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Full Length Research Paper

Variation of resistance to endosulfan in tea mosquito bug, *Helopeltis theivora* waterhouse (heteroptera: miridae) in the tea plantation of the Sub-Himalayan Dooars, northern west Bengal, India

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The tea mosquito bug, *Helopeltis theivora* Waterhouse, from the Dooars tea plantations, North Bengal, India, was tested for susceptibility change and resistance development to endosulfan. Six different *H. theivora* populations collected from six subdistricts of the Dooars and their responses to endosulfan were investigated by probit analysis and compared with those of a susceptible reference strain. Resistance ratio and resistant coefficient for endosulfan ranged from 582.18 - 1504.20 folds and 5.78 - 14.95, respectively (based on LC_{95}). Selection of adults of *H. theivora* in laboratory to various sub-lethal concentrations of endosulfan up to five generations resulted in maximum mortality at highest concentration in every successive generation. The resistance ratio for the fifth generation increased 4.417 fold as compared to first generation. The results indicate that endosulfan should be avoided for managing endosulfan resistance in *H. theivora*.

Key words: *Helopeltis theivora,* Dooars tea plantations, susceptibility change, endosulfan, resistance.

INTRODUCTION

The tea mosquito bug, *Helopeltis theivora* waterhouse (Hemiptera: Heteroptera: Miridae), is one of the most destructive polyphagous sucking pests of tea (*Camellia sinensis*) in North Eastern states of India (Assam and West Bengal - Dooars, Terai and Darjeeling) because it attacks only the young shoots for feeding and egg laying that is the actual crop of tea (Roy et al., 2008e). Though in earlier days (1900 - 1960), it was a minor pest on tea (Das, 1957), due to environmental changes, deforestation and other anthropogenic activities it has become a major threat for tea cultivation in North East India. The problem is more acute as it has rapid multiplication capacity and polyphagous in nature (Roy et al., 2009a, b, c). It has attained the national importance in India and was estimated that 80% of the tea plantations area is affected resulting in crop loss to the tune of 10 - 50% (Bora and Gurusubramanian, 2007; Roy et al., 2009a).

Among the tea growing areas of North East India, the Dooars tea plantations of North Bengal (26° 16" to 27° 0" N latitude and 88°4" to 89° 53" E longitude) comprise six subdisricts (Damdim, Chulsa, Nagrakata, Binnaguri, Dalgong, Kalchini) suffered badly by this pest attack and consumed highest quantity of insecticides (Barbora and Biswas, 1996; Sannigrahi and Talukdar, 2003). The climatic condition 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). An average of 7.5 l/ha of insecticides was used per year in the Dooars tea plantations (Roy et al., 2008b) wherein, endosulfan is the most common insecticide accounted for 73.5% and found to be ineffective in controlling the pest in different parts of the Dooars tea plantations. Season of occurrence of *H. theivora* infestation (May-November) and monsoon coincides in the Dooars condition. Use of insecticide has been

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the main controlling measure against this pest. All Tocklai (Tea Research Association, Jorhat, Assam) released tea clones, tea garden released clones and seed jats are found to be susceptible to *H. theivora* attack at varying degrees (Rahman et al., 2007; Roy et al., 2009a) . In spite of regular application of insecticides, *H. theivora* has turn into a menace all around the year. Decrease in the sus-ceptibility to different classes of insecticides may be one of the causes for their resurgence and persistence on tea crop (Bora et al., 2007; Gurusubramanian and Bora, 2008; Gurusubramanian et al., 2008a; Rahman et al., 2006; Sarker and Mukhopadhyay, 2003, 2006a,b; Sarmah et al., 2006; Roy et al., 2008a,b,c). Added to this, in recent days the situation further worsened as it deve-loped resistance to commonly used insecticides (Roy et al., 2008b; Mukhopadhyay and Roy, 2009). The resistance developed by *H. theivora* populations in North East India ranged from 1.47 - 62.99 folds for males and 1.25 - 62.82 folds for females to different classes of insecticides (Gurusubramanian and Bora, 2008).

Though the distribution of this pest is ubiquitous in all the North East tea plantations, yet no work has been undertaken to assess the level of resistance in this pest to the most commonly used insecticides in the Dooars, in spite of the fact that at many occasions in the past, planters have approached with information of insecticidal control failure against this pest even after spraying repeatedly at much higher dosage of endosulfan than recommended for its control and frequency of sprays, but this irrational use of this insecticide only speeded up the development of insecticide resistance in *H. theivora*. A need was, therefore, realized to quantify the potential of development of resistance to endosulfan of this pest.

