

Resistance to Insecticides in Field-Collected Populations of Tea Mosquito Bug (*Helopeltis theivora* Waterhouse) From the Doars (North Bengal, India) Tea Cultivations

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ABSTRACT

Dose (LC₅₀) and time-mortality response (LT₅₀) parameters are used to compare resistance levels of field-collected populations of the tea mosquito bug, *Helopeltis theivora* (Waterhouse) from Kalchini tea plantations of the Doars, North Bengal, India with the reference strain collected from Darjeeling foothills against eleven insecticides by following leaf-dip technique. Tea plantation of Darjeeling is largely organic, that had never used synthetic pesticides in its history, whereas Kalchini plantation is conventional that routinely uses synthetic pesticides. The field-collected Kalchini strain of *H. theivora* showed a high Resistance Ratio level (> 10 folds) both in LC₅₀ (20.00 - 17564.11 folds) and LT₅₀ (20.20 - 45.99 folds) parameters for the tested insecticides. This is the first report on the reduced susceptibility of *H. theivora* populations to eleven insecticides (one organochlorine, three organophosphorus, two neonicotinoids and five synthetic pyrethroids) from Kalchini tea cultivations in the Northeastern Doars region of India.

Key words: Insecticide resistance, Tea cultivations, Organochlorine, Organophosphates, Synthetic pyrethroids, Neonicotinoids, *Helopeltis theivora*

INTRODUCTION

The tea mosquito bug *Helopeltis theivora* (Waterhouse) (Heteroptera: Miridae) is one of the major sucking pests of tea throughout India. More than 80% of tea cultivation area is being affected by nymphs and adults of *H. theivora*, leading to dark brown shrunken spots in young foliage, no shoot formation, delayed flushing, stunted growth and die back of stems (Rahman *et al.* 2005 and 2007; Roy *et al.* 2008a and 2009a). There exist many reports about *H. theivora* causing significant losses (10-50%) in tea cultivations, when mainly broad-spectrum pesticides-which eliminated natural enemy populations-were used (e. g. Gurusubramanian *et al.* 2009; Hazarika *et al.* 2009).

A major problem in the control of *H. theivora* is its capability to develop resistance quickly to frequently used insecticides (Gurusubramanian and Bora 2008). Their high reproductive potential and numerous annual generations, combined with continued and repeated use of a variety of active substances of insecticides on tea cultivations for many years (7.5 L h⁻¹), have limited the control of *H. theivora* populations by natural

enemies and led to resurgences in bug populations with development of resistance and consequently control failure (Roy *et al.* 2009b,c,d). Such failures are already known in case of organochlorine (OC), organophosphorus (OP) and synthetic pyrethroid (SP) insecticides and more recently for the newer compound such as neonicotinoids (NN) (e.g. Gurusubramanian and Bora 2007; Gurusubramanian *et al.* 2008; Roy *et al.* 2008a, b, c, d).

OC, OP and SP compounds are highly effective broad-spectrum insecticides. Insecticides like endosulfan, monocrotophos, quinalphos, deltamethrin and cypermethrin are being extensively used in the Dooars tea cultivations, West Bengal for the control of tea pests (Roy *et al.* 2008b). Instead of practicing integrated pest management in tea cultivations of the Dooars, producers prefer to use broad-spectrum insecticides at 'no threshold level', which are supposed to be effective against pest insects such as caterpillar pests, thrips, jassids, scale insects and aphids. Thus, *H. theivora* populations are frequently exposed to above mentioned broad-spectrum insecticides. In recent years, control failures have led the growers to question the efficacy of registered insecticides. Although there are bioassay results about the decreasing efficacy of some insecticides against *H. theivora* in tea cultivations of the Dooars, the exact situation in respect to OC, OP SP and NN resistances in *H. theivora* is unknown (Roy *et al.* 2008a,e). The present study focused on resistance of *H. theivora* populations collected from tea cultivations of Kalchini area of the Dooars to the commonly used OC, OP, SP and NN compounds.

