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Research Article

SUSCEPTIBILITY OF *CULEX QUINQUEFASCIATUS* TO THREE COMMONLY USED INSECTICIDES FROM WEST BENGAL, INDIA

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ABSTRACT

Vector control is a useful tool for elimination of lymphatic filariasis which is severely challenged by the emergence of resistance among the vector mosquitoes against used insecticides. Reports on status of insecticide susceptibility from different parts of the country are important for formulation of vector control strategy. The present study was aimed to assess the larval susceptibility of *Culex quinquefasciatus* to temephos and adult susceptibility to DDT, deltamethrin and malathion. Larvae and adult of F1 generation were used for insecticide bio-assay using standard WHO protocol. It was that the adult *Culex quinquefasciatus* population of the study areas were resistant to all three insecticides tested with corrected mortality well below the 90% (1.25 to 9.38% for DDT, 33.33 to 61.25% for deltamethrin and 13.75 to 50.63 % for malathion). High degree of temephos tolerance was recorded among the larval forms. Such study from other parts of the country is highly suggested.

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INTRODUCTION

Lymphatic filariasis (LF), a neglected tropical disease, affects the lymphatic system which leads to the unusual enlargement of body parts, and causes pain, severe disability and social stigma. This disease is commonly known as elephantiasis and found in many regions of the world including the South East Asia, Middle East and Eastern Mediterranean countries (Gyapong *et al*, 2005; Abdel-Hameed *et al*, 2004). World Health Organisation (WHO) estimated that 856 million people in 52 countries worldwide are at risk of LF infection (WHO, 2017). According to WHO, 700 million endemic populations are found in WHO's South Eastern Asian Region (WHO-SEAR) and India contributes about 67% of the endemic populations. In South East Asia, 60 million persons are affected with LF i.e., either harbouring microfilariae (mf) or suffering from clinical manifestations of the disease, of which 82% are found in India (WHO, 2017). India is one of the worst affected countries where 17 states and 6 Union Territories are endemic with about 553 million people are at risk of infection, of which

about 146 million live in urban and the remaining in rural areas. About 31 million mf carriers and over 23 million clinical cases are found in India (WHO, 2005). West Bengal is one of the major filarial endemic states of India and 12 districts are endemic for filariasis and mf prevalence ranged from 1.2% to 8.1% (NVBDCP, 2017).

Lymphatic filariasis is a mosquito-borne disease caused by helminth parasite *Wuchereria bancrofti* and *Brugia malayi*, *Culex quinquefasciatus* is the major vector throughout all Asian countries (Bhaskar, 2000). In north eastern states of India, this species is considered to be an efficient vector of bancroftian filariasis (Mahanta *et al*, 2001). In India, Elimination Lymphatic Filariasis (ELF) programme was launched in 1997 and it mainly based on annual mass drug administration (MDA) i.e., administration of single dose of diethylcarbamazine and albendazole (NVBDCP, 2017). The current strategy to eliminate lymphatic filariasis is unlikely to achieve complete elimination if MDA is not supplemented by transmission-control interventions in some areas where

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persistent transmission occurs even after 5 to 6 rounds of MDA (Sabesan, et al, 2010).

Chemical Insecticides are considered as the most important components in the global mosquito control efforts (McCarroll and Hemingway, 2002) and has been used for vector and pest control programs for many decades. The vector control strategy formulated by the National Vector Borne Disease Control Programme of India (NVBDCP) is mainly based on larval source management by larvicidal agents (temephos, *Bti*), and adult vector control by indoor residual spray (IRS) of organochlorine (DDT) and synthetic pyrethroids, use of insecticide impregnated bed nets with deltamethrin and thermal fogging of malathion. Due to the regular use of these insecticides for vector control measures and in agricultural field for pest management at rural and urban areas, *Culex* mosquitoes, especially *Cx. quinquefasciatus* population are under tremendous pressure of used insecticides. In addition, different physiological mechanisms are also involved for insecticide resistance among vector mosquitoes like reduced sensitivity of sodium channels to insecticides, over-production of detoxifying enzymes which are responsible for detoxification of toxic substances (Brogdon and McAllister, 1998; Feyereisen, 1999; Roberts and Andre, 1994).

