vein clearing, epinasty and hyponasty were observed up to 60 days after spraying of *C. infortunatum* under field conditions. Further, made tea samples prepared after treatment showed taint negative and organoleptic score of tea infusions, leaf and liquor ranged from 6.5 to 7.0 and having good liquor, strength and colour. In a real sense, *C. infortunatum* is considered to be a weed in tea ecosystem and distributed widely in North-east India (Misra and Dutta, 2003). Further, phytosociological analysis of North-east Indian forest zones revealed the distribution and availability in core and buffer zones of forests (Frequency – 30-40; Density – 3-5; Abundant 8-10) (Anon., 2000). The weed is grown luxuriantly throughout the year especially during rainy season (May – August). Then it is easy for the planters to collect the plant, shade dried and preserve it in gunny bags for the ensuing season to combat pest problems.

Pesto-toxic compounds in the leaf extracts of the *C. infortungtum* were water-soluble and could be filtered, in contrast to that of garlic *(Allium sativum)* in which the toxins are water insoluble and cannot be filtered through Whatman filter paper No. 1 (Nath *et al.*, 1982). Present findings revealed that the leaf-extracts of *C.infortunatum* were highly toxic to *H.theivora*. Srikumar *et al* (1989), Sejbac *et al* (1996) and Anon. (2003) observed that Clerodin, Lupane, Clerodone, Uncinatone and Pectolinarigenin present in the leaves of *C. infortunatum* and *C. siphonenthus* were the main insecto- and miti-toxic principles. Apart from the pesticidal, ovicidal, antifeedant and growth inhibitory activities, the leaf extracts of *C. infortunatum* were shown to possess other beneficial effects including fungi-suppressive effects to blister blight causing moulds. Evidently, treatment with *C. infortunatum* based botanicals could control the tea mosquito bug significantly. The role of *C. infortunatum* botanicals in the tropical North East Indian tea ecosystem against different tea pests appeared to be very promising. It is therefore concluded that the incorporation of dried leaf extracts of *C. infortunatum* (screened for their pesticidal effects) could provide a suitable and cheaper alternative means to biologically manage the tea mosquito bug. Although the active pesticidal principles from the different solvent extracts of the *C. infortunatum* are yet to be characterized, these findings will enable us to test the bio-formulations of locally available weed in different geographical regions of the country as tapping the weed source as potent phytopesticides.

Composition of natural enemies in North Bengal

The natural enemy complex in tea earmarked that under three regions of North Bengal conditions (Dooars, Terai and Darjeeling) tea crop was found mainly inhabitant with eight different predators, *viz.*, lady bird beetles, spiders, brown lace wing, syrphid fly, geocoris, green lace wing and predatory pentatomids and four different types of parasites *viz.*, braconids, ichneumonids, tachinids and chalcid (Figs. 2 & 3). Lady bird beetles (61.0%) predominated and outnumbered all predators followed by spiders (28.7%), brown lace wing (4.1%) and syrphid fly (3.1%), while *Geocoris* (0.48%), green lace wing (0.35%) and predatory pentatomids (0.22%) remained the lowest among the observed predatory types (Fig. 2). Forty two species of spiders were observed in North Bengal region wherein *Oxyopes*, *Plexippus*, *Phidippus*, and *Marpissa* were the predominant genera. The spider population was noticed to increase gradually from March to October in Dooars, Terai and Darjeeling and decreased during winter season.

Among the parasites recorded braconids dominated to the extent of 56.5% and the percent share of incidence of other parasites were computed to be 28.9%, 10.3% and 4.4% for ichneumonids, tachinids and chalcids respectively over total percent incidence in North Bengal (Fig. 3).

Selection and usage pattern of insecticides

Dimethoate at normal dose recording 68 percent egg mortality remained significantly superior to other treatments. Profenofos gave 23.2 percent egg mortality followed by Phosalone (21.6%) and Deltamethrin (20.0 %). Neem and quinalphos (8.8 and 16.8 %) were least effective as ovicides. The chosen insecticides were found highly effective in killing the neonate nymphs of hatched *H. theivora* that successfully came out of treated eggs. Deltamethrin and Dimethoate recorded the highest mortality of cent percent in newly hatched neonate nymphs followed by Quinalphos (73%), Phosalone (65%), Profenofos(62%) and Neem (18.3%) (Table 6).

The duration of effectiveness of insecticides was evaluated on the basis of PT values denoting persistence of different insecticides against *Helopeltis* when exposed to tea leaves treated with recommended dilutions (Table 7). Quinalphos, Deltamethrin, Dimethoate, Profenofos and Phosalone persisted on tea foliage against *Helopeltis* for 11-14 days after application. Quinalphos persisted for longer period. On the basis of PT values (in parenthesis) the order of relative efficacy was Phosalone (715), Quinalphos (630), Profenofos (600), Dimethoate (576), and Deltamethrin (411.58) (Table 7).

Normally the peak period of pest attack coincides with rainy and flushing period of tea i.e., from April to August. During this period chemical control measures are being taken to manage *H.theivora*. The main hindrance in control measures is the rain that is intermittent. To manage this situation foliage adsorption time period (minimum time requirement of a pesticide to adsorb on a leaf surface for exhibiting its toxicity) for commonly used insecticides is mandatory to retain the toxicity of insecticides. From the results it was observed that Deltamethrin and Dimethoate required a foliage adsorption time period of 1 and 2 hours after spraying followed by Quinalphos and Profenofos that needed 5 hours to retain their toxicity. More than six hours time was requisite in case of Phosalone (Table 8). As a whole, minimum of an hour or more of foliage adsorption time is compulsory for all the chosen pesticides during rainy period to keep the toxic effect of the insecticides.

Preliminary trial on relative toxicity of Deltamethrin, Profenofos, and Quinolphos was determined against *Helopeltis* populations collected from different sub districts of Dooars *viz.*, Nagrakata, Damdim, Chalsa, Binnaguri, Kalchini and Dalgaon. The relative toxicity in terms of LC_{50} values was so erratic that it ranged from 260 ppm in Kalchini to 7.0 ppm in Chalsa in case of Quinalphos; 0.75 ppm in Kalchini and 0.15 ppm in Chalsa regarding Deltamethrin; and 6.5 ppm in Kalchini and 0.65 ppm in Nagrakata concerning Profenofos (Table 9). The relative toxicity of Deltamethrin in terms of LC_{50} was found to vary between male (40 mg/kg) and female (25 mg/kg) populations of *H.theivora* collected from Jorhat area (Table 10). Variation in relative toxicity was observed between male and female populations of Jorhat (Table 9) and among the populations of *Helopeltis* collected from different sub districts of Dooars in case of all the tested insecticides due to selection pressure by insecticides (Table 10). This baseline data are very helpful in generating regionwise package of practice for the usage of chemicals. Further confirmation and in-depth studies are being in progress to develop a strategy and package for North East India.

In order to combat the situation like mixed infestation of mite and *Helopeltis* normally the planters have been practicing the tank mixing of insecticides with acaricides. Before mixing of chemicals knowledge about the chemical compatibility of pesticides is prerequisite. The present study was undertaken to ascertain the toxic nature of insecticides after mixing with acaricides wherein Fenazaquin, Propargite, Sulfur and neem formulations were mixed at recommended dilutions with different pesticides (Table 11). Propargite in combination with Deltamethrin, Endosulfan, ë–cyhalothrin and ß–cyfluthrin registered a per cent reduction after 4th week (65.41, 41.79, 64.25 and 78.55%) lesser than the chosen insecticides treated alone (84.9, 80.80, 66.49 and 100%), so all the above four insecticides not compatible with Propargite. But when Propargite was mixed with Alphamethrin, the per cent reduction achieved was 87.72% which was more than that treated alone (80.90%). From the above it is clear that only Alphamethrin was compatible with Propargite (Table 11).

Among the seven insecticides tested (Fig. 5) for congruance to Fenazaquin, Deltamethrin, Alphamethrin, â-cyfluthrin and ë-cyhalothrin were not found compatible chemically showing 23.27 – 38.35 %, 38.57 - 43.33 %, 57.61 -8.37 %, and 37.58 - 50.06 % reduction from I week to IV week (Table 11) whereas Thiomethoxam, Endosulfan and Imidacloprid registered 83.01 – 86.41 %, 90.60 – 92.48 %, and 83.48 – 96.62 % reduction respectively and found compatible. Based on the reduction in infestation pattern of *H.theivora,* Endosulfan (79.8%), Alphamethrin (88.93%), Betacyfluthrin (85.4%), Lamdacyhalothrin (89.6%), Thiomethoxam (93.6%) and Imidacloprid (93.0%) were compatible with sulfur (46.42%) except Deltamethrin (46.6%) (Table 11).

Desired *Helopeltis* control was not achieved showing the incompatible nature of Fenazaquin with Alphamethrin, Deltamethrin, ë-cyhalothrin, â-cyfluthrin; Propargite with Deltamethrin, Endosulfan, â-cyfluthrin, Cypermethrin; and Sulfur with Deltamethrin and neem. This is one of the reasons, i.e., by mixing of incompatible insecticides with other pesticides, the menace of *Helopeltis* infestation could not be checked even after repeated applications of chemicals.

Proposed package for *H. theivora* (CIB approved chemicals)

Jan –Feb - I round of spray after infestation in *Helopeltis* prone sections

Unpruned	-	use Dimethoate @1:400 / Deltamethrin @1:2000 /Phosalone @ 1:400
DS/MS	-	Use either systemic (Dimethoate @1:400)/ Contact (Phosalone @1:400 / Deltamethrin @1:2000 / Profenofos @1:1000
LP/MP	-	Use Dimethoate @1:400 / Phosalone @1:400 / Deltamethrin @1:2000 immediately after bud breaking

Mar–Apr	I round - Phosalone	@ 1:400	II round - Neem extract -5% @ 1:1500
May –June	I round - Dimethoate	@ 1:400	II round - Deltamethrin @ 1:2000
July–Aug	I round - Profenofos	@1:1000	II round - Phosalone @1:400
Sept–Oct	I round - Dimethoate	@ 1:400	II round - Neem extract -5% @ 1:1500
Nov –Dec	I round - Diflubenzuron	@ 1:1000	II round – *Beauveria bassiana* @3 kg/ha

Table 1. Effect of cultural practices on the infestation pattern of *Helopeltis theivora*

S.No.	Cultural Practice	Treat ment	Pre treatment Per cent infestation/ plot	Pre treatment Mean crop Yield/plot/ week/kg	Post treatment Per cent infestation /plot	Post treatment Mean crop Yield/plot/ week/kg
1	BP	ILE	76.5	1.80	1.3	21.0
2	BP	LEB	81.6	1.45	1.2	26.0
3	LOS	ILE	79.5	1.50	0.2	27.8
4	LOS	LEB	80.0	1.80	0.2	29.0
5	Normal	ILE	87.0	1.70	9.8	10.0
6	Normal	LEB	80.0	2.20	10.2	10.5
7	Control	Nil	80.0	2.50	82.0	3.2
	CD (P=0.05)				6.54	8.52
	CV (%)				4.88	7.63

ILE – Imidacloprid, Lamda Cyhalothrin and Etofenprox ;
LEB – Lamda Cyhalothrin, Etofenprox and Beta cyfluthrin;
BP – Black Plucking (Removal of infested leaves) with chemical spraying;
LOS – Level of Skiff (Removal of infested leaves along with tender stems below the table) with chemical spraying;
Normal – Normal Plucking with chemical spraying;
Control – No cultural operation and pesticide spraying