With this point in the background, a study was made with three objectives as to determine susceptibility change in the field collected populations of *H. theivora* from six subdistricts (Damdim, Chulsa, Nagrakata, Binnaguri, Dalgong, and Kalchini) of Dooars, a northern part of West Bengal, India to endosulfan.

To demonstrate development of resistance by using laboratory population (Kalchini population – least suscep-tible to endosulfan) to be able to acquire resistance through selection by endosulfan.

MATERIALS AND METHODS

The insect and its rearing

The culture of *H. theivora* was initiated by collecting six populations of about 200 nymphs by using aspirator from the tea plantations from six subdistricts (Damdim, Chulsa, Nagrakata, Binnaguri, Dalgong and Kalchini) of Dooars during May - July, 2007 in rearing jars (20 cm x 15 cm) and brought to the laboratory and maintained at 27 ± 2°C, 70 - 80% RH and 16:8 LD photoperiod in a BOD (Table 1). The larvae were provided with the tea shoots (two and a bud) of TV 1 clone to feed upon till they attained adult stage. After multi-plying the culture in the laboratory for two successive generations, the whole stock was divided into two lots. One lot was named as parental stock and the other was used for exposure to endosulfan.

Hundred nymphs of susceptible strain of *H. theivora* (maintained as a laboratory culture since 2005) were also brought from the Division of Plant Protection Division, Tocklai Experimental Station, Tea Research Association, Jorhat, Assam. These nymphs were also reared till the emergence of adults and thus the culture was further multiplied to be used in the bioassay studies with the same method as that of field collected *H. theivora* (Table 1).

Preparation of insecticidal concentrations

The technical grade of endosulfan (99.9% pure, Thiodan 35 EC, Aventis Crop Science Ltd., UK) was used to prepare 10,000 µg of A.I. /L stock solution in distilled water from which further dilutions were prepared subsequently.

Determination of susceptibility change in the field collected populations of *H. theivora* from six subdistricts of Dooars

Relative toxicity assay was performed by following the standard method recommended by Insecticide Resistance Action Committee (IRAC method No. 7) by using *H. theivora* adults (F_2 generation) from the stock culture maintained, which were collected from six subdistricts of Dooars. Graded concentrations of endosulfan (100, 200, 400, 800, 1200, 1400, 1600, 1800 and 2000 µg of A.I. /L) were prepared in distilled water from the stock solution. TV 1 clone two and bud healthy shoots were collected from the experimental gar-den plots and brought to the laboratory. The leaves were washed thoroughly with distilled water and air-dried. Fifteen tea shoots for each treatment were sprayed with each of the chosen insecticides separately at the respective dilutions using a glass atomizer, and then they were kept in a glass tube containing water and wrapped with cotton. The sprayed tea shoots were kept under ceiling fans for 15 min to evaporate the emulsion. The glass tubes containing tea shoots were placed in glass chimneys (20 x 15 cm). Muslin cloth was tied with the help of rubber bands on top of the glass chim-neys, and the tubes were kept at 27 ± 2°C in culture room. Thirty adults of *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 24 h after the treatment. Moribund insects were counted as dead (Gurusubramanian and Bora, 2007). Nine concentrations of endo-sulfan were tested against six field collected population to obtain a concentration - probit mortality curve. The mortality data was converted to corrected percent mortality by using Abbott's formula (Abbott, 1925) and subjected to probit analysis (Finney, 1971) to obtain LC_{50} values, LC_{95} values and a regression equation.

To assess the resistance of a given population of *H. theivora*, the resistance coefficient was calculated as follows: Resistance coeffi-cient (RC) = LC_{95} value/recommended field dose of endosulfan (250 µg of A.I. /L) - Gurusubramanian et al., 2005. The following criteria for resistance assessment were assumed (W gorek et al., 2009): RC 1 - The lack of resistance; RC = 1.1-2 - Low resistance; RC = 2.1 - 5 - Medium resistance; RC = 5.1 - 10 - High resistance; RC > 10 - Very high resistance.

Demonstration of development of resistance by using laboratory population (Kalchini) to be able to acquire resistance through selection by endosulfan

Sub-lethal concentrations of endosulfan prepared on the basis of susceptibility experimental results were applied to the thoracic dorsum of each adult of susceptible as well as field collected strain (Kalchini) of *H. theivora* separately with the help of a Merck micro-pipette @ 1.0 l per adult. Six concentrations of endosulfan (Table 3) were utilized for exposure in each generation. Besides, a set of

Table 1. Origin of *Helopeltis theivora* populations.