Successful implementation of vector control strategies requires definite knowledge on vector distributions, biology and changing trends on susceptibility status to used insecticide. So, regular monitoring of the susceptibility status of vectors mosquitoes against different insecticides is the most important element for vector control programmes (Nauen, 2007) Insecticides susceptibility bioassay is the primary tools for surveillance and monitoring of the insecticide resistance, though it is also monitored by studying the insecticide detoxifying enzyme and molecular markers (WHO, 2011). In spite of huge disease burden and vector abundance, the susceptibility status of *Cx. quinquefasciatus* against different insecticides has not been monitored in a regular basis and very few reports are available from different parts of India (Sarkar et al, 2009a; Sarkar et al, 2009b., Mukhopadhyay et al, 1993; Thavaselvam et al, 1993)

The present study was designed to assess the insecticide susceptibility status of adult *Cx. quinquefasciatus* to DDT, deltamethrin, and malathion, in three northern districts of West Bengal, India. This study also assessed the susceptibility of temephos, the major larvicide against *Cx. quinquefasciatus* in the study areas.

MATERIALS AND METHODS

Study sites

The present study was conducted during April, 2017 to October, 2017 in Darjeeling, Jalpaiguri and Uttar Dinajpur districts of West Bengal. These three districts are located at the northern part of West Bengal. In consultation with the respective district health authorities, three blocks of Darjeeling, two blocks of Jalpaiguri and one block of Uttar Dinajpur were selected as study sites. The sites were Phansidewa, Matigara, and Khoribari block of the Darjeeling district; Dhupguri and Malbazar block of the Jalpaiguri district and Chopra block of the Uttar Dinajpur. The study sites of Matigara, Malbazar were

sub-urban in nature, whereas study sites of Phansidewa, Khoribari, Dhupguri and Chopra were rural in nature (Fig 1).

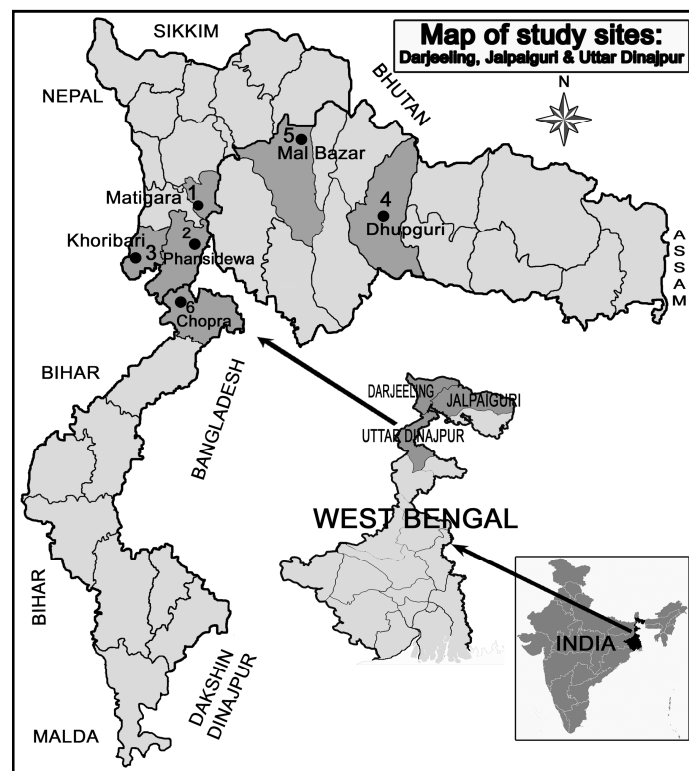


Figure 1 Map showing the study sites

Mosquito collection, rearing and identification

The immature stages of mosquito collected from the natural breeding places such as septic tanks, streams, abandoned buildings, drainages and construction sites. The larvae and pupae were collected with the help of different sized dippers. The larvae and pupae collected from each dip were gathered in a plastic contain. Then the containers were transported to the laboratory on the same day and the larvae and pupae are transferred in the larvae rearing tray along with water collected from the field. The larvae rearing tray was supplied with artificial food such as yeast and fish food. The optimum temperature (25^oC-30^oC) and optimum relative humidity (80%-90%) was maintained in the laboratory. The immature stages were reared to the adult stages and after emergence, the adult mosquitoes were anesthetized with ethyl ether and identified morphologically according to the keys of Rattanarithikul et al, 2005 (Rattanarithikul et al, 2005) and Tyagi et al, 2012 (Tyagi et al, 2012). The identified *Cx. quinquefasciatus* were allowed to breed under laboratory conditions. The larvae and adults of the F1 generation were used for larval and adult insecticide bioassays.