Table 2. Effect of shade status on *Helopeltis theivora* infestation

Site	Shade intensity	% infestation Pre treatment	% infestation Post treatment	Treatments (June- Aug.)	Mean crop/ month (kg.) Jul- Oct.)
I Unshaded	Full Sunshine	59.0	10.0	BP, three rounds of pesticides *	18.2 (12 harvests
II Moderate shade	60% shade	35.4	3.5	One round of pesticide	23.0 (12 harvests)
III Unshaded	89% shade	90.0	3.0	BP, LOS, two rounds of pesticides	20.2 (11 harvests)
CD (P=0.05)			2.89		2.91
CV (%)			1.42		1.06

* Including one round of acaricide

Table 3 . Ovicidal activity of water extract of *Clerodendron infortunatum* against *Helopeltis theivora* Waterhouse under laboratory condition

Treatments	Concentration (%)	Pre-treatment counts	No. of eggs hatched	% unhatched eggs over control
T1	2	18	18	0.0 ± 0.00
T2	5	16	14	12.5± 3.47
T3	10	18	15	16.6 ± 2.15
T4-Control	-	23	23	0.0
CD at P=0.05			16.94	
CV%			11.74	

Each mean (± SD) represents three replicates; T1,T2, and T3 – Water extracts of *Clerodendron infortunatum*

Table 4 . Antifeedant activity of water extract of *Clerodendron infortunatum* against *Helopeltis theivora* under laboratory condition

Treatments	Concen-tration (%)	No. of feeding spot/24 hrs.	% reduction of feeding spots over control
T1	5	600	61.52 ± 0.47
T2	10	450	71.14 ± 0.52
T3-Control	untreated	1560	-
CD at P=0.05		4.92	
CV%		4.30	

Each mean (± SD) represents three replicates; T1 and T2– Water extracts of *Clerodendron infortunatum*

Table 5. Bioefficacy of different concentrations of water extract of *Clerodendron infortunatum* against *Helopeltis theivora* Waterhouse in the field

Treatments	Conc. (%)	MPTI (%)	I week		II week		III week		IV week	
			1	2	1	2	1	2	1	2
Water extract	2	57.00	42.80	24.38	39.00	30.57	29.50	48.45	31.00	45.31
	5	62.30	29.00	53.12	42.00	32.28	27.30	55.45	34.30	44.47
	10	68.30	26.60	60.90	34.00	50.19	23.16	66.11	29.30	57.09
Neem (Standard)	10	66.30	36.00	45.67	31.00	53.20	26.00	60.57	36.00	45.60
Control		67.60	69.00	-5.20	78.40	-19.52	76.00	-16.23	75.60	-15.80
C.D. at P= 0.05			9.34		12.33		7.56		7.75	
CV%			14.40		28.13		8.91		10.46	

Conc - Concentration; MPTI – Mean Pre Treatment Infestation;
1 – Percent infestation; 2 - Percent reduction.

Table 6. Insecticides against eggs of *Helopeltis theivora*

Insecticide	Dose	Per cent hatching	Percent mortality of neonate nymphs	Corrected Egg mortality (%)
Deltamethrin	1:2000	50	100.0	20.0
Dimethoate	1:400	20	100.0	68.00
Azadirachtin	1:1500	57	18.3	8.80
Phosalone	1:400	49	65.0	21.60
Quinalphos	1:400	52	73.0	16.80
Profenofos	1:1000	48	62.0	23.20
Control	Untreated	62.5	-	
CD (P=0.05)				5.43
CV (%)				2.34

Table 7. PT values and order of relative efficacy of different insecticides against adults of *Helopeltis theivora*

Insecticides	Concen-tration (%)	Period (P) days	% average residual toxicity	PT value	Order of relative efficacy (ORE)
Dimethoate	1:400	12	48.00	576.00	4
Deltamethrin	1:2000	13	31.66	411.58	5
Phosalone	1:400	11	65.00	715.00	1
Profenofos	1:1000	12	50.00	600.00	3
Quinalphos	1:400	14	45.00	630.00	2

Product (PT) of average residual toxicity (T) and the period in days (P) for which the toxicity persisted.

Table 8. Tea foliage adsorption time for different commonly used insecticides during rainy period

Insecticide	Tea foliage adsorption time (hrs)/ per cent mortality					
	1	2	3	4	5	6
Dimethoate	80	100	100	100	100	100
Phosalone	0	0	0	0	0	20
Deltamethrin	100	100	100	100	100	100
Quinalphos	20	40	60	60	100	100
Profenofos	80	90	90	90	100	100
Pesticide	CD - 3.39 ; CV – 8.04					
Absorption time	CD - 1.92 ; CV - 6.35					
Interaction	CD - 0.76 CV - 4.78					

Table 9. Variation in relative toxicity of commonly used insecticides against *Helopeltis theivora* collected from different sub-districts of Dooars

Insecticide	LC_{50} values (ppm)					
	Nagrakata	Damdim	Chalsa	Binnaguri	Kalchini	Dalgaon
Deltamethrin	0.75	0.4	0.15	0.5	0.75	0.8
Quinalphos	37.5	-	7.0	40.0	260.0	25.0
Profenofos	0.65	-	-	1.5	6.5	-

Table 10. Variation in relative toxicity of Deltamethrin between male and female populations of *Helopeltis theivora* collected from Jorhat

Insecticide	LC_{50} values (ppm)	
Deltamethrin	Male	Female
	40.0	25.0

Table 11. Chemical Compatibility of Agrochemicals

S.No.	CHEMICAL	Compatible with	Incompatible with
1	Propargite	Alphamethrin	Deltamethrin Endosulfan β-cyfluthrin λ-Cyhalothrin
2	Fenazaquin	Thiomethoxam Endosulfan Imidacloprid	Deltamethrin Alphamethrin β-cyfluthrin λ-Cyhalothrin
3	Sulfur	Endosulfan β-cyfluthrin λ-Cyhalothrin Alphamethrin Thiomethoxam Imidacloprid	Deltamethrin
4	Cypermethrin	Fenazaquin Fenpyroximate MOP Urea Zinc Sulphate	Propargite
5	Neem		Ethion Sulfur

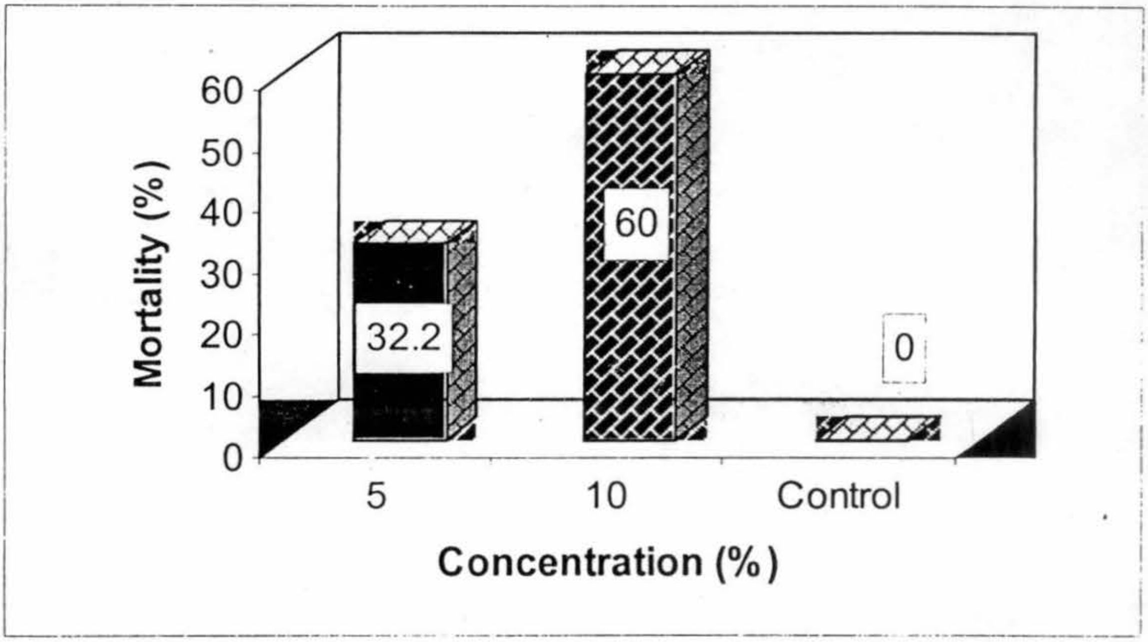

Fig.1 Mortality of *Helopeltis theivora* adults in water extract of *Clerodendron infortunatum* under laboratory conditions.

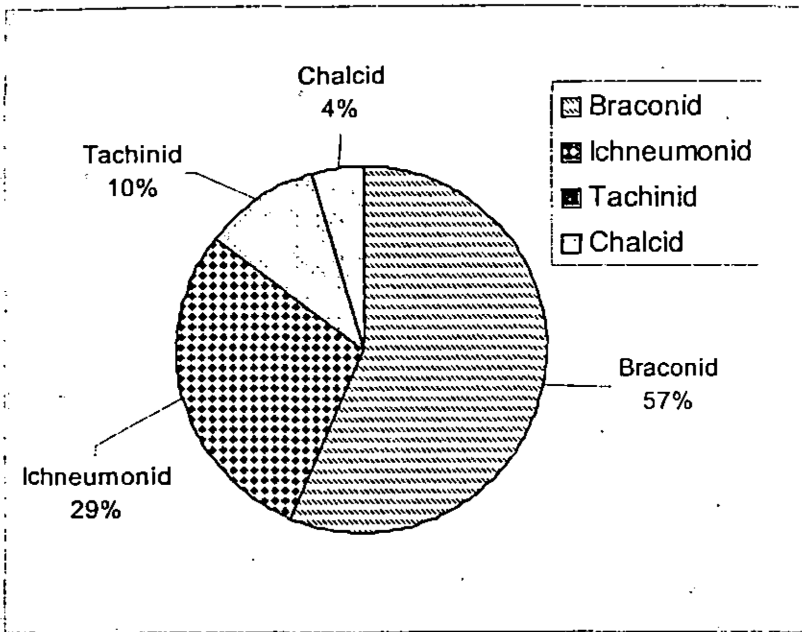

Fig. 2 Composition of predators in tea in North Bengal

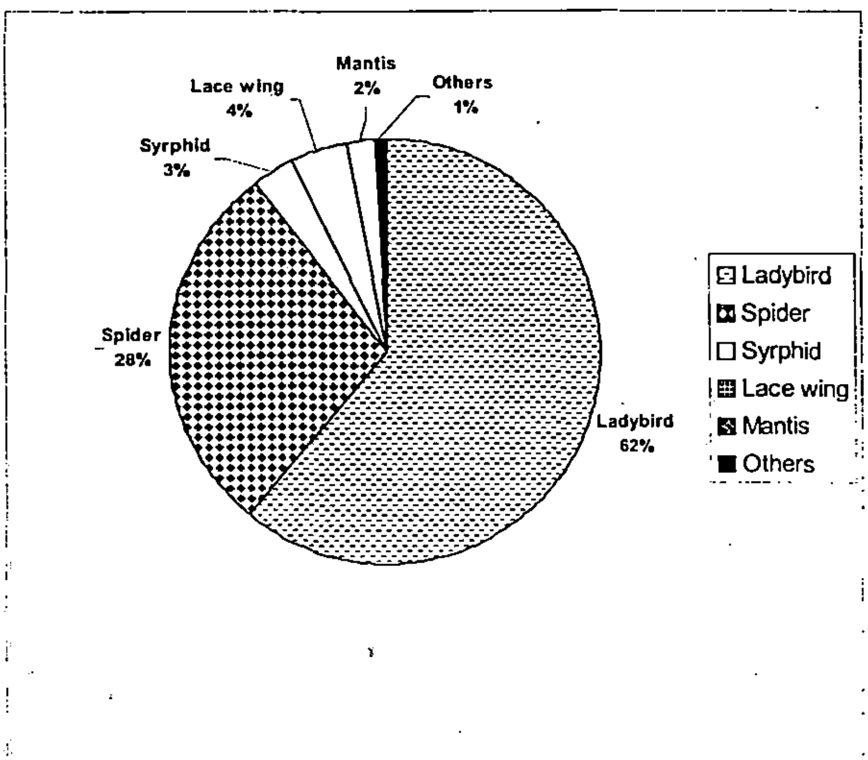

Fig. 3 Composition of parasites in tea in North Bengal

Conclusions

- Black Plucking and Level of Skiff cultural operations were prerequisite in severely *Helopeltis* infested sections before chemical spraying as remedial measures to alleviate the problem of complete shut down in shoot production and eliminate the eggs and further decrease infestation of *Helopeltis* and increase productivity.
- Moderate shade status condition to the tune of 60% suffered from less attack of *Helopeltis*. Unshaded condition was prone to *Helopeltis* infestation and needs more rounds of chemical spray to bring down the infestation level.
- Cultural operations (BP & LOS) coupled with moderate shade status increased the shoot production and decreased the *Helopeltis* infestation level with lesser rounds of chemical spray compared to shaded and unshaded conditions.
- Leaf extracts of *Clerodendron infortunatum* were shown to possess strong antifeedant and insecticidal activities against *Helopeltis* and could provide a suitable and cheaper alternative means as plant based insecticide.
- Potent predators and parasites are available in tea ecosystem for *Helopeltis* which can be conserved and augmented with judicious use of pesticides
- Mixing of incompatible pesticides lead to decrease the toxicity of chemicals thereby not attaining desired control of *Helopeltis*.

