Development of Tolerant Traits in Tea Mosquito Bug (*Helopeltis Theivora* Waterhouse) (Hemiptera: Miridae) under Insecticide Stress

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Abstract

Despite continuous application of synthetic insecticides of different kinds, the sucking pest of tea, *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) has become most damaging to tea crop in Darjeeling Terai-Dooars and North East India. In conventionally managed tea plantations, specimens of *H. theivora* get a regular exposure to synthetic insecticide showing significantly enhanced values of median lethal concentration or $LC_{50.}$ Such pesticide-stressed populations of *H. theivora* mainly sported changes in (i) colouration (ii) development and fitness (iii) body lipid quantity (iv)detoxifying enzyme levels and (v) egg-laying behavior implying adaptive changes of certain fitness components in the biology of the tolerant/ resistant strains. This study reveals a reduced efficacy of insecticides (viz. endosulfan, quinalphos, oxydemeton methyl, imidacloprid, monocrotophos, thiametoxam, cypermethrin and lambda cyhalothrin) against conventional field populations of *H. theivora*, possibly due to the changed traits in the insecticide- stressed but tolerant populations of *H. theivora.* Such developments throw up serious challenges for management of this major sucking pest of tea in Terai-Dooars and the North East India.

1. Introduction

The tea mosquito bug, *Helopeltis theivora* Waterhouse (Heteroptera: Miridae), has become a major pest of tea (*Camellia sinensis* O'Kuntz) in the last few decades in North East India, including Terai and the Dooars areas of Himalayan foothills. It is estimated that 80% of the tea plantations in India is affected by this pest alone, which often results in crop loss to the extent of 10-50% (Bora and Gurusubramanian, 2007). Among the tea growing regions of India, pest activity has always been reported to be high in the Dooars (Borbora and Biswas, 1996 and Sannigrahi and Talukdar, 2003). The agro-climatic conditions of this region, high moisture of the tea ecosystem along with thick double-hedged monoculture of tea over vast stretches of land probably contribute largely to an enhanced pest incidence. A survey conducted during 1998 to 2004 by Roy et al. (2008a) reported that on an average 7.499 $l\ kg^{-1}$ of insecticide was used per hectare per year in the Dooars. Endosulfan, monocrotophos, deltamethrin and cypermethrin were extensively used in the entire region. The requirement of synthetic pyrethorid gradually increased in the recent past.The control measure taken so far to keep the population of major tea pests down is by applying synthetic chemical pesticides. But much dependence and repeated use of these chemicals have rendered them less functional and sometimes counterproductive. Relative susceptibility values (LC_{50}) of *H. theivora* to different insecticides varied region wise. The pests due to their changed coloration, life style, behavior, enhanced levels of defense enzymes, body lipid content showed certain features of pesticide resistance.

The LC_{95} values of the experiments when compared with the recommended dosages, suggest medium to high resistance coefficient values for endosulfan, low to medium resistance coefficient for cypermethrin, lambda cyhalothrin, imidacloprid and quinalphos. However, there is not much change in case of other registered insecticides, which therefore are still found effective at recommended dose (Roy et al., 2009a, Roy et al., 2011). The present study, gives an in site into the pesticide tolerance levels in *H. theivora* vis-a-vis their different life traits. The study also gives us an opportunity to understand how this insect pest is fast adapting to high insecticide stress and becoming a chemically unmanageable one in tea crop.

2. Materials and Methods

2.1. Determination of pronotal colour variation

The colour variation in pronotal area of males and females in the Dooars population of the subHimalayan Terai was observed by collecting the adults from different insecticide-treated conventional tea plantations and by studying them under an advanced research microscope. One hundred male and one hundred female specimens were examined from each plantation for pronotal colour variation.

2.2. Determination of fitness traits of insecticide resistant and susceptible strains

Newly emerged 30 adults of the resistant (of the Dooars) and susceptible (of Makaibari organic tea plantation) population of *H. theivora* were kept at 25±2°C, 70-80% relative humidity and under photoperiod conditions of 16 h light: 8 h dark (LD 16:8) separately (n=10) in three groups inside hurricane lamp glass chimneys with the mouth covered with a nylon mesh for aeration.