Larval susceptibility tests

Larval bioassays were conducted as per the standard WHO bioassay protocol (WHO, 2005) Technical-grade formulations of temephos (50EC; Nitapol Industries Pvt Ltd., Kolkata) was employed for the larval bioassay study. Six different concentrations (0.01, 0.05, 0.1, 0.5, 1.0, and 2.0 ppm) of insecticides were prepared from the stock temephos solution and used in the susceptibility bioassay. Susceptibility tests were performed using 20–25 third instar to early fourth instar larvae

in 200 ml disposable paper cups filled with the required concentration of insecticide solution and double distilled water at room temperature (25°C ± 2°C). Each concentration had four sets of replicates and each set of experiment was accompanied by two sets of controls containing equal concentration of 95% ethanol. After 24 hours of experiment larval mortality was recorded and the larvae that were motionless or convulsive upon a sharp stimulation were counted as dead (WHO, 2005). Larval mortality was determined by dividing the number of dead larvae by the total number tested. A test was considered as invalid if pupation rate was greater than 10%, or mortality rate in the control was greater than 20% (WHO, 2005).

Adult susceptibility bioassay

Adult susceptibility bioassay was performed on 2 to 3-day old laboratory emerged (F1 generation) unfed female *Cx. quinquefasciatus* mosquitoes as per WHO protocol (WHO, 2016). The tests were carried out using 4% DDT, 0.05% deltamethrin, and 5% malathion impregnated papers which were obtained from Universiti Sains Malaysia, Malaysia. Five different holding tubes were used for each set of the experiment of which four were a test and one was a control. In each holding tube, 20-25 adult female mosquitoes were kept for one hour and then mosquitoes from four tubes marked as test were exposed to insecticide-impregnated papers. Silicone oil, olive oil, and risella oil pre-impregnated papers for deltamethrin, malathion, and DDT, respectively were used in control sets. Mosquitoes were allowed in the exposure tube for one hour and cumulative knock down was recorded after 10, 15, 20, 30, 40, 50, and 60 minutes. After that the mosquitoes were transferred to holding tubes and fed on a 5% sucrose solution for the next 24 Hours. Mortality was scored after 24 hours to determine the susceptibility status as per WHO recommendation (WHO, 2016).

Data analysis

The results of larval bioassay were analyzed using Log dose probit (Ldp) Line computer software (Ehabsoft, Cairo Egypt) according to the Finney's method (Finney, 1972).

Lethal concentrations (LC₁₀, LC₅₀, and LC₉₉) along with the slope were estimated at 95% confidence intervals (CI) by using the Ldp line software. The resistance ratio (RR₉₉) was calculated by comparing the lethal concentration (LC₅₀/LC₉₉) value for a population with the LC₅₀/LC₉₉ value for the insecticide for a laboratory colony. The RR₉₉ ≤ 3 was considered as susceptible, and 3 < RR₉₉ ≤ 5 as low resistance, 5 < RR₉₉ ≤ 10 as moderate resistance, and RR₉₉ > 10 as high resistance (Mazzarri and Georghiou, 1995).

In case of adult bioassays, observed mortality was calculated by the formula: observed mortality (%) = (Total no. of dead mosquitoes / Total mosquitoes exposed) x 100. The observed mortality was corrected using Abbott's formula when the mortality rate of control was within 5% - 20%. Corrected Mortality (CM) (%) = [(% of observed mortality - % of control mortality) / (100 - % of control mortality)] x 100. According to WHO, mosquitoes were considered susceptible (S) if the corrected mortality (CM) rate was greater than 98% and resistant (R) if mortality rate was less than 90%. Mortality rate between 90-98% was considered as possible resistance (PR) and needs verification by alternative methods like enzyme bioassay and molecular marker studies (WHO, 2016). The cumulative knock down rates (KDR) were calculated by observing the number of knocked down mosquitoes after 10, 15, 20, 30, 40, 50 and 60 minutes during the hour-long exposure period. Knockdown time (KDT₁₀, KDT₅₀, and KDT₉₅) is the time required for knockdown of a particular proportion of mosquitoes following exposure to any insecticide. KDTs were determined using Ldp Line computer software (Ehabsoft, Cairo Egypt) programme according to the Finney's method (Finney, 1972).