Future plan of work

- Inclusion of biologically based pest management tools as predators and parasites and their augmentation and conservation.
- Search for safe, effective and native microbial insecticides.
- Development and production of indigenous phytochemicals and animal products as environment friendly pesticides.
- Integration of semiochemicals, ultrasound and sex pheromones into pest management.
- Genetic engineering of plants for *Helopeltis* resistance – resistant transgenic plants expressing inhibitors of insect digestive enzymes, lectins, hydrolytic and oxidative enzymes and lipid oxidases.

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formulated, so that mite pest can be managed efficiently to obtain profitable yields. Additionally, acaricides with high ovicidal action may be incorporated in the control programmes keeping in mind the peak oviposition period of this pest.

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Susceptibility status of *Helopeltis theivora* Waterhouse (Heteroptera: Miridae) to the commonly applied insecticides in the tea plantation of the Sub-Himalayan Dooars area of North Bengal, India

ABSTRACT

Among various biotic stresses that tea plants face, insect attack, especially from the tea mosquito bug (*Helopeltis theivora*), has been a major challenge in recent years. Difference in relative toxicity of different commonly used insecticides to *Helopeltis theivora* was observed in the plantation of the tea subdistrict of the Dooars, located in the region of northern West Bengal, India. The study indicated that relative susceptibility values (LC_{50}) of *H.theivora* to different insecticides varied region wise. The populations of Kalchini tea subdistrict showed less susceptibility to all insecticides tested as compared to the high susceptibility of those from the Damdim and Chulsa subdistricts to most of the tested insecticides. The populations of the Nagrakata, Dalgong and Binnaguri subdistrict represented intermediate level of susceptibility to different insecticides tested. Endosulfan showed the lowest susceptibility against *H.theivora* in all tea growing sub-districts in the Dooars with a high LC_{50} value in all locations in the Dooars tea plantation. The effective field dosages of these insecticides were computed based on LC_{50} values, and when compared with the recommended dosages, it suggested a significant decrease in the susceptibility of the test population was observed against six insecticides like endosulfan, deltamethrin, λ-cyhalothrin, imidacloprid, quinalphos and oxydemeton methyl. The decrease in susceptibility of *H. theivora* to endosulfan (12.33 to 72.26 fold), deltamethrin (4.02 - 22.40 folds) and imidacloprid (13.61 to 29.16 folds) was the highest. However, there was not much change in case of monocrotophos and fenpropathrin, which therefore was found effective even at a lower dose than the recommended dose.

KEY WORDS: *Helopeltis theivora*, insecticides, LC_{50}, relative toxicity, resistance factors, effective field dose.

INTRODUCTION

The tea mosquito bug (*Helopeltis theivora*) is considered as one of the major pests of tea in Assam, Dooars, Terai and Darjeeling because it attacks only the young shoots that is the actual crop of tea. Planters from different parts of the Dooars tea plantation have been reporting control failure of the notorious pest with the use of insecticides. All Tocklai released tea clones, garden released clones and seed jats are found susceptible to *H. theivora* attack at varying degrees. It was estimated that 80% of the tea plantations area in India is affected by this pest alone, which often result in crop loss to the tune of 10-50% (Bora and Gurusubramanian 2007). Among the tea growing region of India, pest activity has always been reported to be high in the Dooars (Borbora & Biswas 1996 and Sannigrahi and Talukdar 2003). The climatic conditions of this region and monoculture of tea over vast stretches of land contribute largely to high pest incidence. With increases in the quantity of pesticide being applied every passing year, the problem has been aggravated and the cost of pest control is increasing day by day (Sannigrahi and Talukdar 2003). A survey conducted during 1998 to 2004 by Roy *et al.* (2008a) reported that on average 7.499 l/kg of insecticide was used per hectare per year in the Dooars, of which the organo-chlorine, organo-phosphate and carbamate (Non-pyrethroid) accounted for 73.5% and pyrethroids 36.6%. Among the different subdistrict in the Dooars, the lowest consumption was noted in Damdim subdistrict (5.799 kg/L per hectare) followed by Chulsa (6.433 kg/L per hectare), Binnaguri (7.399 kg/Lper hectare), Nagrakata (7.655 kg/L per hectare), and Dalgong (7. 920 kg/L per hectare) with the highest consumption noted in Kalchini (9.793kg/L per hectare). The requirement of synthetic pyrethorid gradually increased with every passing year in the Dooars. Endosulfan, monocrotophos, deltamethrin and cypermethrin were extensively used in the entire subdistrict.

In spite of regular application of insecticides, *H. theivora* has turn into a menace all around the year. Decrease in the susceptibility to different classes of insecticides may be one of the causes for their resurgence and persistence on tea crops (Sarker and Mukhopadhyay, 2003, 2006, 2006a; Rahman *et al.*, 2006; Sarmah *et al.*, 2006; Bora *et al.*, 2007, 2007a and 2008; Roy *et al.*, 2008,).

In order to confirm the above fact, a study was conducted to assess the relative toxicity at effective concentrations of twelve commonly used pesticides: organochlorine (endosulfan); organophosphates (monocrotophos, quinalphos, profenophos and oxydemeton methyl); synthetic pyrethroids (deltamethrin, cypermethrin, λ- cyhalothrin, alphamethrin and fenpropathrin,); and neonicotinoids (imidaclopyrid, thiomethoxam) against *H. theivora* populations of six tea growing subdistricts of the Dooars.

Materials and Methods

Insecticide Susceptibility

Helopeltis theivora adults were collected from the tea plantations of the six tea subdistrict of the Dooars region. They were placed in rearing jars (20cm x 15 cm) in laboratory for conditioning at a temperature of 27± 2°C, 70-80% RH and a 16:10 LD photoperiod for seven days. Insecticides used in the studies were imidaclopyrid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25EC, λ-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37SL, endosulfan 35 EC, quinalphos 25 EC, profenophos 50 EC and oxydemeton methyl 25 EC. Graded concentrations of insecticides were prepared in distilled water from 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). Healthy shoots of TV1 were collected from the experimental garden. 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 welting and stem part of the sprayed shoot was inserted 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. This arrangement was caged in a glass chimney. The mouth of which was covered with muslin cloth. The whole set up was kept at 27 ± 2°C in culture room. Ten field-collected and preconditioned *H. theivora* were released separately into each glass chimney containing tea shoots. Observations of adult mortality were recorded in all the three replications of each concentration after 24 hours of the treatment. Moribund insects were counted as dead (Bora and Gurusubramanian, 2007). 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, 1973; Busvine, 1971) to obtain LC_{50} values. Resistance factors (RFs) were determined based on LC_{50} values of an insecticides in reference to the lowest LC_{50} value of the same insecticides from a subdistrict. This method was adopted 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).

$$\text{Expected effective concentration } (LC_{100}) (\%) = 2 \times LC_{50}\%$$

$$\text{Expected effective dose (g a.i./ ha)} = ED / 100 \times EC \times 20 \text{ fold}$$

$$ED = \% \text{ concentration} / EC \times 1000 \times 400 \text{ liters of spray fluid/ha.}$$

Result and Discussion:

Resistance factors (RFs) were determined based on LC_{50} values relative to the corresponding lowest LC_{50} value of monocrotophos, oxydemeton methyl, thiomethoxam, imidaclopyrid, fenpropathrin and alphamethrin for the Damdim strain's and of endosulfan, deltamethrin, cypermethrin, λ-cyhalothrin, quinalphos, profenophos for the Chulsa strain, due to the unavailability of a suitable reference, a susceptible strain was normally used to calculate resistance factors.

Endosulfan:
The Kalchini population recorded the highest LC_{50} value to endosulfan (1580.77 ppm) followed by the population from Dalgong (952.715 ppm), Binnaguri (938.213 ppm), Nagrakata (884.95 ppm) and Damdim (544.722 ppm). The lowest LC_{50} value was observed in the population from Chulsa (269.744 ppm). While the Kalchini strain showed the highest resistance to endosulfan (5.86 folds), followed by Dalgong (3.53 folds), Binnaguri (3.47 folds), Nagrakata (3.28 folds) and the least resistance was observed in the population of Damdim (2.02-folds) (Table 1).

Table 1. Relative toxicity values of Endosulfan 35 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location–Sub-district in Dooars	Endosulfan 35 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.16	y = 3.902 x - 17.384	544.72	0.011	607.753 480.324	2.02
Chulsa	4.95	y = 2.375 x - 7.899	269.744	0.010	328.181 221.712	1.00
Nagrakata	5.51	y = 4.270 x - 20.369	884.95	0.010	988.899 791.936	3.28
Binnaguri	2.38	y = 5.621 x - 28.568	938.213	0.006	1017.63 864.995	3.47
Dalgong	2.45	y = 3.501 x -15.934	952.715	0.012	1099.37 825.689	3.53
Kalchini	2.38	y = 4.428 x - 22.455	1580.77	0.009	1756.22 1422.48	5.86

- Susceptible Chulsa Population.

Monocrotophos:
The Dalgong population recorded the highest LC_{50} value to monocrotophos (18.046 ppm) followed by the population from Nagrakata (17.903 ppm), Kalchini (16.270 ppm), Chulsa (7.378 ppm), and Binnaguri (4.592 ppm). The lowest LC_{50} value was observed in the population from Damdim (3.025 ppm). The Dalgong strain showed the highest resistance to monocrotophos (5.97-folds) and the least resistance was observed in the population of Binnaguri (1.52-folds) (Table 2).

Table 2. Relative toxicity values of Monocrotophos 37SL against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub-district in Dooars	Monocrotophos 37SL					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	4.97	y = 3.942 x - 8.722	3.025	0.008	3.382 2.706	1
Chulsa	4.26	y = 7.026 x - 22.179	7.378	0.006	7.895 6.895	2.44
Nagrakata	5.30	y = 4.230 x - 12.991	17.903	0.010	20.172 15.890	5.92
Binnaguri	1.31	y = 1.723 x - 1.309	4.592	0.025	6.101 3.457	1.52
Dalgong	0.49	y = 3.976 x - 11.926	18.046	0.011	20.421 15.946	5.97
Kalchini	2.99	y = 2.004 x - 3.441	16.270	0.017	20.045 12.056	5.38

- Susceptible Damdim Population.