Population	Location of collection	District and state	Host	Date of collection
Damdim	Damdim	Western Dooars, West Bengal	*Camellia sinensis* TV 1, TV12, TV23 and TS653 clones	21.05.2007
Chulsa	Chulsa			24.05.2007
Nagrakata	Nagrakata	Central Dooars, West Bengal		29.05.2007
Binnaguri	Binnaguri			10.06.2007
Dalgong	Dalgong			13.06.2007
Kalchini	Kalchini	Eastern Dooars, West Bengal		18.06.2007
Susceptible	Tea Research Association, Tocklai, Jorhat	Jorhat, Assam	*Camellia sinensis* TV1 clone	12.07.2007

control (with acetone only) was also maintained with each exposure to work out the correct mortalities. Thirty adults per replicate were treated with one concentration and three replications were maintained for each concentration. The treated adults were housed in the glass chimney containing tea shoots individually and were kept in B.O.D. incubator (27 ± 2°C; 12: 12 L/D period). The mortality data was recorded 24 h after the treatment. The survivals obtained at higher concentrations were shifted to clean rearing glass vial each and provided with fresh TV 1 tea leaves. The progeny of the first surviving lot was termed the F1 generation and the exposures and the selections were conducted subsequently up to 5 genera-tions. The parental and susceptible strains were also maintained through without exposure to observe the biological parameters. Increasing concentration of endosulfan was used for each selection [700 - 1500, 1000 - 2000, 1800 - 3500, 2500 - 5000, 3500 - 6000 µg of active ingredient (A.I.) /L of distilled water] so that 20 to 40% of females survived for the succeeding generations. Survivors after 48 h were used to initiate the next generation. At least 540 adults were used in each selection. The intervals between selections varied from 20 to 25 days.

Quantification of insecticidal resistance

The degree of development of resistance through different generations was determined by working out LC_{50} values in each generation by computer aided statistical programme SPSS 8.0 and computing the resistance ratio. The resistance ratio for any generation was worked out by dividing LC_{50} for that generation with LC_{50} value of the parental strain.

RESULTS

The change of endosulfan susceptibility

The LC_{50} values, resistance coefficient and resistance factor of each population varied (Table 2). The LC_{50} values of all the six populations of *H. theivora* (269.74 - 1580.77 µg of A.I. /L) from the Dooars region exceeded the recommended field concentration of endosulfan. However, based on lethal concentration results, there was no significant decrease in the susceptibility levels of any population against endosulfan. Resistance factors relative to the response of the susceptible population ranged from 582 to 1504 folds for endosulfan (Table 2). The Kalchini population showed the highest LC_{50} value, very high resistance coefficient and resistance ratio to endosulfan than did the other populations (Table 1).

Development of resistance by laboratory population of *H. theivora* (Kalchini) through selection by endosulfan

Laboratory culture of Kalchini population of *H. theivora* adults were selected for five generations to various discriminating concentrations to obtain maximum mortality at the highest concentration in each generation. The minimum survival of *H. theivora* adults obtained in the 1st generation at 1500 µg of A.I. /L concentration (Table 3) was 17.0% among all the concentrations used. Similarly, the lowest survival of 16.67, 13.00, 12.00 and 10.00% were obtained in the 2nd to 5th generation, respectively. After subjecting the data to probit analysis, the results obtained in the Pearson Goodness of fit chi-square revealed that homogeneity in the population of the test insect was significant ($p < 0.05$) in the 1st generation as well as in the successive generations as was evident from the observed and expected responses. The regression lines in the 1st generation (slope 3.88 ± 0.01) were significantly different from the other log concentration - mortality lines obtained in the 2nd to 5th generation. The LC_{50} value (Table 3) recorded in the 1st generation was 896.51 µg of A.I. /L which subsequently increased to 3960.17 µg of A.I. /L in the 5th generation and the insect developed 4.41 fold resistance in the 5th generation as compared to 1st generation. It is clearly indicated that the *H. theivora has* propensity to develop more resistance to endosulfan in Dooars area of North Bengal.

DISCUSSION

In North East India, Tocklai Experimental Station, 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 pesticides for controlling tea pests (Anonymous, 1993, 1999) . Review of literature suggests that from the early forties onwards DDT (organochlorine) was routinely used to combat the problem of *H. theivora*. Endosulfan (cyclodiene: organochlorine) was introduced in the Dooars tea plantation from the year 1968 in form of Thiodan 35 EC (Mukerjea, 1968). As use of DDT was restricted

Table 2. Probit statistics and susceptibility level of *Helopeltis theivora* population collected from different subdistricts of the Dooars tea plantations to endosulfan 35 EC.