MATERIAL AND METHODS

Tea mosquito bug populations: The susceptible strain (SS) of *H. theivora* was originally collected in 2007 from Makibari Tea Estate, Darjeeling (an organic tea plantation that had never used synthetic pesticides in its history) (Gurusubramanian and Bora 2008). Field populations were collected from, Chuapara commercial tea estate in Kalchini area (26° 16' and 27° 0' N, 88° 4' and 89° 53' E) of District Jalpaiguri (Dooars), West Bengal during 2007. They were cultured in separate cages on young tea foliage (variety - TV1, named after Tocklai vegetative clone) in a BOD at 27 ± 2°C, 80 % r.h. and a photoperiod of 16L/8D. Synchronized cultures of *H. theivora* were produced from stock populations and the SS population for use in bioassays. Adults were transferred from stock populations to young tea leaves in small plastic containers.

Insecticidal compounds belonging to OC, OP, SP and NN groups used in this study along with their details, regarding trade name, formulations, manufacturers and field recommended dose (ppm) are summarized in Table 1.

Dose and time mortality bioassays

Commercially available insecticides were diluted with demineralized water in five selected concentrations, expressed in parts per million (ppm).

Adult tea mosquito bugs (*H. theivora*) were exposed initially to a wide range of concentrations, and on the basis of the resultant mortality a series of concentrations with narrower ranges were further tested. The second series was determined on the

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basis of the concentrations causing insect mortality above 20% and below 100%.

Toxicity assays were conducted using the standard 'leaf dipped method' recommended by the Insecticide Resistance Action Committee (IRAC) of GIFAP (Anonymous 1990). Healthy shoots (three leaves and a bud) of TV1 clone were collected from an experimental plot, washed thoroughly with distilled water and air-dried. Fifteen tea shoots for each treatment were dipped for 5 seconds in the various insecticide solutions to ensure complete wetting, their bases were wrapped with wet cotton wool, and then they were kept in glass vials (2 cm diameter x 2 cm length) containing water. The vials were held under a ceiling fan for 15 min to allow drying of the emulsion, then placed in a Petri dish (10 cm diameter) lined with blotting paper. The assembly was covered with a hurricane lamp glass chimney (12.5 cm diameter mouth, 9.0 cm base opening and 18.0 cm height), and maintained in a BOD incubator ($27 \pm 2^\circ\text{C}$, 16 h light-8 h dark photoperiod, 80% humidity). Thirty adults of *H. theivora* were released using a camel's hair brush separately into each glass chimney containing treated tea shoots. To prevent the escape of bugs, the glass chimneys were closed by muslin cloth with the help of rubber bands on top.

Each concentration comprised three replications and the control. A series of five doses was tested for each of the insecticides and the control. The entire procedure was replicated three times. Final assessment (lethal effects of the active substances of insecticide) was determined after 48 hours of the insecticide application and expressed as per cent mortality of the insect at each dose, in relation to untreated control mortalities using Abbott's formula (Abbott, 1925) as needed (mortality above 10%).

Lethal concentrations for 50 % (LC_{50}) were calculated using the statistical package for social sciences (SPSS) version 10.0 SPSS Inc., USA, based on the Finney probit analysis method (Finney, 1973) and expressed in ppm.

The median lethal exposure time (LT_{50} = time required to mortality 50% mirids tested) of each insecticide was determined by using recommended dilution (mentioned in table 1) in leaf dipped bioassay method (anonymous 1990). Mortality was recorded at 12 hours interval from 1-10 days for lethal time mortality relationship. LT_{50} were calculated using the statistical package for social sciences (SPSS) version 10.0 SPSS Inc., USA, based on the Finney probit analysis method (Finney, 1973) and expressed in day.

Resistance ratio

Resistance ratios (RR) were calculated by dividing the LC_{50} or LT_{50} values of the field strain by the corresponding LC_{50} or LT_{50} values of susceptible Darjeeling strain (SS). According to Cochran (1989), Resistance Ratio (RRs: i.e. LC_{50} field strain/ LC_{50} susceptible strain) of 1.0-2.0 indicate little, if any, resistance; values between 2.0 and 10.0 indicate moderate resistance; and RRs >10 indicate high levels of resistance.