Profenophos:
The Kalchini population recorded the highest LC_{50} value to profenophos 50 EC (29.713 ppm) followed by the population from Damdim (12.328 ppm), Dalgong (12.112ppm), Nagrakata (11.786 ppm) and Binnaguri (11.235 ppm). The lowest LC_{50} value was observed in the population from Chulsa (7.633 ppm). The Kalchini strain showed the highest resistance to profenophos (3.89 folds) and the least resistance was observed in the population of Binnaguri (1.47 folds) (Table 3).

Table 3. Relative toxicity values of Profenophos 50 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub-district in Dooars	Profenophos 50 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	5.79	y = 3.973 x - 11.255	12.328	0.011	13.972 10.878	1.61
Chulsa	6.23	y = 4.007 x - 10.56	7.6336	0.011	8.654 6.733	1.00
Nagrakata	4.72	y = 5.359 x - 16.818	11.786	0.007	12.856 10.805	1.54
Binnaguri	0.69	y = 6.142 x - 19.88	11.235	0.008	12.215 10.332	1.47
Dalgong	5.68	y = 3.806 x - 10.541	12.112	0.011	13.760 10.661	1.59
Kalchini	1.37	y = 3.568 x - 10.963	29.713	0.012	33.967 25.991	3.89

- Susceptible Chulsa Population.

Quinalphos:
On the basis of LC_{50} values, the descending order of toxicity of quinalphos 25 EC was observed in six different subdistricts in the Dooars was Chulsa (6.560 ppm), Damdim (18.335 ppm), Dalgong (29.051 ppm), Nagrakata (38.836 ppm), Binnaguri (43.764 ppm) and Kalchini (214.471 ppm). The Kalchini strain showed the highest resistance to quinalphos (32.69-folds) followed by Binnaguri (6.67 folds), Nagrakata (5.92 folds), Dalgong (4.42 folds). The least resistance ratio was observed in the population of Damdim (2.79 folds) (Table 4).

Table 4. Relative toxicity values of Quinalphos 25 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub-district in Dooars	Quinalphos 25 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.94	y = 3.048 x - 7.995	18.335	0.011	21.140 15.903	2.79
Chulsa	4.04	y = 2.267 x - 3.654	6.560	0.016	7.943 5.418	1.00
Nagrakata	4.41	y = 3.581 x - 11.435	38.836	0.010	44.005 34.274	5.92
Binnaguri	8.03	y = 3.595 x - 11.688	43.764	1.119	49.927 38.362	6.67
Dalgong	2.99	y = 2.927 x - 8.061	29.051	0.012	33.754 25.003	4.42
Kalchini	7.10	y = 2.564 x - 8.672	214.471	0.014	254.542 180.708	32.69

* Susceptible Chulsa Population.

Oxydemeton Methyl:

The population of *H.theivora* collected from Kalchini sub-district was comparatively less susceptible to oxydemeton methyl 25 EC which registered a LC_{50} value of 74.076, whereas the Damdim population showed a relatively higher degree of susceptibility to the same insecticides. Considering the LC_{50} values of oxydemeton methyl 25 EC, the order of susceptibility was as Damdim (18.309 ppm) > Chulsa (19.362 ppm) > Binnaguri (30.051 ppm) > Nagrakata (30.997 ppm) > Dalgong (58.346 ppm) > Kalchini (74.076 ppm). The resistance factor (RF) against susceptible strain was found to be highest for population of Kalchini (4.05 folds) followed by Dalgong (3.19 folds), Nagrakata (1.69 folds), Binnaguri (1.64 folds). The least resistance ratio was observed in the population of Chulsa (1.06 folds) (Table 5).

Table 5. Relative toxicity values of Oxydemeton Methyl 25 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub-district in Dooars	Oxydemeton Methyl 25 EC					
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	Resistance Factors (RF)
Damdim	2.27	y = 7.088 x - 25.216	18.309	0.006	19.571 17.128	1
Chulsa	3.42	y = 6.816 x - 24.219	19.362	0.006	20.735 18.081	1.06
Nagrakata	8.48	y = 2.122 x - 4.5324	30.997	0.015	37.973 25.303	1.69
Binnaguri	4.00	y = 2.831 x - 7.678	30.051	0.014	35.212 25.646	1.64
Dalgong	1.27	y = 3.083 x - 9.696	58.346	0.010	66.358 51.301	3.19
Kalchini	3.57	y = 3.968 x - 14.325	74.076	0.008	82.082 66.850	4.05

• Susceptible Damdim Population.

Imidaclopyrid:

The Kalchini population recorded the highest LC_{50} value to imidaclopyrid (19.907 ppm) followed by the population from Binnaguri (18.496 ppm), Nagrakata (15.052 ppm), Dalgong (15.173 ppm), and Chulsa (15.052 ppm) and the lowest LC_{50} value was observed in the population from Damdim (10.162 ppm). The values of relative resistance to imidaclopyrid when calculated taking Damdim populaton as base showed that the resistance factor (RF) to be highest in population of Kalchini (1.99 folds) followed by Binnaguri (1.82 folds), Nagrakata (1.52 folds), Dalgong (1.49 folds). The least resistance ratio was observed in the population of Chulsa (1.48 folds) (Table 6).

Table 6. Relative toxicity values of Imidaclopyrid 17.8 SL against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub-district in Dooars	Imidaclopyrid 17.8 SL					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	3.75	y = 4.032 x - 11.155	10.162	0.008	11.313 9.1284	1.00
Chulsa	0.88	y = 5.180 x - 16.640	15.052	0.009	16.510 13.722	1.48
Nagrakata	6.65	y = 5.186 x - 16.737	15.537	0.007	16.998 14.202	1.52
Binnaguri	2.70	y = 7.378 x - 26.483	18.496	0.005	19.7394 17.3323	1.82
Dalgong	3.59	y = 2.955 x - 7.353	15.173	0.012	17.654 13.041	1.49
Kalchini	1.52	y = 3.641 x - 10.654	19.907	0.010	22.499 17.616	1.99

• Susceptible Damdim Population.

Thiomethoxam:

Populations from Kalchini, Nagrakata, Binnaguri and Dalgong showed more or less the same LC_{50} value against thiomethoxam that ranged from 5.346 to 5.761, but Damdim and Chulsa showed relatively lower LC_{50} (i.e. 4.599 ppm and 4.737 ppm respectively). The values of relative resistance when calculated taking the LC_{50} of thiomethoxam in Damdim as base ranged from 1.03 folds to 1.25 folds (Table 7).

Table 7. Relative toxicity values of Thiomethoxam 25 WG against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub-district in Dooars	Thiomethoxam 25 WG					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	4.14	y = 2.248 x - 3.234	4.599	0.828	5.631 3.756	1.00
Chulsa	2.92	y = 2.087 x - 2.671	4.737	0.022	5.924 3.788	1.03
Nagrakata	1.50	y = 2.247 x - 3.449	5.761	0.016	6.996 4.744	1.25
Binnaguri	6.64	y = 1.883 x - 2.052	5.557	0.023	7.139 4.326	1.21
Dalgong	1.27	y = 1.941 x - 2.237	5.346	0.022	6.834 4.182	1.16
Kalchini	2.73	y = 2.032 x - 2.638	5.738	0.021	7.284 4.520	1.25

• Susceptible Damdim Population

Deltamethrin:

On the basis of the LC_{50} value (Table 8), the descending order of toxicity of deltamethrin 2.8 EC in six different subdistricts in the Dooars was Chulsa (0.131ppm), Damdim (0.289 ppm), Binnaguri (0.326 ppm), Dalgong (0.678 ppm), Nagrakata (0.691 ppm), and Kalchini (0.731 ppm). It was noticed that the relative resistance factor (RF) of deltamethrin against *H.theivora* from Kalchini, Nagrakata, Dalgong, Binnaguri and Damdim populations were found to be 5.58, 5.27, 5.17, 2.49 and 2.21 folds in respect to Chulsa population (Table 8).

Table 8. Relative toxicity values of Deltamethrin 2.8 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Deltamethrin 2.8 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	5.13	y = 2.555 x - 1.288	0.289	0.016	0.346 0.241	2.21
Chulsa	0.32	y = 2.240 x + 0.255	0.131	0.018	0.162 0.106	1
Nagrakata	4.75	y = 4.042 x - 6.476	0.691	0.010	0.775 0.616	5.27
Binnaguri	5.67	y = 1.774 x + 0.539	0.326	0.024	0.426 0.250	2.49
Dalgong	6.40	y = 4.324 x - 7.241	0.678	0.009	0.756 0.608	5.17
Kalchini	3.65	y = 5.509 x - 10.781	0.731	0.008	0.818 0.683	5.58

* Susceptible Chulsa Population.

Cypermethrin:
The Kalchini population recorded the highest LC_{50} value to cypermethrin (7.475 ppm) followed by the population from Nagrakata (3.813 ppm), Dalgong (3.026 ppm), Binnaguri (2.222 ppm), and Damdim (1.276 ppm) and the lowest LC_{50} value was observed in the population from Chulsa (0.802 ppm). Resistance to cypermethrin was very variable, ranging from 1.59-fold in the Damdim strain to 9.32-fold in the Kalchini (Table 9).

Table 9. Relative toxicity values of Cypermethrin 25 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Cypermethrin 25 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	2.59	y = 2.510 x - 2.796	1.276	0.212	1.581 1.029	1.59
Chulsa	6.32	y = 2.688 x - 2.691	0.802	0.016	0.966 0.666	1.00
Nagrakata	2.75	y = 1.971 x - 2.057	3.813	0.020	4.795 3.032	4.75
Binnaguri	4.35	y = 2.147 x - 2.186	2.222	0.017	2.739 1.805	2.77
Dalgong	7.77	y = 2.368 x + 3.245	3.026	0.018	3.714 2.466	3.77
Kalchini	6.57	y = 2.896 x - 6.218	7.475	0.014	8.756 6.381	9.32

* Susceptible Chulsa Population

Alphamethrin:
The Kalchini population recorded the highest LC_{50} value to alphamethrin 10 EC (1.532 ppm) followed by the population from Nagrakata (0.759 ppm), Binnaguri (0.593 ppm), Dalgong (0.435 ppm) and Chulsa (0.287 ppm). The lowest LC_{50} value was observed in population from Damdim (0.231 ppm). Resistance to alphamethrin ranged from 1.24 fold in the Chulsa strain to 6.63-fold in the Kalchini strain (Table 10).

Table 10. Relative toxicity values of Alphamethrin 10 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Alphamethrin 10 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	1.58	y = 5.558 x - 11.071	0.231	0.009	0.853 0.711	1.00
Chulsa	5.06	y = 3.391 x - 3.333	0.287	0.013	0.332 0.248	1.24
Nagrakata	0.73	y = 6.995 x - 15.151	0.759	0.006	0.814 0.708	3.29
Binnaguri	4.35	y = 2.893 x - 3.206	0.593	0.015	0.702 0.502	2.57
Dalgong	1.58	y = 3.423 x - 4.032	0.435	0.131	0.503 0.375	1.88
Kalchini	1.59	y = 4.748 x - 10.124	1.532	0.007	1.673 1.404	6.63

- Susceptible Damdim Population

Lamda cyhalothrin:
The Kalchini population recorded the highest LC_{50} value for lamda cyhalothrin (5.324 ppm) followed by the population from Nagrakata (3.175 ppm), Dalgong (2.405 ppm), Binnaguri (1.335 ppm), and Damdim (0.560 ppm). The lowest LC_{50} value was observed in the population from Chulsa (0.474 ppm). The Kalchini strain showed the highest resistance to λ- cyhalothrin (11.23-fold) followed by Nagrakata (6.70), Dalgong (5.07) and Binnaguri (2.82), The least resistance ratio was observed in the population of Damdim (1.18) (Table 11).