The life history traits of pre-oviposition period (days), oviposition period (days), postoviposition period (days), fecundity (no. of eggs laid/female), incubation period (days), hatchability (% of egg hatch), total nymphal duration (days), total developmental time i.e. egg to adult (days), sex ratio (female: male) and average longevity of both male and female (days) were recorded from such a culture.

2.3. Estimation of total body lipid content

Body fat content of *H. theivora* was extracted using glass Soxhlet apparatus (AOAC, 1990). For this purpose, round bottomed flask was oven dried and kept in desiccators for cooling. The weight (W_1) of the round bottom flask was taken. A cellulose thimble (dry and fat free) was taken in which 1-2 g of *H. theivora* as sample (Collected from different conventional plantations of subdistricts of the Dooars) was placed and subjected to the Soxhlet distillation using petroleum ether (with boiling range 40-60^0 C) for 5 h. The round bottom flask with the extract was dried for 1 h. at 100^0 C to evaporate the solvent ether and moisture, then cooled in desiccator and weighed (W_2). Fat content was calculated in percentage by using the following formula:

Fat (%)=(W_2-W_1/sample weight)×100

2.4. Determination of detoxifying enzyme levels

2.4.1. Extraction and estimation

Single adult (female) *H. theivora* were homogenized in 500µl ice cold 0.1 M sodium phosphate buffer (pH 7.0) using Teflon homogenizer in 1.5 ml centrifuge tube. The homogenate was centrifuged at 12,000 g for 20 min at 4^{0}C in high speed refrigerated centrifuge (SIGMA 3K30). The resultant post-mitochondrial supernatant was aliquoted 100 µl each in 0.5 ml centrifuge tube and was stored at -80^{0}C to be used as enzyme source for GEs, GSTs and CYP450s activity assay and to estimate the amount of total protein. For GST assay the pH of the homogenization buffer was 6.5. General esterase activity was measured using α-naphthyl acetate (α-NA) as substrate according to the method of van Asperen (1962) with slight modifications for using in microplate reader. GST activity was estimated using the method of Kao et al. (1989) with minor adaptations. Heam-containing enzymes of insects, which include the cytochrome oxidase enzymes, were measurable (Brogdon et al., 1997). So, Cytochrome P450 activity was estimated by measuring heam peroxidase activity (Penilla et al., 2007; Tiwari et al., 2011).

2.5. Change in egg-laying strategy on tea shoot on exposure to pesticides

The adults of *H. theivora* were collected from the commercial tea fields of the Dooars (Kalchini subdistrict). The population was divided into three groups and kept in laboratory on tea clone TV_1. Among them two groups were treated with sublethal concentrations of endosulfan 35 EC and deltamethrin 2.8 EC. The bugs that survived after treatments were used for oviposition experiments along with the untreated bugs, maintained in the laboratory as control. Differences in egg laying pattern of the three treatments (i.e. endosulfan and deltamethrin exposed, and control) were assessed using analysis of variance by the Tukey's multiple comparison test (Bland and Altman, 1995).

3. Results and Discussion

3.1. Determination of pronotal colour variation

In the male *H. theivora* three colour variants and in females, six colour variants could be recorded (Table 1). As per the findings of Eastop (1973) and Russel (1978) the biotypes usually differ based on diurnal or seasonal activity patterns, size, shape, color, insecticide resistance, migration and dispersal tendencies, pheromone differences or disease vector capacities.

Colour variability in populations collected from Vietnam, South India and Assam with special reference to head and pronotum was reported by Stonedahal (1991). Variation in colour pattern *in H. theivora* was reported in different seasons by Mann (1907) and Stonedahl (1991), but in the present observation, colour variation was found both in males and females in the same season. The colour variation in the Dooars condition is presumably due to pesticide selection pressure (Roy et al., 2009a, 2010, 2011).