RESULTS

Toxicities of OC, OPs, SPs and NNs regarding lethal dose (LC) and time (LT) mortality to the investigated *H. theivora* populations (field collected – Kalchini tea

cultivation and susceptible strain - Darjeeling) are summarized in Tables 2 and 3. In all bioassays, control mortality did not exceed 10%.

In the LC bioassays, LC₅₀'s ranged from 0.06 to 1580.77 ppm and a significantly higher LC₅₀ was obtained with the field populations of *H. theivora* compared with the susceptible strain (Table 2). Field populations of *H. theivora* had a significantly very high LC₅₀ (74.08 - 1580.77 ppm) for endosulfan, quinalphos and oxydemeton-methyl, followed by high (16.27 - 19.91 ppm) for imidacloprid and monocrotophos, moderate (5.32 - 7.48 ppm) for cypermethrin, thiametoxam and lambda-cyhalothrin and low (0.73 - 1.53 ppm) in alphamethrin and deltamethrin and very low (0.06 ppm) for fenpropathrin. Low LT₅₀ values were observed in monocrotophos and thiomethoxam which required less time (3 days) to kill 50% populations while, deltamethrin, oxydemeton-methyl, quinalphos and endosulfan required 6-8 days (Table 3).

Basing on LC₅₀ and LT₅₀ results (Table 2 and 3), Kalchini field populations could be marked to be of 'high-resistance' to the tested insecticides. Their RR values (resistance ratio) ranged between 1016.87 - 2680.87 folds with OPs; 20 - 7480 folds, with SPs; 331.83 -1435 folds, with NNs and 17564.11 folds for OC using LC method (Table 2). Whereas based on LT method the values were between 21.74 - 44.90 folds with OPs; 28.32 - 43.17 folds with SPs; 20.20 – 27.76 folds with NNs; and 45.99 folds with OC (Table 3).

Kalchini field strain was 2660 - 17564.11 folds and 43.17 - 45.99 folds resistant to the commonly used insecticides (endosulfan, cypermethrin, quinalphos and lambda-cyhalothrin) at the LC₅₀ and LT₅₀ levels respectively, the slopes of the dose-response plots for those insecticides being lower than for the susceptible strain. Deposits of tested insecticides failed to kill 100% of the Kalchini field strain with in 24h. Chi-square values were not significant at p<0.05 level for both LC and LT parameters. The slopes of the dose-response lines of the insecticides tested were not quite steep and the differences between the highest and lowest concentrations were high.

Table 1. List of commercial formulated insecticides tested, their active ingredient, trade name, class and manufacturer.

Active ingredient	Trade name	FRD* [ppm]	Class	Manufacturer
Endosulfan	Thiodan 35 EC	350	Organochlorine	Aventis Crop Science
Monocrotophos	Monocil 36 EC	250	Organophosphate	De-Nocil Crop Protection
Oxydemeton methyl	Metasystox 25 EC	250		Bayer India
Quinalphos	Flash 25 EC	370		Indofil
Alphamethrin	Tata Alpha 10 EC	24		Rallis India
Cypermethrin	Cymbush 25 EC	50	Synthetic pyrethroid	Syngenta India
Deltamethrin	Decis 2.8 EC	6		Bayer Crop Science
Fenpropathrin	Meothrin 30 EC	10		Sumitomo Chemical India
Lambda-cyhalothrin	Karate 5 EC	10		Syngenta India
Thiametoxam	Actara 25 WG	10	Neonicotinoid	Syngenta India
Imidacloprid	Confidor 17.8 SL	75		Bayer India

*FRD - field recommended dose.

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Table 2. Lethal Concentration (LC50 in ppm) probit-mortality data of insecticides on the susceptible (Darjeeling) and field-collected populations (Kalchini tea cultivation) of *Helopeltis theivora* in the year 2007.