Table-11. Relative toxicity values of Lamda cyhalothrin 5 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.

Location - Sub- district in Dooars	Lamda cyhalothrin 5 EC					Resistance Factors (RF)
	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	
Damdim	4.45	y = 3.284 x - 4.025	0.560	0.012	0.646 0.487	1.18
Chulsa	0.93	y = 4.023 x - 5.765	0.474	[illegible]	0.532 0.422	1.00
Nagrakata	3.71	y = 2.038 x - 2.136	3.175	0.017	3.945 2.556	6.70
Binnaguri	2.01	y = 2.396 x -2.491	1.335	[illegible]	1.600 1.114	2.82
Dalgong	3.39	y = 2.129 x - 2.198	2.405	0.017	2.951 1.959	5.07
Kalchini	2.80	y = 2.441 x - 4.107	5.324	0.017	6.438 4.402	11.23

* Susceptible Chulsa Population

Fenpropathrin:
Populations from Chulsa, Nagrakata and Binnaguri showed more or less same LC_{50} value against fenpropathrin that ranged from 0.040 to 0.048 ppm, but Kalchini and Dalgong showed relatively higher LC_{50} (i.e. 0.064 ppm and 0.058 ppm respectively). The lowest LC_{50} value was observed in Damdim (i.e. 0.033 ppm). The values of relative resistance when calculated taking LC_{50} of fenpropathrin in Damdim as base showed that the resistance factor (RF) against susceptible ranged from 1.23 fold to 1.98 fold (Table 12).

Table 12. Relative toxicity values of Fenpropathrin 30 EC against *Helopeltis theivora* in different tea growing Sub district in Dooars.						
	Fenpropathrin 30 EC					
Location - Sub- district in Dooars	χ^2	Regression equation	LC_{50} (ppm)	S.E.	Fiducial limits (95%)	Resistance Factors (RF)
Damdim	2.70	$y = 1.007x + 3.473$	0.0328	0.040	0.051 0.020	1.00
Chulsa	1.105	$y = 0.976x + 3.382$	0.0454	0.043	0.074 0.028	1.38
Nagrakata	1.947	$y = 0.827x + 3.606$	0.0485	0.317	0.085 0.028	1.48
Binnaguri	4.19	$y = 0.842x + 3.647$	0.0404	0.044	0.069 0.024	1.23
Dalgong	2.02	$y = 1.044x + 3.155$	0.0582	0.067	0.093 0.036	1.77
Kalchini	7.53	$y = 0.796x + 3.557$	0.0648	0.045	0.112 0.037	1.98
• Susceptible Damdim Population						

The data on the dosage-mortality response of *H. theivora* collected from different subdistricts in the Dooars revealed that chi-square values indicated a good fit of probit responses in all the bioassays showing that there was no heterogeneity between the observed and expected responses.

The present study suggested that the LC_{50} value of *H.theivora* at Chulsa and Damdim (low pesticide applied area) were low as compared to other locations. Kalchini subdistrict (high pesticide applied area) registered the highest LC_{50} value for almost all tested insecticides except monocrotophos and thiomethoxam. This differential response to the insecticides in the populations of the Dooars could be due to indiscriminate use of pesticides. It is clear from the table that susceptibility was comparatively low in all subdistricts that used endosulfan.

Generally, it is accepted that field application rates of insecticides should at least be 20 fold or more of the LC_{50} value (determined through bioassay methods) to achieve satisfactory control of the pest in agriculture (Misra, 1989). Following this simple logic, the expected effective dosages of various insecticides were worked out in subdistrict wise and are presented in Table 13. Among the chosen insecticides the comparison of expected effective dosages of seven insecticides (endosulfan, Oxydemeton methyl, λ-cyhalothrin, quinalphos, imidaclopyrid, thiomethoxam and deltamethrin) based on their LC_{50} values with the recommended dosages revealed a pronounced shift in the level of susceptibility of *H. theivora* in all different subdistricts in the Dooars. In case of endosulfan when the computed expected dosages was compared with the recommended dosages of the insecticide, it was observed that 11.05 – 72.27 times more of the recommended dosage of endosulfan might be required to achieve desirable control of the pest. The change in susceptibility of *H. theivora* against deltamethrin in the order of 4.02, 8.75, 9.99, 20.75, 21.15 and 22.40 fold for Chulsa, Damdim, Binnaguri, Dalgong, Nagrakata, and Kalchini subdistrict populations respectively; similarly against imidaclopyrid the resistance levels were recorded as 13.61, 20.16, 20.32, 20.81, 24.77 and 26.66 fold for Damdim, Chulsa, and Dalgong, Nagrakata, Binnaguri and Kalchini regions respectively. In case of oxydemeton methyl, λ-cyhalothrin, quinalphos, thiomethoxam when compared with the recommended dosages, a 1.17 to 4.74, 1.06 to 11.93, 1.17 to 13.73 and 1.47 to 1.84 fold decrease in the susceptibility were evident. The usual recommended dose of synthetic pyrethroids (deltamethrin and λ-cyhalothrin), neonicotinoids (Imidaclopyrid, thiomethoxam), organophosphates (quinalphos) and organochlorines (endosulfan), however, was practically ineffective against this pest.

However, there was no major development of resistance in the case of monocrotophos (0.13 to 0.78 fold) and fenpropathrin (0.010 to 0.012 fold), which therefore held promise to being effective even at a lower dose than the recommended dose. In some subdistricts (Chulsa, Damdim, Nagrakata, Dalgong, Binnaguri) profenophos, cypermethrin and alphamethrin proved effective even at a lower dose than the recommended dose, but in Kalchini sub-district, these insecticides required 1.22 to 2.39 times more of than the recommended dosage for effective control of the pest (Table 13).

Table 13. Comparison of effective field dosage with recommended dosage of different insecticides against *Helopeltis theivora* in different tea growing sub districts in Dooars.							
Insecticide	Recom-mended dose (g a.i/ha)	Location					
		Nagrakata	Binnaguri	Damdim	Chalsa	Kalchini	Dalgong
		Expected effective dose (g a.i/ha) / Recommended dose					
Endosulfan 35 EC	350.00	40.45	42.89	24.90	12.33	72.26	43.55
Monocrotophos 37SL	370.00	0.77	0.20	0.13	0.32	0.70	0.78
Quinalphos 25 EC	250.00	2.49	2.80	1.17	0.42	13.73	1.86
Profenophos 50 EC	200.00	0.94	0.90	0.99	0.61	2.38	0.97
Oxydemeton Methyl 25 EC	250.00	1.98	1.92	1.17	1.24	4.74	3.73
Thiomethoxam 25 WG	50.00	1.84	1.78	1.47	1.52	1.84	1.71
Imidaclopyrid 17.8 SL	23.49	20.81	24.77	13.61	20.16	26.66	20.32
Deltamethrin 2.8 EC	5.60	21.15	9.99	8.57	4.02	22.40	20.75
Fenpropathrin 30 EC	75.00	0.01	0.01	0.01	0.01	0.01	0.012
Alphamethrin 10EC	20.00	0.61	0.47	0.19	0.23	1.23	0.35
Cypermethrin 25 EC	50.00	1.22	0.71	0.41	0.42	2.39	0.97
λ-cyhalothrin 5EC	10.00	7.11	2.99	1.25	1.06	11.93	5.39

A comparison of LC_{50} values of most insecticides used against the *H. theivora* population of the Dooars with other tea growing parts of the North East India

(Darjeeling and Assam) revealed that the insecticides were 2 to 350 times less toxic to the Dooars population than other regions (Bora *et al.*, 2007 and Bora and Gurusubramanian, 2007). *H. theivora* showed the lowest susceptibility to endosulfan in all tea growing subdistrict of the Dooars with high LC $_{50}$ value ranging from 269.744 ppm to 1580.77 ppm. The poor performance of endosulfan has also been reported by Roy *et al.* (2008), and on other insect pests by Singh and Deol (1998), Peter and Sundararajan (1990), Kalra *et al.*, (1997) and Singh *et al.* (2005), who observed the falling efficacy of this insecticide against the larvae of *Mythimna separata, Heliothis armigera, Plutella xylostella* and *Henosepilachna vigintioctopunctata* respectively. The present study suggests that the usual recommended doses of organochlorines (endosulfan), synthetic pyrethroids (deltamethrin and λ-cyhalothrin), neonicotinoids (imidaclopyrid) and organophosphates (quinalphos and oxydemeton methyl) were practically ineffective against *H. theivora* population of the Dooars. The change towards less susceptibility of *H. theivora* against endosulfan was found to be remarkable. Similar findings were reported against *H. theivora* population from Jorhat tea plantations of South Assam, India, where 1.54 – 82.85 fold increases in resistance caused control problems on tea (Bora and Gurusubramanian, 2007 and Bora *et al.*, 2008). According to Borbora and Biswas (1996), Sannigrahi and Talukdar (2003) and Roy *et al.* (2008), organochlorines (endosulfan), synthetic pyrethroids (deltamethrin) and organophosphates (quinalphos) were extensively used for tea pest management in Dooars for a long period of time whereas molecules like imidaclopyrid and λ-cyhalothrin were introduce in tea very recently. Such high levels of resistance to these compounds may be mediated through different mechanisms. Mechanisms of pyrethroid resistance in pests include reduced penetration (Armes *et al.*, 1992; Kranthi *et al.*, 2000 and 2001), decreased nerve sensitivity and enhanced metabolism (Ahmad and McCaffery, 1991). The absence of a common resistance mechanism that could confer cross-resistance between these compounds suggests that the use of the compounds in rotations or sequences for resistance management should be explored. However, there was no change in the case of monocrotophos, profenophos and fenpropathrin that may prove effective even at a lower dose than the recommended dose.

However, there is a great deal of variation in insecticide resistance from location to location within the Dooars tea ecosystems. The variations in insecticide susceptibility status from different geographical populations of pest were also reported by Kranth *et al.*, (2000), Chaturvedi (2004) and Fakrudin *et al.*, (2004) with Cotton Bollworm, *H. armigera* from Central and South Indian Cotton Ecosystem and Zhou *et al.*, (2000) from Northern China with same pest.

The resistance levels in Kalchini, Dalgong, Binnaguri and Nagrakata region is high due to heavy dependence on insecticides. The synthetic pyrethroids are being used widely in tea plantations, and their consumption is about 3-5 liters/ha in North East India (Gurusubramanian *et al.*, 2005), and a recent survey by Roy *et al.* (2008) revealed that on average 7.499 l/kg of insecticides were used per hectare per year in Dooars of which the organochlorine, organophosphate and carbamate (nonpyrethroid) accounted 73.5% and pyrethroid represent 36.6% during 1998 to 2004. Endosulfan, monocrotophos, deltamethrin and cypermethrin were extensively used in all the regions of the Dooars and it was noted that the requirement of synthetic pyrethorid gradually increased with every passing year in all subdistricts. The reasons are i) per hectare requirement was less (100 ml/ha), ii) having knockdown effect and iii) cost effectiveness. Against the tea mosquito bug, planters using insecticides as prophylactic, due to it being a wet season pest and their peak season (May-July) coinciding with the rainy season (June-July), caused the consumption of pesticides to increase, with about 8-16 applications per year of synthetic pyrethroids on top of other chemical applications.

This clearly explains that resistance levels were proportionate with the usage of pesticides. The study conducted by Forrester (1990) also clearly revealed that resistance levels rose when pyrethroids were used but fell significantly when they were withheld. Thus the pesticides were creating very high selection pressure for resistant genotypes. This suggests that indiscriminate use and heavy dependence on pesticides will further complicate the already worsened situation and this hints at aiming for insecticide resistance management strategies.