Population	Total number of adults used	Chi squire value (X^2)	Degrees of freedom	Slope ± SE	LC_{50} [µg of A.I./L] (confidence interval, p =0.95)	LC_{95} (µg of A.I./L)	RC#	RF*
Damdim	810	2.16	7	3.902 ± 0.011	544.72 (480.324 - 607.753)	1446.72	5.78	582.18
Chulsa	810	4.95	7	2.375 ± 0.010	269.744 (221.712 - 328.181)	1940.64	7.76	780.94
Nagrakata	810	5.51	7	4.270 ± 0.010	884.95 (791.936 - 988.899)	2160.51	8.64	869.42
Binnaguri	810	2.38	7	5.621 ± 0.006	938.213 (864.995 - 1017.63)	1848.53	7.39	743.87
Dalgong	810	2.45	7	3.501 ± 0.012	952.715 (825.689 - 1099.37)	2829.97	11.31	1138.32
Kalchini	810	2.38	7	4.428 ± 0.009	1580.77 (1422.48 - 1756.22)	3737.95	14.95	1504.20
Susceptible	810	9.42	7	6.756 ± 0.153	0.749 (68.963 – 80.946)	2.485	0.009	-

[#] RC- Resistance coefficient - LC_{95} of the field collected or susceptible strain /recommended Dose (250 µg of A.I. /L]., *RF- Resistance factor = LC_{95} of the field-collected/ LC_{95} of the susceptible population.

for pest management in tea (Anonymous, 1970), the use of endosulfan for control of *H. theivora* has been the common practice and some other pests has increased rapidly in tea industry due to its economic viability in field management practice. During the last several decades, the control of pests, diseases and weeds in tea fields is predominantly by the use of synthetic chemicals. Though broad-spectrum pesticides (endosulfan) offer powerful incentives in the form of excellent control, increased yield and high economic returns, they have serious drawbacks such as development of resistance to pesticides, resurgence of pests, outbreak of secondary pests, harmful effects on human health and environment and presence of undesirable residue (Das, 1965; Gurusubramanian et al., 2005; Sarnaik et al., 2006).

In a recent survey, synthetic pesticides constituted 85% of the total pesticides used, while 15% were of organic and inorganic origin in tea gardens of the Dooars. In which, insecticides accounted for 60% (8.46 litre ha^{-1}) and within the synthetic insecticides, organochlorine (26% - 2 rounds $year^{-1}$) were most preferred (Sannigrahi and Talukdar, 2003; Roy et al., 2008a, b, c). It has been estimated that tea industry in India are currently facing serious problem to tea for pest control (Gurusubramanian et al., 2008a) to with this pest and therefore, extreme care must be exercised before a pesticide is introduced avoid residue build-up. Organochlorine insecticide, endosulfan has been in use on tea in North-East India for the past 100 years. Much of the efficacy and sustainability of this group of organochlorine insecticide in tea pest management would depend on the susceptibility of the *H. theivora*. Variation in relative toxicity was observed between male and female populations of Jorhat and Darjeeling and among the populations of *H. theivora* collected from different sub districts of Dooars to the tested insecticides due to selection pressure by insecticides (Rahman et al., 2005; Bora et al., 2007; Gurusubramanian and Bora, 2008). A comparison of expected effective dose of thirteen insecticides against tea mosquito bug based on their LC_{50} values with recommended dose revealed a pronounced shift in the level of susceptibility of *H. theivora* to all the chosen insecticides except acephate (Gurusubramanian et al., 2008b). Gurusubramanian and Bora (2008) observed that

Table 3. Selection of residual population of *Helopeltis theivora* (Kalchini population) in various generations to endosulfan and their probit statistics and resistance ratio.

Generation	Concentration of endosulfan used (µg of A.I./L]	Mortality (%)	Residual population rejected (X) / Selected (+)	Slope ± SE	LC $_{50}$ [µg of A.I./L] (confidence interval, p = 0.95)	Chi square value (Df)	Resistance Ratio (F_n / F_1)
F_1	700	33	X	3.8879 ± 0.010	896.51 (799.71- 1005.03)	0.594 (6)	1
	900	47	X				
	1000	63	X				
	1200	70	X				
	1400	77	X				
	1500	80	+				
F_2	1000	43	X	3.7045 ± 0.011	1290.27 (1145.70 - 1453.08)	1.587 (6)	1.439
	1200	50	X				
	1500	57	X				
	1700	63	X				
	1900	70	X				
	2000	83	+				
F_3	1800	40	X	4.3168 ± 0.010	2129.01 (1915.77 – 2365.00)	0.892 (6)	2.374
	2200	53	X				
	2600	60	X				
	3000	73	X				
	3200	77	X				
	3500	87	+				
F_4	2500	30	X	5.3931 ± 0.006	3286.57 (3022.83 - 3573.33)	1.282 (6)	3.665
	3000	37	X				
	3500	57	X				
	4000	63	X				
	4500	78	X				
	5000	88	+				
F_5	3500	40	X	5.8036 ± 0.007	3960.17 (3659.41- 4285.65)	0.931 (6)	4.417
	4000	50	X				
	4500	63	X				
	5000	70	X				
	5500	76	X				
	6000	90	+				