Insecticides	df	Slope \pm SE	FCS LC ₅₀ [ppm]	95% confidence limit (ppm)		χ^2 NS	SS LC ₅₀ (ppm)	95% confidence limit (ppm)		RR
				Lower	Upper			Lower	Upper	
Endosulfan	4	1.428 \pm 0.009	1580.77	1422.48	1756.22	2.38	0.090	0.0200	0.1400	17564.11
Quinalphos	4	1.564 \pm 0.014	214.47	180.71	254.54	7.10	0.080	0.0730	0.0870	2680.87
Oxydemeton- methyl	4	1.968 \pm 0.008	74.08	66.85	82.08	3.57	0.050	0.0380	0.0650	1481.60
Monocrotophos	4	2.004 \pm 0.017	16.27	12.06	20.05	2.99	0.016	0.0054	0.0252	1016.87
Imidacloprid	4	5.641 \pm 0.010	19.91	17.62	22.50	1.52	0.060	0.0050	0.0640	331.83
Thiametoxam	4	2.032 \pm 0.012	5.74	4.52	7.28	2.73	0.004	0.0031	0.0051	1435.00
Deltamethrin	4	5.509 \pm 0.008	0.73	0.68	0.82	3.65	0.006	0.0054	0.0065	121.66
Cypermethrin	4	1.896 \pm 0.014	7.48	6.38	8.76	6.57	0.001	0.0007	0.0012	7480.00
Alphamethrin	4	4.748 \pm 0.007	1.53	1.40	1.67	1.59	0.001	0.0007	0.0012	1530.00
Lambda cyhalothrin	4	1.441 \pm 0.017	5.32	4.40	6.44	2.80	0.002	0.0018	0.0022	2660.00
Fenpropathrin	4	4.796 \pm 0.648	0.06	0.04	0.11	7.53	0.003	0.0021	0.0041	20.00

Values were based of five concentrations and three replications with 30 adults in each.

NS Non-significant at $p < 0.05$ level.

df – degrees of freedom; SE – standard error; FCS – Field Collected Strain (collected from Kalchini T.E.); SS – Susceptible stain (collected from Darjeeling); RR – Resistance Ratio = LC50 field strain/ LC50 suitable strain

Table 3. Lethal time (LT in days) mortality data of insecticides against the susceptible (Darjeeling) and field-collected populations (Kalchini tea cultivation) of *Helopeltis theivora* at field recommended dose in the year 2007.

Insecticides	FRD [ppm]	df	χ^2 NS	Slope \pm SE	FCS LT ₅₀ [days]	SS LT ₅₀ [days]	RR
Endosulfan	350	4	1.25	3.254 \pm 0.002	8.292	0.1803	45.99
Quinalphos	250	4	3.26	1.265 \pm 0.008	7.106	0.1613	44.05
Oxydemeton -methyl	250	4	2.21	2.347 \pm 0.005	6.979	0.1554	44.90
Monocrotophos	370	4	2.95	1.067 \pm 0.005	2.925	0.1345	21.74
Imidacloprid	24	4	3.87	1.657 \pm 0.019	4.723	0.1701	27.76
Thiametoxam	50	4	3.65	2.279 \pm 0.008	3.027	0.1498	20.20
Deltamethrin	6	4	1.59	1.008 \pm 0.007	6.916	0.1602	43.17
Cypermethrin	10	4	4.67	1.678 \pm 0.006	4.348	0.1535	28.32
Alphamethrin	10	4	2.47	2.456 \pm 0.010	4.982	0.1435	34.71
Lambda cyhalothrin	10	4	2.89	2.987 \pm 0.024	4.564	0.1097	41.60
Fenpropathrin	75	4	5.74	3.349 \pm 0.011	4.112	0.1328	30.96

Values were based of five concentrations and three replications with 30 adults in each.

NS Non-significant at $p < 0.05$ level.

df – degrees of freedom; SE – standard error; FCS – Field Collected Strain (collected from Kalchini T.E.); SS – Susceptible stain (collected from Darjeeling); RR – Resistance Ratio = LT50 field strain/ LT50 suitable strain.