Thus, *H. theivora* insecticide-resistance issues in the Dooars are becoming ever more acute. Pyrethroid resistance is widespread in populations in almost all geographic populations. It is likely that few refugia of susceptible populations remain to dilute the build-up of resistant populations. Resistant management strategies appropriate for the region should be implemented immediately. These should include greater control over insecticide application and use. Unless this happens, the areas affected by resistant *H. theivora* populations will continue to increase and could ultimately result in the abandonment of tea growing in large areas of North east India. By some strategy, the allelic frequencies for major insecticide resistance genes need to be diluted.

Since the shift in level of susceptibility to insecticides was noticed in *H. theivora* certain measures can be initiated for combating and delaying the problem of resistance so that it does not assume unmanageable proportions. The measures may be: a) restricted use of deltamethrin, imidaclopyrid, quinalphos and endosulfan since their effective dose was higher than the recommended dose, in *H. theivora* prone areas, and reviewing of the recommended dose b) Judicious use of these insecticides only if there use is essential, d) no prophylactic spraying of chemicals, e) timing and frequency of applications should be such which does not create selection pressure, and f) altering of the insecticides in such a way that their modes of action are different. Further in-depth studies are needed to explore the possibility of determining the resistance level by using resistance enzyme studies and biotypes identification through molecular techniques besides the log dose probit assays.

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Detection of Insecticide Resistance in *Helicoverpa armigera* (Hubner) larvae collected from two locations in Madhya Pradesh, India

Abstract

The levels of resistance developed in a field stain of *Helicoverpa armigera* (Hubner) against five insecticides was assessed in two locations in Madhya Pradesh in laboratory conditions using a discriminating dose assay. The larvae of the test insect exhibited a wide spread resistance from 100 percent in cypermethrin and fenvalerate at the Khandwa location to above 50 percent at the Chhindwara location. High resistance of 46 percent to quinalphos was found in the field strains of Khandwa as compared to the Chhindwara strains (12.51%). The level of resistance against methomyl and Endosulfan was found to be lowest. Development of insecticide resistance in *H. armigera* is an important feature among the most commonly used group of insecticides.

Key words: Discriminating dose assay, *Helicoverpa armigera*, insecticides resistance

Introduction

Over the past one and a half decades, insecticide resistance in cotton pests has emerged as a key area of concern in cotton pest management in India. Most of the pest management difficulties in recent times, especially in the year of the bollworm outbreak, have been traced to insecticide resistance. The problem of resistance has rendered insecticides a less useful and reliable tool. If cotton pest management is to be effective, it is necessary to address the problem of resistance to insecticides and devise appropriate proactive management strategies to ensure that it does not continue to impair pest management in the field.

Helicoverpa armigera Hubner (Lepidoptera; Noctuidae), generally known as cotton bollworm or American bollworm, is the most dreaded pest of many agricultural crops worldwide. It has been recorded on more than 200 hosts in India (Pawar, 1998). It causes 24 to 68 percent losses of seed cotton yield at a national level (Vadodaria *et al* 1998). Cotton occupies only 5 percent of the total cultivable area in India but consumes more than 55 percent of the total insecticide used in the country (Puri, 1995). Dhingra *et al.* (1988) and Mc Caffery *et al.* (1989) first reported on the development of resistance by *H. armigera* to pyrethroids and attributed field control failures to the resistance. Subsequently, high levels of pyrethroid resistance were reported in several cotton and pulse growing regions of the country (Mehrotra and Phokela, 1992). Kranthi *et al* (2001) and Choudhary *et al.* (2004) also reported increased resistance of *H. armigera* to conventional insecticides such as methomyl, endosulfan and quinalphos in India.

Materials and methods

The experiment was carried out in a laboratory at the JNKVV Research Stations at Khandwa and Chhindwara during the 2003-04 crop season. The stock solutions of technical grade insectide (namely cypermethrin, fenvalerate, endosulfan, quinalphos and methomyl) were obtained from CICR, Nagpur. Eggs of *Helicoverpa armigera* were collected on a weekly basis from cotton fields. A discriminate dose assay was performed on the larvae at the third instar stage with 30-40 mg of body weight. The discriminate dose used was cypermethrin 0.1 μg, fenvalarate 0.2 μg, endosulfan 10 μg, quinalphos 0.75 μg and methomyl 1.2 μg/larva for the monitoring of resistance. An insecticide dose of 1μl was applied on thorasic dorsam of the third instar larvae by a Hamilton hand micro-applicator (Armes *et al*; 1996). Larvae treated with acetone only were used as a control. Larvae were held individually in 12-well tissue culture plates containing has the present program that now includes the use of glyphosate. Estimates of direct financial impact are $56-$78/hectare for additional herbicides alone in cotton (Bryant, 2007), with the additional expectation of as yet unquantified crop yield losses.

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INSECTICIDE PERSISTENCE AND RESIDUAL TOXICITY MONITORING IN TEA MOSQUITO BUG, *HELOPELTIS THEIVORA* WATERHOUSE (HETEROPTERA: HEMIPTERA: MIRIDAE) IN DOOARS, WEST BENGAL

ABSTRACT. Persistence (PT values) and residual toxicity (LT_{50} values) of imidacloprid 17.5 SL, thiomethoxam 25 WG, deltamethrin 2.8 EC, alphamethrin 10 EC, cypermethrin 25 EC, λ-cyhalothrin 5 EC, fenpropathrin 30 EC, monocrotophos 37 SL, oxydemeton methyl 25 EC, quinalphos 25 EC and endosulfan 35 EC against *Helopeltis theivora* Waterhouse were studied by exposing field collected tea mosquito bug adults for 24 hours to TV1 tea leaves treated with three concentrations, (0.05, 0.10 and 0.25 percent) for a period of 4-28 days by following probit analysis and product (PT) of average residual toxicity methods.

Persistence of neonicotinoids (thiomethoxam and imidacloprid), synthetic pyrethroids (alphamethrin, deltamethrin, cypermethrin, λ-cyhalothrin, fenpropathrin) and monocrotophos (organophosphate) last for a longer duration (18 - 28 days) with increased PT values (938.25 – 1423.13) at 0.25% concentration whereas, oxydemetonmethyl, endosulfan, and quinalphos persisted for a relatively short duration (7-11 days) with lesser PT values (307.00 – 513.87). But at a lower concentration (0.05%), recommended dose by TRA, the thiomethoxam, imidacloprid, λ-cyhalothrin, fenpropathrin and monocrotophos exhibited persistence toxicityfor 14-16 days along with higher PT values (689.50 – 806.00) while persistence was 7-11 days with 124.00 – 573.37 PT values in alphamethrin, deltamethrin, cypermethrin, oxydemetonmethyl, endosulfan, and quinalphos respectively.

Higher LT_{50} values of 10.59 – 11.27 days were observed at 0.25% concentration of thiomethoxam, λ-cyhalothrin, imidacloprid and fenpropathrin followed by 8.29 – 9.04 days in deltametrhrin, alphamethrin and monocrotophos, moderately by 4.11 – 6.99 days in cypermethrin and oxydemetonmethyl and least by 2.92 – 3.02 days in endosulfan and quinalphos. Nevertheless, lesser LT_{50} values (1.11 – 4.98 days) were noted in the recommended dose of λ-cyhalothrin, imidacloprid, alphamethrin, deltamethrin, cypermethrin, oxydemetonmethyl, endosulfan, and quinalphos and those insecticides are commonly used in tea. Thus, for combating and delaying the problem of resistance either the TRA must reassess the dose or the planters must change over their strategies in the light of above findings.

Key words: Insecticide, LT_{50}, Persistence, Residual toxicity, *Helopeltis theivora*

INTRODUCTION. Tea mosquito bug (*Helopeltis theivora* Waterhouse) is considered as one of the major pests of tea in Assam, Dooars, Terai and Darjeeling because it attacks only to the young shoots that is the actual crop of tea. Many Tocklai released clones, garden released clones; and seed jats are susceptible to *H. theivora* attack at varying degrees. 80% of the tea plantations are being affected by this pest alone which in turn is reducing the productivity 10-50% (Gurusubramanian and Bora, 2007). The nymphs and adults of *H. theivora* suck the sap of the young leaves, buds and tender stems, and 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 attacks, bushes virtually cease to form shoots, and the affected area may not flush for weeks together. In addition, due to oviposition, the tender stems develop cracks and over-callousing which led to blockage of vascular bundles thereby affecting the physiology causing stunted growth and sometimes die-back of the stems (Rahman *et al.*, 2005).

In North East India, Tocklai Experimental Station, Tea Research Association (TRA), Jorhat, Assam, India is the premier institute to test and certify the plant protection chemicals for use in tea plantations. Earlier TRA recommended different pesticides [endosulfan, quinalphos, phosphomidon, phosalone, acephate, dimethoate, chlorpyrifos, monocrotophos, oxydemetonmethyl, λ-cyhalothrin, β-cyfluthrin, ethofenprox, cartap hydrochloride, alphamethrin, cypermethrin, deltamethrin, profenophos, thiomethoxam, imidacloprid, dicofol, ethion, propargite, fenazaquin, sulfur and neem formulations] for controlling tea pests (Anonymous, 1993, 1999). During the last several decades, the control of pests, diseases and weeds in tea fields is predominantly by the use of synthetic chemicals. From the recent survey in tea gardens of Dooars, it was observed that synthetic pesticides constituted 85% of the total pesticides used, wherein, acaricides and insecticides accounted for 25% (3.60 l/ha) and 60% (8.46 l/ha) respectively. 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, 2003). The latest surveillance report of the European Community (EC) indicated that the presence of residues in Indian tea is a cause of great concern. Assam and Darjeeling teas continue to record high numbers of positive values for organochlorine and synthetic pyrethroids pesticide residues, very few of which exceeded the EU maximum residue level. Thus, use of DDT (10.4 to 47.1%), endosulfan (41.1 to 98.0%), dicofol (0.0 - 82.4%) and cypermethrin (6.0 - 45.1) remain comparatively high during 2001 to 2004 in different tea growing areas of the North-East states of India (Anonymous, 2001, 2002, 2003 and 2004). Due to regular application of insecticides, *H. theivora* has been realized as a menace year round and had a noticeable decrease in susceptibility to different classes of insecticides (Sarker and Mukhopadhyay, 2003, 2006, 2006a; Rahman *et al.*, 2006 and 2007; Sarmah *et al.*, 2006; Bora *et al.*, 2007, 2007a and 2008; Gurusubramanian *et al.*, 2008).

Thus, the tea mosquito bug constituted a major constraint in obtaining maximum tea yield. It was, therefore, considered imperative to assess residual toxicity of some commonly used insecticides at variable concentrations for their efficacy against *H. theivora* for economic and effective management.

MATERIALS AND METHODS. The TV 1 clones were 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 (0.05, 0.1 and 0.25 percent) for evaluating the persistence of residual toxicity against *H. theivora*. Each treatment contained 150 bushes with three replications. One plot was not treated and used as an 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 a 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 27 ± 2°C in a culture room. Ten 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 24 hours after the release of *H. theivora* adults. Moribund insects were counted as dead. The same procedure was repeated every day until insect mortality declined to ten percent (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), which used the product (PT) of average residual toxicity (T) and the period in days (P) for which the toxicity persisted. 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.

$$T = \frac{\text{Sum of percent mortality of tea mosquito bug on different days}}{\text{No. of observations}}$$

The LT_{50} values for different concentrations of insecticides were calculated by probit analysis (Busvine, 1971; Finney 1973) for all of the 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, the "t" value for testing the difference between the log LT_{50} values of endosulfan 0.05% and 0.25% was calculated as follows.

$$\text{Sum SEM} = ?\,(\text{SEM of endosulfan } 0.25\,\%)^2 + (\text{SEM of endosulfan } 0.05\,\%)^2$$

$$\frac{\log LT_{50}\ \text{value of endosulfan } 0.25\% - \log LT_{50}\ \text{value of endosulfan } 0.05\%}{\text{SumSEM}}$$

The table value of "t" is 2.0369 and 2.7385 at 5 and 1 percent level respectively.