RESULTS

Larval susceptibility status

The LC₁₀, LC₅₀, and LC₉₉ values of different study sites did not follow a normal distribution for mortality to the log dose ($\chi^2 \leq 28.57$; $p \leq 0.0001$).

Table 1 Temephos sensitivity status of *Culex quinquefasciatus* in three districts of West Bengal

Values	Study sites					
	Darjeeling		Khoribari (n = 180)	Jalpaiguri		Uttar Dinajpur
	Phansidewa (n = 180)	Matigara (n = 180)		Dhupguri (n = 180)	Malbazar (n = 180)	Chopra (n = 180)
LC ₁₀ (lower limit - upper limit) [mg/L]	0.044 (0.026-0.059)	0.089 (0.063-0.112)	0.071 (0.04-0.095)	0.057 (0.022-0.077)	0.028 (0.014-0.038)	0.041 (0.019-0.055)
LC ₅₀ (lower limit - upper limit) [mg/L]	0.159 (0.109-0.228)	0.194 (0.147-0.252)	0.249 (0.168-0.369)	0.255 (0.135-0.446)	0.112 (0.071-0.173)	0.146 (0.089-0.236)
LC ₉₉ (lower limit - upper limit) [mg/L]	1.639 (1.265-3.109)	0.784 (0.631-1.107)	2.446 (1.907-5.187)	3.914 (3.207-12.593)	1.365 (1.089-3.379)	1.466 (1.212-3.912)
X ² (p)	46.96 (<0.0001)	28.57 (0.0001)	39.58 (<0.0001)	79.89 (<0.0001)	46.51 (<0.0001)	58.46 (<0.0001)
Slope	2.29 ± 0.11	3.83 ± 0.19	2.35 ± 0.11	1.96 ± 0.09	2.14 ± 0.11	2.32 ± 0.12
R	0.97	0.93	0.97	0.96	0.97	0.96
G	0.08	0.07	0.1	0.16	0.12	0.15
RR ₅₀ /RR ₉₉ *	8.93/9.46	10.89/4.53	13.99/14.12	14.33/14.12	6.29/22.59	8.20/8.46
Status [#]	MR	LR	HR	HR	HR	MR

n = number; LC₁₀/LC₅₀/LC₉₉ = lethal concentration 10%/50%/99%, RR = resistance ratio, g = 'g' is a factor used for fiducial limit calculations

* The LC₅₀ and LC₉₉ values of laboratory strain was 0.0178mg/L and 0.1732mg/L, respectively

#Classification adapted from Mazzarri and Georghiou (1995): S = Susceptible (RR₉₉< 3), LR = Low Resistance (3 < RR₉₉< 5), MR = Moderate Resistance (5 < RR₉₉< 10), HR = High Resistance (RR₉₉>10)

The LC₅₀ values of Phansidewa, Matigara, Khoribari, Dhupguri, Malbazar and Chopra ranged from 0.112 (0.071-0.173) to 0.255 (0.135-0.446) mg/L, whereas LC₉₉ values ranged from 0.784 (0.631-1.107) to 3.914 (3.207-12.593) mg/L. The calculated RR₅₀ and RR₉₉ values in different study sites were ranged from 6.29 to 14.33 and 4.53 to 22.59, respectively (Table 1). *Cx. quinquefasciatus* larval population of Matigara exhibited low level of resistance to temephos whereas moderate level of temephos resistance was observed among the Phansidewa and Chopra population. The high level of temephos resistance was recorded among the larval population of Khoribari, Dhupguri and Malbazar.

Adult susceptibility status

The adult susceptibility bioassay results *Cx. quinquefasciatus* against three different insecticides are presented in Table 2. After 24 hours of initial exposure, the corrected mortality rates for 4% DDT were ranged from 1.25% to 9.38% in different study sites, which is well below the WHO recommended 90% mortality rate for resistance. So, results suggested that the *Cx. quinquefasciatus* population of the study areas was highly resistant to DDT. The corrected mortality rate for 0.05% deltamethrin and 5% malathion were ranged from 33.33% to 61.25% and 13.75% to 50.63%, respectively in different study sites. Therefore, the adult *Cx. quinquefasciatus* population of all the study sites were also highly resistance to deltamethrin and malathion (Table 2).