DISCUSSION

This is the first report on the level of susceptibility of *H. theivora* populations from Kalchini tea cultivations for eleven insecticides (OC-1, OP-3, NN-2, SP-5) in the Dooars region of Northeastern India. Compared to the susceptible strain (Darjeeling), the field strain (Kalchini tea cultivation) varied considerably in susceptibility to insecticides. Field collected strain was found to have developed resistance to the tested insecticides.

These variations might reflect history of the commonly sprayed insecticides or other insecticides from similar groups. Resistance ratio can be closely related to the number of chemical applications with the same mechanisms of action (Campos *et al.* 1995). Although we do not have the history of chemical applications for each tea cultivation area, it is well known from local chemical companies and the recent survey report on use pattern of insecticides in tea estates of the Dooars (Roy *et al.* 2008b) that endosulfan, monocrotophos, deltamethrin and cypermethrin insecticides are extensively used in six sub-districts of the Dooars and highest consumption was noted in Kalchini region (SP-2.19 L h⁻¹; non-SPs- 7.59 L h⁻¹; total insecticide used- 9.79 L h⁻¹). As resistance was previously reported for *H. theivora* Jorhat, Nagrakata and other strains using these compounds (Bora *et al.* 2007; Gurusubramanian and Bora 2007 and 2008; Gurusubramanian *et al.* 2008b; Roy *et al.* 2008c,e), our results indicated that resistance to these insecticides might have developed in many *H. theivora* populations in the tea cultivations of the Dooars region. LC₅₀'s of field strains (Jorhat and Nagrakata) reported by Gurusubramanian and Bora (2008) were 278.84 to 1978.43 folds lower than the highest LC₅₀ (Kalchini, Dooars) observed in the bioassays reported herein.

Fenpropathrin was the most toxic to Kalchini field populations among the tested insecticides with lowest LC₅₀ value and low RR, while endosulfan was not toxic with highest LC₅₀ value and RR. While monocrotophos and thiometoxam had a some what intermediate LC₅₀ (5.74 – 16.27 ppm) and provided 50% control in 3 days. Fenpropathrin and deltamethrin however showed quite low LC₅₀ values (0.06 – 0.73 ppm) that required 4-7 days to obtain 50% control. The slopes of the dose-response lines of the insecticides tested were not quite steep and the differences between the highest and lowest concentrations were high. If, the population is phenotypically heterogeneous, and with a small increase in insecticide concentration, the mortality would not increase considerably. This necessitates more careful use of these insecticides in the field to avoid a high selection pressure that could eliminate the susceptible individuals and lead to selection of resistant individuals (Robertson and Preisler 1992). Levels of defense enzymes, esterases, GSTs (glutathione-s-transferases) (Sarker and Mukhopadhyay 2003, 2006a, 2006b) and P450 enzyme complex (Roy *et al.* 2009e) and increase in total body lipid content (Roy *et al.* 2008c), have been shown to be involved in the detoxification of several chemical classes of insecticides, i.e., organophosphates, pyrethroids, carbamates, and chlorinated hydrocarbons. So, the presence of enhanced quantity of these defense enzymes or their newer isozymes may be to a large extent responsible for development of resistance in this pest.

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In pest management programs, treatment decisions are based on economic thresholds or aesthetic injury levels. However, insecticide resistance reduces the efficacy of insecticide treatments, thereby influencing the decision process by reducing the number of viable treatment options. In the recent years, it has become a major concern of the tea industry, as the importing countries are imposing stringent restrictions for acceptability of the made tea with pesticide residues. In Kalchini field populations, endosulfan, quinalphos oxydemeton-methyl and deltamethrin fail to kill 95% of the bugs which also take 6-8 days for killing 50% of the populations. These conditions warrant a review of the levels and use patterns of the insecticides which would impose the necessity of rotation of insecticides of different mode of action in order to prevent resistance and reduce insecticides load on tea and environment.

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