Table 1. PT values and order of relative efficacy of different insecticides at variable concentration against adults of *Helopeltis theivora* Waterhouse

Insecticide	Concentration (%)	Period (P) (Days)	Percent average residual toxicity (T)	PT value	Order of relative efficacy (ORE)
Endosulfan 35EC	0.05	4	31.000	124.00	33
	0.10	6	45.800	274.80	29
	0.25	10	43.500	435.00	25
Quinalphos 25EC	0.05	5	36.750	183.75	32
	0.10	6	39.333	236.00	30
	0.25	7	43.857	307.00	27
Monocrotophos 37SL	0.05	14	49.25	689.50	20
	0.10	15	51.500	772.50	17
	0.25	18	52.125	938.25	11
Oxydemeton methyl 25EC	0.05	6	38.00	228.00	31
	0.10	7	39.333	275.33	28
	0.25	11	46.714	513.87	24
Deltamethrin 2.8EC	0.05	11	50.500	555.50	23
	0.10	17	51.500	875.50	12
	0.25	23	57.375	1319.63	4
Cypermethrin 25EC	0.05	8	49.142	393.14	26
	0.10	14	49.142	688.00	21
	0.25	18	56.000	1008.00	9
Alphamethrin 10EC	0.05	11	52.125	573.37	22
	0.10	14	62.000	868.00	13
	0.25	20	56.000	1120.08	10
Fenpropathrin 30EC	0.05	16	49.625	794.00	16
	0.10	19	61.375	1166.13	8
	0.25	28	49.875	1396.50	3
λ-cyhalothrin 5EC	0.05	14	49.625	694.75	19
	0.10	16	51.125	818.00	14
	0.25	21	61.125	1283.63	5
Imidacloprid 17.5SL	0.05	15	48.750	731.25	18
	0.10	20	58.375	1167.50	7
	0.25	24	59.250	1422.00	2
Thiomethoxam 25WG	0.05	16	50.735	806.00	15
	0.10	21	57.000	1197.00	6
	0.25	23	61.175	1423.13	1

Mean of three observation

RESULTS AND DISCUSSION. The duration of effectiveness (persistent toxicity) and residual toxicity of eleven commonly used insecticides under four different classes (organochlorine (endosulfan), organophosphates (quinalphos, monocrotophos and oxydemetonmethyl), synthetic pyrethroids (deltamethrin, cypermethrin, alphamethrin, fenpropathrin and λ-cyhalothrin) and neonicotinoids (imidacloprid and thiomethoxam)) were evaluated on the basis of PT (persistence) (Table 1) and LT_{50} (residual toxicity) (Table 2) values respectively against adults of *H. theivora* when exposed to tea leaves (TV1) treated with three different concentrations (0.05, 0.10 and 0.25%) as foliar sprays. It was evident from Table 1 that the higher concentration (0.25%) of fenpropathrin (28 days), imidacloprid (24 days), thiomethoxam (23 days), deltamethrin (23 days), λ-cyhalothrin (21 days), alphamethrin (20 days), cypermethrin (18 days) and monocrotophos (18 days) persisted for a longer duration (18 - 28 days) against *H. theivora*. Imidacloprid and thiomethoxam at 0.10% concentration caused tea mosquito bug mortality for 20 and 21 days respectively. Oxydemetonmethyl, endosulfan, and quinalphos at 0.25% persisted for a relatively short duration, 11, 10 and 7 days respectively (Table 1). Lower concentration of all the chosen insecticides (0.05%) exhibited persistence toxicity for4-8 days in endosufan, quinalphos, oxydemetonmethyl and cypermethrin and 11-15 days in alphamethrin, deltamethrin, λ-cyhalothrin, monocrotophos, fenpropathrin, thiomethoxam and imidacloprid. As a whole, it was observed that the toxicity of organochlorine, organophosphates, synthetic pyrethroids and neonicotinoids persisted for 4-10 days, 5-18 days, 8-28 days and 15-24 days respectively (Table 1). Among the synthetic pyrethroids, shorter persistence duration (8 - 11 days) was recorded in the lower concentration (0.05%) of cypermethrin, alphamethrin and deltamethrin.

Table: 2. Relative efficacy of different insecticides against Tea mosquito bug, *Helopeltis theivora* Waterhouse.

Insecticide	Concentration (%)	X^2	Regression equation	LT_{50} (Days)	Fiducial limit (Days)	Relative residual toxicity	ORE
Endosulfan 35EC	0.05	1.68	y = 12.505 - 2.187x	1.115	1.521 - 0.813	1.00	33
	0.10	4.07	y = 12.505 - 2.187x	2.705	3.497 - 2.148	2.42	28
	0.25	3.02	y = 14.627 - 2.766x	3.077	3.617 - 2.533	2.71	26
Quinalphos 25EC	0.05	1.50	y = 12.117 - 2.205x	1.689	2.198 - 1.298	1.51	32
	0.10	1.69	y = 13.056 - 2.403x	2.248	2.724 - 1.856	2.01	30
	0.25	3.47	y = 16.217 - 3.257x	2.925	3.385 - 2.544	2.62	27
Monocrotophos 37SL	0.05	3.94	y = 13.424 - 2.257x	5.502	6.625 - 4.569	4.93	19
	0.10	5.04	y = 12.893 - 2.071x	6.468	7.936 - 5.303	5.80	16
	0.25	4.69	y = 15.566 - 2.696x	8.292	9.729 - 7.067	7.43	10
Oxydemeton methyl 25EC	0.05	0.09	y = 13.444 - 2.567x	1.946	2.376 - 1.595	1.74	31
	0.10	1.05	y = 12.826 - 2.332x	2.269	2.760 - 1.862	2.03	29
	0.25	4.55	y = 14.274 - 2.569x	4.112	4.908 - 3.449	3.69	24
Deltamethrin 2.8EC	0.05	0.72	y = 15.662 - 2.930x	4.348	5.160 - 3.663	3.90	23
	0.10	0.85	y = 13.942 - 2.387x	5.667	6.427 - 4.745	5.08	17
	0.25	2.15	y = 14.761 - 2.467x	9.046	10.811 - 7.570	8.11	7
Cypermethrin 25EC	0.05	3.53	y = 13.253 - 2.375x	3.532	4.242 - 2.940	3.17	25
	0.10	4.85	y = 11.617 - 1.762x	5.633	7.169 - 4.426	5.05	18
	0.25	4.84	y = 13.766 - 2.283x	6.916	8.314 - 5.745	6.20	15
Alphamethrin 10EC	0.05	1.97	y = 14.769 - 2.674x	4.564	5.319 - 3.908	4.09	22
	0.10	6.59	y = 15.084 - 2.595x	7.686	9.091 - 6.498	6.89	11
	0.25	4.50	y = 13.714 - 2.207x	8.881	10.714 - 7.361	7.96	8
Fenpropathrin 30EC	0.05	3.50	y = 15.110 - 2.367x	6.979	8.195 - 5.944	6.26	14
	0.10	4.63	y = 18.808 - 3.524x	8.287	9.436 - 7.257	7.43	9
	0.25	2.10	y = 14.707 - 2.412x	10.599	12.654 - 8.878	9.50	4
λ-Cyhalothrin 5EC	0.05	2.86	y = 14.494 - 2.508x	4.982	5.872 - 4.226	4.47	20
	0.10	3.94	y = 14.908 - 2.566x	7.263	8.553 - 6.168	6.51	12
	0.25	4.71	y = 10.762 - 2.571x	10.762	12.778 - 9.099	9.65	2
Imidacloprid 17.5SL	0.05	5.86	y = 11.054 - 1.648x	4.723	5.990 - 3.719	4.23	21
	0.10	6.70	y = 11.479 - 1.628x	9.521	12.213 - 7.422	8.53	5
	0.25	5.15	y = 12.433 - 1.846x	10.608	13.281 - 8.473	9.51	3
Thiomethoxam 25WG	0.05	7.73	y = 14.609 - 2.495x	7.106	8.397 - 6.013	6.37	13
	0.10	3.30	y = 13.523 - 2.146x	9.371	11.381 - 7.716	8.40	6
	0.25	6.19	y = 14.331 - 2.278x	11.272	13.597 - 9.344	10.10	1

Mean of three observations, Y = Probit kill, ORE = Order of relative efficacy, X = log (time x 1000)

Higher percent average residual toxicity (T) was observed in synthetic pyrethroids (49.14 – 62.00%) followed by neonicotinoids (48.75 – 61.17%), organophosphates (36.75 – 52.12%) and finally by organochlorine (31.0 - 45.8%). Average residual toxicity was noted between 49.14% and 62.0% in monocrotophos, cypermethrin, deltamethrin, alphamethrin, fenpropathrin, λ-cyhalothrin, imidacloprid and thiomethoxam whereas in endosulfan, quinalphos and oxydemetonmethyl, it was between 31.0% and 46.71% (Table 1).

PT values (persistence of insecticides) ranged between 124 and 435, 183.75 and 938.25, 393.14 and 1396.50 and 731.25 and 1423.13 for organochlorine, organophosphate, synthetic pyrethroids and neonicotinoids respectively. The highest PT was observed in 0.25% thiomethoxam (1423.13) whereas endosulfan at 0.05% registered lowest PT value. On the basis of PT values the order of relative efficacy of the chosen eleven insecticides is shown in Table 1. Based on PT values, it was noticed that thiomethoxam, imidacloprid, fenpropathrin, deltamethrin, λ-cyhalothrin, alphamethrin, cypermethrin and monocrotophos persisted for a longer period and effectively check the tea mosquito population (PT value - 1008.00 – 1423.13) at 0.25% concentration, and oxydemetonmethyl, endosufan and quinalphos showed a shorter duration and lesser average residual toxicity (PT value - 307.00 - 513.87). However, at 0.05% concentration the order of persistence of insecticides (PT value: 124.00 – 806.00) was thiomethoxam, fenpropathrin, imidacloprid, λ-cyhalothrin, monocrotophos, alphamethrin, deltamethrin, cypermethrin, oxydemetonmethyl, quinalphos and endosulfan (Table 1).

The LT_{50} value increased with the concentration as well as varied with the class of insecticides that showed the relative efficacy of the selected eleven insecticides against tea mosquito bug. Neonicotinoids (4.72 – 11.27 days) and synthetic pyrethroids (3.53 – 10.76 days) registered relatively higher LT_{50} value than organophosphate (1.68 – 8.29) and organochlorine (1.11 – 3.02). Higher LT_{50} values of 10.59 – 11.27 days were observed in thiomethoxam, λ-cyhalothrin, imidacloprid and fenpropathrin followed by 8.29 – 9.04 days in deltamethrin, alphamethrin and monocrotophos, moderately by 4.11 – 6.99 days in cypermethrin and oxydemetonmethyl and least by 2.92 – 3.02 days in endosulfan and quinalphos (Table 2) at 0.25% concentration. Further, the order of LT_{50} values changed at the lower concentration (0.05%) of the insecticides. The relative residual toxicity of different insecticides was calculated by considering the LT_{50} value of these insecticides at three different concentrations and accordingly the order of relative efficacy was assigned accordingly. It may be highlighted here that the relative residual toxicity of monocrotophos(0.25%), thiomethoxam (0.05%), fenpropathrin (0.05%), λ-cyhalothrin (0.05%), imidacloprid (0.05%), alphamethrin (0.05%), deltamethrin (0.05%), oxydemetonmethyl (0.25%), cypermethrin (0.05%), endosulfan (0.25%) and quinalphos(0.25%) at the recommended concentrations by TRA was 7.43,6.37, 6.26, 4.47, 4.23, 4.09, 3.90, 3.69, 3.17, 2.71 and 2.62 times higher than the residual toxicity of endosulfan at 0.05% against *H. theivora* (Table 2).