3.2. Determination of fitness traits of insecticide resistant and susceptible strains

Insecticide-stressed specimens utilize substantial amount of body resources for building up various tolerance characters

Table 1: Incidence of colour variation in *H. theivora* specimens collected from sub Himalayan tea plantations of Terai-Dooars

Colour variants of *H. theivora*		Description	% incidence of colour variant in the field (Mean±SE)
M	CVM I	Pronotal area completely black	80.25±1.24
	CVM II	Pronotal area black with one trench having brown colouration and two brownish dots at lateral side just below the trench	15.00±1.17
	CVM III	Pronotal area black with two trenchs having brown colouration one in top and other in middle at the base of scutellum	4.75±1.09
F	CVF I	Centre area of pronotal area brownish orange with lateral side black and green trench in anterior end.	20.35±1.42
	CVF II	Half pronotal area brownish orange (anterior end) and rest half totally black (distal end).	40.50±0.99
	CVF III	Centre area of pronotal area brownish orange with two black spots at the distal end.	5.55±0.41
	CVF IV	Pronotal area totally brownish orange.	4.00±1.23
	CVF V	Major part of pronotal area black with "U" shaped brownish spot at the frontal end	17.75±1.16
	CVFVI	Pronotal area black with two trenches having brown colouration at lateral side.	11.85±0.81

M: Male; F: Female; CVM: colour variant male; CVF: colour variant female

and thus overcoming the toxic effects of insecticides. This may often result in some biological deficiencies in the tolerant/resistant population when compared to a susceptible population. When the biological traits of insecticide-resistant field populations of *H. theivora* were compared with those of the susceptible population, only four (oviposition period, fecundity and nymphal and total developmental duration) out of eleven parameters observed were found to be adversely affected in the resistant population of *H. theivora* (Table 2).

The reduction in the rate of egg laying in the resistant population might have resulted from less energy partitioning for reproduction. The major share of the metabolic energy possibly gets allocated and used in developing biochemical and physiological defenses related to detoxification of the insecticide (Price, 1974; Ribeiro et al. 2001). Significantly longer larval development in the resistant strain of Colorado potato beetle than that in its susceptible strain is well known (Trisyono and Whalon, 1997). Further, Georghiou and Taylor (1977) reported that resistant strains of arthropods often have longer developmental time than their susceptible counterparts.

3.3. *Estimation of total body lipid content*

In a study on variation in total body lipid content in *H. theivora* populations collected from different tea growing subdistricts of the Dooars, the Kalchini population showed significantly high level of body lipid (6.215 %±0.124) followed by population from Dalgong (5.793%±0.084), Nagrakata (5.366%±0.208), Binnaguri (4.760%±0.112) and Damdim (4.633%±0.040). The Chulsa population indicated lowest value (4.553%±0.036).

The findings suggest that higher lipid content in the body of the specimens having higher insecticide-exposure possibly provides protection and insulation to pesticide action, which as a consequence helps develop a higher insecticide resistance, evident through enhanced LC_{50} values. Similarly the total fat body content in resistance strains of houseflies was more than susceptible one Srivastava (2004).

Table 2: Difference in some fitness parameters between resistant and susceptible populations of *H. theivora*

Strain	Oviposition period (days)	Fecundity (eggs $female^{-1}$)	Total nymphal duration (days)	Total developmental time (days)
R	22.7±1.47 (13-30)[b]	135.4±9.26 (70-171)[b]	14.9±0.73 (12-17)[b]	23.7±0.24 (18-25)[b]
S	20.3±0.91 (17-27)[a]	170.2±13.02 (103-201)[a]	11.7±0.26 (10-13)[a]	19.0±0.33 (17-21)[a]

R: Resistant; S: Susceptible; Means followed by different letter in a column are significantly different (p=0.05)

3.4. *Determination of detoxifying enzyme levels*

The activity of three principal detoxifying enzymes of *H. theivora* collected from conventional (Kalchini Tea Estates) and organic (Mokaibarie T.E.) is summarized in Table 3. The α-esterase activity of *H. theivora* populations of the conventional plantation was 6.59 fold higher than the organic populations. Similarly, monooxygenases and GST activity was 2.37 and 1.88 fold higher, respectively for the *H. theivora* populations of conventional tea plantations as compared to the organic population of the Darjeeling. The change in susceptibility may be related to the history of the commonly sprayed insecticides or other pesticides of similar groups (Zhu et al., 2011). Resis-