Nagarkata population showed high level of resistance than Jorhat population in both males (7.998 folds in Jorhat and 8.522 folds in Nagrakata) and females (4.739 folds in Jorhat and 8.326 folds in Nagrakata) to endo-sulfan during 2006 - 2007. But in the present study, the resistance level was registered to be 582 to 1504 folds. The recommended dose of organochlorine (endosulfan), however, was practically ineffective against this pest collected from North East India (Gurusubramanian and Bora, 2008) . Roy et al. (2008c) reported that *H. theivora* populations of Kalchini subdistrict showed less suscep-tibility to endosulfan (12.33 to 72.26 fold) as compared to the high susceptibility of those from the Damdim and Chulsa subdistricts to endosulfan during 2006 with lesser LT_{50} values (1.11 - 2.70 days) were noted in the recom-mended dose of endosulfan in tea (Roy et al., 2008d) and 11.05 - 72.27 times more of the recommended dose of endosulfan might be required to achieve control of the pest. The expected dose of endosulfan required for effec-tive control of *H. theivora* in Dooars tea plantation ranged between 1446.72 - 3737.95 µg of A.I. /L (LC_{95} value) than the recommended dose of 250 µg of A.I. /L. Hence, it was observed that 5.78 - 14.79 times more of endosulfan might be required to achieve desirable control of this pest. Therefore usual recommended dose of endosulfan was practically ineffective against this pest in this region.

The presence of various oxido-reductase enzymes in the salivary and mid gut along with the basic hydrolyzing enzymes enable *H. theivora* to become one of the most destructive pests of tea by depredating the young leaves and growing shoots of tea (Sarker and Mukhopadhyay, 2006a). In addition, qualitative and quantitative changes were recorded in the enzymes pattern of the tea mos-quito bug (General esterase - Sarker and Mukhopadhyay, 2003; glutathione S-transferase and acetylcholinesterase – Sarker and Mukhopadhyay, 2006b), indicated a higher tolerance/resistance status due to formation of greater amount of esterases, glutathione S-transferase and acetylcholinesterase. One of the main reasons for higher tolerance or resistance by tea mosquito bug and red spider mite to different pesticides was due to mixing of incompatible insecticides with acaricides to combat mixed infestation which, not only decreased the insecticide toxi-city but also shifted the level of relative toxicity (Rahman et al., 2005b).

During the development of resistance by laboratory population of *H. theivora* (Kalchini) through selection by endosulfan, LC_{50} value (Table 3) recorded in the 1^{st} generation was 896.51 µg of A.I. /L which subsequently increased to 3960.17 µg of A.I. /L in course of selection up to 5^{th} generation. The insect summarily developed 4.417 folds resistance. The data reasonably explains the propensity of *H. theivora* to develop higher resistance with repeated use of endosulfan in tea. So an increase in the use of this group of insecticide for control of this pest may lead to control failures. Though several workers (Dzolkhifli et al., 1986; Liew et al., 1992; Ho, 1994; Muhamad and Omar, 1997; Bora et al., 2007; Gurusubramanian and Bora, 2008 and Roy et al., 2008b) have indicated development of levels of resistance against chlorinated hydrocarbons in *H. theivora*, however information is scanty on generation- wise accruing of resistance in this pest. In view of these, it is expected that the field recommended dose of endosulfan may become sub-lethal and less effective or develop resistance, hence the use of endosulfan may be discontinued or field recommended dose may be reviewed. Piperonyl butoxide (PB) blended with endosulfan at a ratio of 1:5 increased the toxicity significantly ($p < 0.01$) to the tune of 4.45 - 44.60 folds than endosulfan alone. Higher synergism of PB with endosulfan indicates that PB may be effective in preventing or retarding the tea mosquito bug from deve-loping resistance of these insecticides in North Bengal tea plantation (Roy et al., 2009b). Further in-depth studies are needed to explore the possibility of deter-mining the resistance level by using resistance enzyme studies and biotypes identification through molecular techniques besides the log dose probit assays. The pre-sent findings have practical implications in the insecticide resistance management (IRM) programme of *H. theivora*.

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