Based on "t" values calculated for testing the difference between $\log_{50}$ values of different concentrations of eleven insecticides against *H. theivora* (Table 3), thiomethoxam, imidacloprid, fenpropathrin and λ-cyhalothrin were significantly superior in imparting higher residual toxicity *vis-à-vis* other insecticides used in this study. Among the three concentrations, 0.25% was significantly in high order. Thiomethoxam proved to be highly toxic to *H. theivora* as evidenced by significant difference in "t" values with respect to organophosphates and organochlorine. Toxicity of imidacloprid, λ-cyhalothrin, and fenpropathrin were at par. Endosulfan and quinalphos manifested low residual toxicity (Table 3).

Table 3: 't' values of three concentrations of different insecticides assessed against *Helopeltis theivora* Waterhouse

[illegible]	[illegible]

The value of 't' is above 2.0357 significant at 5.0 percent level and above 2.7385 significant at 1 percent level.

It has been estimated that tea industry in India harbour about 300 species of pests and therefore, extreme care must be exercised before a pesticide is introduced to tea for pest control (Gurusubramanian *et al.*, 2005) to avoid residue build-up. Organophosphate, organochlorine, carbamate, and synthetic pyrethroid insecticides have been in use on tea in North-East India for the past 100 years. Much of the efficacy and sustainability of these groups of insecticides in tea mosquito bug management would depend on the susceptibility. Endosulfan, quinalphos, cypermethrin, alphamethrin, deltamethrin and oxydemetonmethyl at recommended dose persisted for shorter durations of 7-11 days in the Dooars area. In this situation, either the planters may reduce the frequency of the above mentioned chemicals or TRA may reassess the dose of cypermethrin, alphamethrin, and deltamethrin to 0.10%, which showed 14-17 days of persistence with increased PT values (688.0 – 875.0) (Table 1) and LT_{50} values (5.63 – 7.68 days) (Table 2), instead 0.05%. Earlier, Rahman *et al* (2005) observed the PT and LT_{50} values of different insecticides against tea mosquito bug in Jorhat, Assam and found the persistence of 10 – 23 days with 300 – 1771 PT values. Variation in relative toxicity was observed between male and female populations of Jorhat (Gurusubramanian and Bora, 2007) and Darjeeling (Bora *et al.*, 2007) and among the populations of *H. theivora* collected from different sub-districts of Dooars (Rahman *et al.*, 2005) due to selection pressure by insecticides. A comparison of expected effective doses of different classes of insecticides against tea mosquito bug collected from Jorhat, Assam based on their LC_{50} values with recommended dose revealed a pronounced shift in the level of susceptibility of *H. theivora.* The recommended dose of synthetic pyrethroids (fenpropathrin, cypermethrin, λ-cyhalothrin, and deltamethrin), organophosphates (profenophos, dimethoate, oxydemetonmethyl, phosalone, and quinalphos) neonicotinoids (thiomethoxam and imidacloprid), and organochlorine (endosulfan), however, was practically ineffective against this pest (Bora *et al.*, 2008; Gurusubramanian *et al.*, 2008). The presence of various oxido-reductase enzymes in the salivary and mid gut along with the basic hydrolyzing enzymes enable *H. theivora* to be one of the most destructive pests of tea by depredating the young leaves and growing shoots of tea (Sarker and Mukhopadhyay, 2006). In addition, qualitative and quantitative changes were recorded in the enzyme patterns of the tea mosquito bug indicating a higher tolerance/resistance status due to the formation of greater amounts of esterases (Sarker and Mukhopadhyay, 2003), glutathione S-transferase and acetylcholinesterase (Sarker and Mukhopadhyay, 2006a). One of the main reasons for higher tolerance or resistance by tea mosquito bug to different pesticides was due to mixing of incompatible insecticides with acaricides to combat mixed infestation of tea mosquito bug and red spider mite which, not only decreased the insecticide toxicity but also shifted the level of relative toxicity (Rahman *et al.*, 2005).

Because of a lesser requirement (100 ml/ha) having knock down effect and cost effectiveness, the synthetic pyrethroids are being used widely in tea plantations, and their consumption is about 3-5 litres/ha (Gurusubramanian *et al.* 2005). Planters using insecticides as a prophylactic against the tea mosquito bug, due to it being the wet season pest and their peak season (May-July) coinciding with the rainy season (June-July), caused the consumption of pesticides to increase, with about 8-16 applications per year of synthetic pyrethroids on top of other chemical applications. Hence, irrespective of the group to which insecticides belong, evidence of the development of resistance to synthetic pyrethroids, organophosphates, organochlorines and neonicotinoids has been experimentally proved in the tea mosquito populations of Dooars, West Bengal and Jorhat, Assam (Rahman *et al.*, 2005; Sarker and Mukhopadhyay, 2006a; Gurusubramanian and Bora, 2007; Bora *et al.*, 2008; Gurusubramanian *et al.*, 2008).

In the recent years, it has become a major concern to the tea industry as the importing countries are imposing stringent restrictions for acceptability of the "made" tea due to pesticide residues. Changes in pest management tactics are resulting from environmental and human safety concerns, development of insect pest susceptibility change against a few insecticides is now a reality, and increases in pesticide cost and availability. Thus, before spraying any chemicals, the tea planters must i)consider the impact of pesticides on non target organisms, human health, wild life habitat and environment and ii) adopt IPM strategies to reduce the pesticide load to produce residue free tea, increase the exports and meet out the consumers' demand. Potential cultural practices for conserving and enhancing the natural enemies need to be integrated with our current crop management strategies for developing sustainable tea crop protection. Therefore, the following integrated resistant management practices must be followed for combating and delaying the problem of resistance so that it does not assume unmanageable proportions:

1. After infestation remove all the infested shoots to rejuvenate the shoot growth as well as to remove the laden eggs before spraying.
2. In severe infestations, LOS (level of skiff) operations should be followed to minimize the infestation of the next generation.
3. Shade status neither should be overshaded nor unshaded.
4. Alternate hosts must be eliminated (Guava (*Psidium guajava),* oak (*Quercus* spp.), melastoma (*Melastoma* sp.), Thoroughwort (*Eupatorium* sp.), fragrant thoroughwort (*Eupatorium odoratum),*Dayflower (*Commelina* spp.), Sesbania (*Sesbania cannibina),* Jackfruit (*Artrocarpus heterophylla*), Bortengeshi (*Oxalis acetocello*), Ornamental jasmine (*Gardenia jesminoid*), Mulberry (*Morus alba*), Kadam (*Enthocephalus cadamba*), Jamun (*Eugenia jambolana*), Boal (*Ehretia acuminata*), Mikania (*Mikania micrantha*), *Acacia moniliformis* and *Premna latifolia).*
5. By following proper monitoring detect the *H. theivora* at an early stage and manually collect the adults (Morning – 06.30 h – 08.30 h; Evening – 16.00 h – 18.00 h) by use of a trained labour force.
6. Unpruned sections must be monitored regularly during December-February and proper care should be taken to kill the residual population.
7. Care needs to be adopted in skiffed and pruned sections during bud breaking (March – April) which are prone to *H. theivora* attack.
8. Underperformance of spraying equipments should be avoided.
9. Toxicity persistence of different insecticides at recommended dose falls between 7 – 16 days. Hence interval between two subsequent rounds must be 7-15 days.

10. Avoid spraying of endosulfan, quinalphos and cypermethrin in severely infested sections due to shorter persistence.
11. Selection and usage of chemicals, assurance of the quality, required spraying fluid, and trained man power for overall good coverage.
12. Under dense tea bush population care must be taken for good and uniform coverage of chemicals.
13. Incompatible chemicals must be avoided in tank-mix formulations in severely affected sections.
14. Recommended dose of chemicals should be followed and avoid sub- and supra- lethal doses to minimize the chances of susceptibility change in *H. theivora* populations.
15. Avoid spraying during hot sunny days which degrade the chemical activity, cause phytotoxicity and has no direct contact with insects. Hence, spray in the early morning and late afternoon.
16. With prior knowledge about *H. theivora* infestation patterns, mark the infested bushes in the early stages and go for spot application to check the pest as well as to reduce the chemical load instead of blanket application.
17. Conserve and preserve the natural enemies present in the natural tea ecosystem by minimizing the load of chemicals for their natural regulation.

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Application of bi-PASA and development of PCR-REN for detection of point mutation 980A>G in *AChE* gene of Colorado Potato Beetle in South Ural's local population

Key words: Colorado potato beetle, resistance, acetylcholinesterase, mutation, PCR, South Urals.

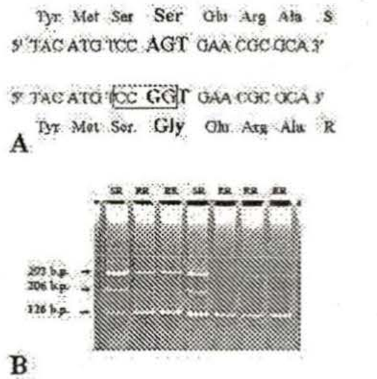

Fig. 1. Nucleotide mutation in *AChE* gene of Colorado potato beetle.
A - comparison of nucleotide and amino acid sequences for *AChE* gene fragment (Zhu et al., 1996). S - wild type of allele, susceptibility to the organophosphorus insecticides. R - mutant allele, resistance to organophosphorus insecticides. Mutant triplet is bold, *Msp*I restriction site is marked by frame.
B – analysis of the bi-PASA reaction of the *AChE* mutation in Colorado potato beetle. RR – homozygous resistant genotypes, SR – heterozygous genotypes. Allele-nonspecific fragment (293 b.p.) – product of nonspecific PCR with two outer allele-nonspecific primers 3 and 4.

Acetylcholinesterase *(AChE)* is the target site of inhibition by organophosphorus and carbamate insecticides in insects (Smallman and Mansing, 1969). In the Colorado potato beetle *AChE* insensitivity and resistance to azinphos-methyl is associated with a transition 980A>G and serine to glycine amino acid substitution (Fig. 1, A) in its active center and with the insensitivity of this *AChE* form to the organophosphorus insecticides action (Zhu, Clark, 1995).

For detection of this point mutation in *AChE* of Colorado potato beetles, we have used bi-directional PCR amplification of specific alleles (bi-PASA) (Clark et al., 2001). This method allows the separation of homozygous resistant RR or susceptible SS genotypes and heterozygous genotype SR. The Bi-PASA reaction contains four separate primers. The two inner primers (No 1 and 2 in Table 1) are allele-specific at the mutation site. The two outer primers (No 3 and 4) are allele-nonspecific. Under the following PCR and electrophoresis, susceptible homozygotes (wild type, SS) are determining by the presence of 206 b.p. fragment, resistant homozygotes (RR genotype) - by the presence of 126 b.p. fragment, and heterozygous genotype (SR) by the presence of both 206- and 126 b.p. fragments (Figure. 1b). Primers for bi-PASA realization were synthesized in Sintol (Russia, Moscow) by (Clark et al., 2001).

The PCR was run for 35 cycles, each consisting of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 1 minute after initial denaturation of the DNA template at 94°C for 1 minute. A final extension at 72°C lasts for 5 minutes (Clark et al., 2001). Following PCR, the bi-PASA product were resolved by size using 5% PAAG electrophoresis in lxTAE buffer and visualized by ethidium bromide staining.