Table 3: Detoxifying enzyme activity of *H. theivora* populations collected from organic (Mokaibarie T. E.) and pesticide-treated conventional (Kalchini T. E.) tea plantations

Populations	General Esterases (GEs)		Monooxygenases (CYP450)		Glutathione-S-transfereases (GSTs)	
	(µmol^{-1} min^{-1}mg protein)	Activity Ratio*	Mean values±SE (µmol^{-1} min^{-1}mg protein)	Activity Ratio*	Mean values±SE (µmol^{-1} min^{-1}mg protein)	Activity Ratio*
Organic (Mokaibarie T. E.)	0.32±0.02^{a}	1	0.62±0.11^{a}	1	0.24±0.05^{a}	1
Conventional (Kalchini T.E.)	2.11±0.12^{b}	6.59	1.47±0.08^{b}	2.37	0.45±0.11^{b}	1.88

*Activity ratio=enzyme activity of the conventional field-collected population/the enzyme activity of the susceptible population; Means followed by the different letter significantly different using Tukey's multiple comparison test (HSDa)

tance ratio can be closely related to the number of chemical applications with the same mechanisms of action (Campos et al., 1995). Earlier reports indicate that field populations of insects, continuously getting exposure to synthetic insecticides, usually showed enhance activities of all the three principal detoxifying enzymes (Zhu et al. 2011 and Saha et al., 2012). The present findings also emphasize the role of the three defense enzymes in detoxification in order to impart greater tolerance in the pest population of *H. theivora* taken from conventional plantation, especially one of 'Kalchini' sub-district.

3.5. Study on change in egg-laying strategy on tea shoot due to pesticide exposure and stress

The spatial distribution of an insect species depends to a large extent on the oviposition behavior of females, because their larvae have limited dispersal capabilities (Huignard et al., 1986; Hanks, 1999). Normally the most frequent and preferred site for egg laying by *H. theivora* is at the stalk (internode) between 1st and 2nd leaves. This portion of the shoot harbor 28.49% of the total number of laid eggs, which is followed by the number of eggs laid in the stalk between the 2nd and 3rd leaves (24.92%), and that between the 3rd and 4th leaves (24.90%) .

An interesting departure from normal preference of oviposition sites could be noted in *H. theivora* when they were subjected to insecticide-stressed condition. Female specimens when topically treated with sub lethal concentration (LC_{20}) of endosulfan and deltamethrin, started laying most eggs below the pluckable level (60% and 58% respectively for the two insecticides). Under this insecticide-stressed conditions the female preferred the stalk between (internode) 3rd and 4th leaves for laying (50% of total eggs, i.e., almost double that of normal condition) instead of using the internode between 1st and 2nd leaves and 2nd and 3rd leaves, for notionally saving their eggs from exposure to pesticide spray and consequent safety of the neonates, as *in situ* the same is supposed to be received more in the table of the tea bush (Roy and Mukhopadhay, 2011). This is the possibly the first example of behavioral resistance to insecticides sported by a tea pest from sub Himalayan plantation. That oviposition preference, induced by previous experience of females has been demonstrated in many insect species (Groot et al., 2003). The ability to learn about a new situation/stimulus and changing the egg laying site, appears to be a new response in face of a novel problem.

4. Conclusion

Treatments of the crop by non-pyrethroid and pyrethroid insecticides are failing to control the pest adequately in the sub-himalayan tea plantations of the Dooars and Terai, mainly due to the bug's multi-pronged adaptive changes resulted from repeated exposure to insecticidal sprays. Under prolonged insecticide-stress, it is expected that resistant strains have got selected in some populations especially those of the Dooars plantations. Under the various degree of tolerance developed and absence of susceptible form in the conventional plantations, management of this pest appears to be a difficult proposition unless some non-conventional pest management practices are adopted.

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