The observed KDT₅₀ values were 94.97 (75.97-132.36) to 210.93 (124.55-600.38) mins for DDT, 16.52 (15.45-17.56) to 35.55 (32.55-39.18) mins for deltamethrin, and 23.75 (18.27-29.66) to 121.08 (81.91-252.23) mins for malathion. The KDT₉₅ values for DDT were 955.09 (514.69-2492.45) to 10214.51 (2283.32-215991.32) mins, for deltamethrin 46.02 (41.91-51.48) to 220.38 (167.08-319.31) mins and for malathion 108.58 (96.12-199.39) to 6814.03 (1753.78-98413.03) mins. The knockdown rate (KDR) of *Cx. quinquefasciatus* against DDT, deltamethrin, and malathion over an exposure time of 1 hour is given in Fig 2(A – C).

During 1 hour of exposure, the knock down rate (KDR) varied from 28.75% - 40.63% for DDT, 74.38% to 93.56% for deltamethrin, and 42.50% - 78.63% for malathion.

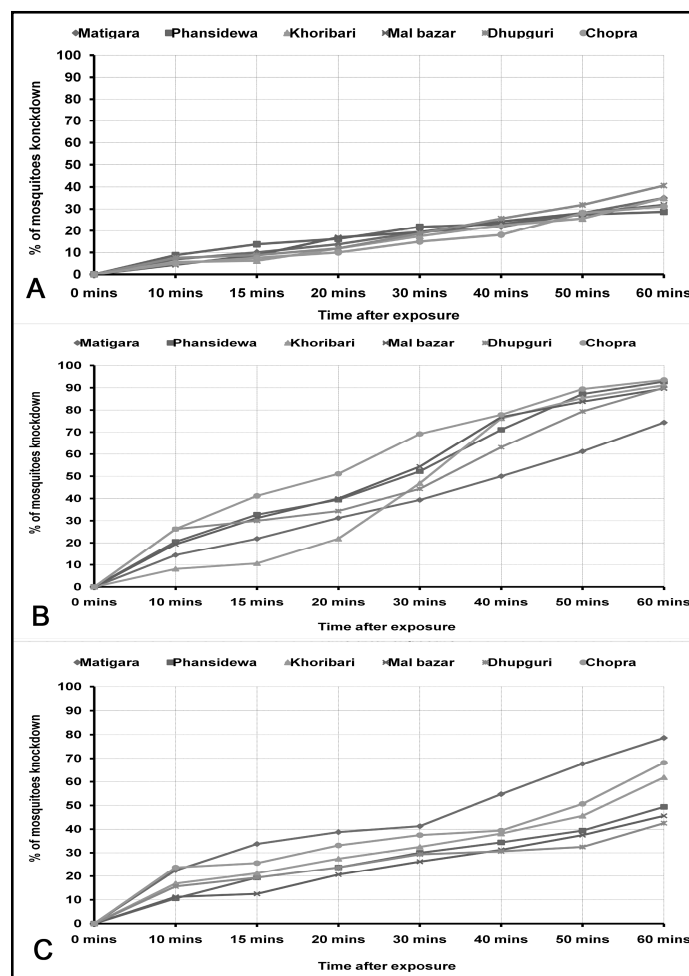


Figure 2 Knock down rate of *Cx. quinquefasciatus* against 4% DDT (A), 0.05% deltamethrin (B), 5% malathion (C) in West Bengal

Table 2 Insecticides susceptibility status of *Culex quinquefasciatus* against 4% DDT, 0.05% deltamethrin and 5% malathion in West Bengal

Insecticides	Districts	Blocks	Mosquito exposed		Mosquito died		Observed Mortality (%)		CM (%)	KDT ₁₀ [95% CI]	KDT ₅₀ [95% CI]	KDT ₉₅ [95% CI]	χ ² (p)	Slope	Status #
			T*	C*	T*	C*	T*	C*							
4 % DDT	Darjeeling	Phansidewa	160	40	2	0	1.25	0	1.25	10.26 [5.56-14.22]	210.93 [124.55-600.38]	10214.51 [2283.32-215991.32]	0.65 (0.99)	0.98±0.17	R
		Matigara	160	40	15	0	9.375	0	9.38	14.84 [10.86-18.20]	128.55 [93.62-215.93]	2053.07 [861.88-8900.77]	1.09 (0.96)	1.37±0.18	R
		Khoribari	160	40	10	0	6.25	0	6.25	18.12 [14.33-21.36]	116.92 [89.19-178.24]	1279.72 [628.43-4024.80]	2.08 (0.84)	1.58±0.19	R
	Jalpaiguri	Dhupguri	160	40	14	0	8.75	0	8.75	15.72 [12.33-18.65]	94.97 [75.97-132.36]	955.09 [514.69-2492.45]	2.99 (0.70)	1.64±0.18	R
		Malbazar	160	40	15	0	9.375	0	9.38	15.59 [11.71-18.88]	122.08 [90.75-196.34]	1712.69 [765.16-6508.67]	3.13 (0.68)	1.43±0.18	R
		Chopra	160	40	7	0	4.375	0	4.38	19.37 [15.35-22.80]	131.75 [97.41-213.81]	1543.69 [711.36-5548.43]	1.94 (0.86)	1.54±0.19	R
0.05% deltamethrin	U. Dinajpur	Phansidewa	180	45	60	0	33.33	0	33.33	8.11 [5.22-9.18]	22.68 [18.01-27.49]	84.83 [74.97-132.20]	29.58 (<0.001)	2.87±0.16	R
		Matigara	160	40	71	0	44.38	0	44.38	8.58 [6.77-10.27]	35.55 [32.55-39.18]	220.38 [167.08-319.31]	5.93 (0.31)	2.08±0.16	R
	Darjeeling	Khoribari	160	40	63	0	39.38	0	39.38	14.12 [10.59-15.81]	27.48 [22.95-32.51]	64.61 [57.93-87.66]	35.05 (<0.001)	4.43±0.21	R
		Dhupguri	160	40	73	0	45.63	0	45.63	7.02 [3.24-7.55]	25.22 [18.39-33.26]	130.15 [123.51-307.74]	32.91 (<0.001)	2.31±0.16	R
	Jalpaiguri	Malbazar	160	40	78	0	48.75	0	48.75	8.31 [5.86-9.65]	22.58 [18.82-26.44]	81.46 [70.17-115.55]	18.37 (0.003)	2.95±0.17	R
		Chopra	160	40	98	0	61.25	0	61.25	7.44 [6.46-8.35]	16.52 [15.45-17.56]	46.02 [41.91-51.48]	8.79 (0.12)	3.69±0.20	R
5% malathion	U. Dinajpur	Phansidewa	160	40	79	0	49.38	0	49.38	8.39 [5.62-10.93]	70.37 [57.68-94.20]	1076.98 [549.19-3117.19]	2.24 (0.82)	1.39±0.16	R
		Matigara	160	38	81	0	50.63	0	50.63	7.27 [4.09-8.24]	23.75 [18.27-29.66]	108.58 [96.12-199.39]	26.68 (0.0001)	2.49±0.16	R
	Darjeeling	Khoribari	160	40	78	0	48.75	0	48.75	6.94 [4.62-9.13]	52.71 [45.24-64.82]	710.86 [404.29-1676.95]	8.51 (0.13)	1.46±0.16	R
		Dhupguri	160	40	22	0	13.75	0	13.75	5.24 [2.19-8.27]	121.08 [81.91-252.23]	6814.03 [1753.78-98413.03]	2.16 (0.83)	0.94±0.15	R
	Jalpaiguri	Malbazar	160	40	57	0	35.63	0	35.63	10.29 [7.24-12.99]	80.19 [64.72-110.34]	1118.84 [569.17-3249.18]	2.16 (0.83)	1.44±0.17	R
		Chopra	160	40	56	0	35	0	35.00	4.69 [1.07-5.41]	43.95 [34.57-76.99]	777.57 [664.99-10462.35]	15.64 (0.008)	1.32±0.15	R

*T = Test, C = Control, CM = Corrected Mortality #S = Susceptible (CM ≥98%), R = Confirmed Resistance (CM <90%); PR = Possible Resistance (CM = 90 - 97%)

DISCUSSION

During the last few decades, synthetic insecticides were widely used in agricultural field and recently is being used in public health programmes, which has led to the development of resistance among vector mosquitoes in many countries (Kamgang *et al*, 2011; Dusfour *et al*, 2011; Singh *et al*, 2011; Dhiman *et al*, 2013). The susceptibility status of *Cx. quinquefasciatus* population to temephos, DDT, deltamethrin and malathion were investigated in three districts of northern West Bengal. The study revealed low to higher level of resistance to temephos among the larval population of *Cx. quinquefasciatus*. Highest level of temephos resistance was recorded in Malabazar of the Jalpaiguri district and lowest level in Matigara of the Darjeeling district. The RR values at LC₅₀ and LC₉₉ were greater than 4.53, which indicated that larval population of *Cx. quinquefasciatus* were resistant to temephos. The difference in RR₅₀ and RR₉₉ values might be due to natural variations in toxicity ratios rather than to resistance selection (Araujo *et al*, 2013). Similar kind of resistance to temephos has been reported from Delhi (Katyal *et al*, 2001), north-western (Suman *et al*, 2010) region and the north-eastern region of India (Sarkar *et al*, 2009; Tikar *et al*, 2009). Conversely, susceptibility to temephos have also been reported in populations of *Cx. quinquefasciatus* and *Aedes aegypti* from Rajahmundry town in Andhra Pradesh, South India (Mukhopadhyay *et al*, 2006) and Karnataka (Shetty *et al*, 2013). Very recently a study from northern part of West Bengal reported susceptible to moderate level of temephos resistance among the *Ae. albopictus* population (Chatterjee *et al*, 2018).

Adult bioassay revealed that adult *Cx. quinquefasciatus* population were highly resistant to DDT, deltamethrin and malathion. Highest level of DDT and deltamethrin resistance was noted in Phansidewa of Darjeeling district, whereas highest level of malathion resistance was recorded in Dhupguri of Jalpaiguri district. The high level of DDT resistance in *Cx. quinquefasciatus* might have been developed over time due to prolonged use in public health programmes for many years. The use of DDT is discontinued in most parts of India due to development of resistance in vector populations. However, it is still being used for control of Kala-azar vector and some parts of north-eastern India for malaria vectors. Nevertheless, persistence of DDT in the environment may have resulted in the continued selection for resistance. Similarly, high level of DDT resistance in *Cx. quinquefasciatus* were also reported from different parts of India such as, north eastern India (Sarkar *et al*, 2009b; Sarkar *et al*, 2009a), Patna (Bihar) (Mukhopadhyay *et al*, 1993) and Panaji (Goa) (Thavaselvam *et al*, 1993). There are many other reports of high levels of DDT resistance in *Cx. quinquefasciatus* in different parts of the world (Duran and Stevenson, 1983; Majori *et al*, 1986; Somboon *et al*, 2003).

In the study areas, pyrethroids were widely used in agricultural fields as well as in public health programmes (for impregnation of bed nets) to reduce the transmission of malaria. A significant level of pyrethroid resistant mosquito was found to be associated with agricultural activity indicating that resistance level increases with increased use of the insecticides in agricultural fields. Two blocks, Khoribari and Phansidewa are endemic for kala-azar. In 2016 the NVBDCP introduced synthetic pyrethroid for IRS to reduce kala-azar transmission.

So the vector mosquitoes were under tremendous pressure of pyrethroid for a long period which is reflected by the higher level of resistance among the *Cx. quinquefasciatus* population of the study area. Similarly, reports are available from north eastern part of the country (Kumar *et al*, 2011).

In the study area, malathion fogging was done sporadically to manage the vector borne disease outbreaks during last few years. Malathion was used widely in paddy fields and also in tea gardens for pest control. Insecticide residues used in paddy fields or tea gardens are washed into mosquito breeding sites thus exerting a huge selection pressure on mosquito larval populations, which resulted in the emergence of insecticide resistance. This might be the cause for recording high level of malathion resistance among the *Cx. quinquefasciatus* population of the study area. Similarly malathion resistance was reported from different parts of the country against different species of *Anopheles* (Dhiman *et al*, 2016), *Aedes* (Yadav *et al*, 2015) and *Culex* (Kumar *et al*, 2011; Dhiman *et al*, 2013).

CONCLUSION

The present study showed that the prevailing *Cx. quinquefasciatus* population of the study areas were highly resistant to all three classes of insecticides. The data generated from this study will be useful for the development of future insecticide resistance management strategies and will also help the public health policy makers to select the insecticides for formulation of effective vector control measures. Such study from other parts of the country is highly suggested.

Authors' Contribution

NB, AKM and PS designed the study and supervised all activities; PS, MC, SB, NB, DKB collection and identification of mosquitoes; MC, SB, PS, AKB, TP assessed the insecticide bioassay; NB, AKM, PS, MC, AKB, TP, perform the data analysis and interpretation; NB, AKM, PS, MC, prepare the manuscript

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Conflicts of Interest

We have no conflicts of interest concerning the work reported in this article.

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