Original Article

The Status of Susceptibility of Japanese Encephalitis Vectors to Insecticides in Endemic Areas of Northern Districts of West Bengal, India

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SUMMARY: Emergence and spread of resistance among vectors toward different insecticides is a serious problem for the Japanese encephalitis (JE) control program. Regularly monitoring the status of susceptibility of vector species to insecticides is important for formulating proper vector control measures. In this study, we studied the susceptibility status of major JE vectors from northern West Bengal, toward 4% DDT, 0.05% deltamethrin, and 5% malathion. Two- to three-day-old unfed female mosquitoes were subjected to a susceptibility bioassay using a World Health Organization kit. Corrected mortality (CM) and knockdown times were estimated. *Culex tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* were the major JE vectors present in the study areas. All 4 vector species were highly tolerant to DDT with CM < 90%. *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* were tolerant to deltamethrin with CM < 90%, except for *Cx. gelidus* of Darjeeling and Malbazar. At most of the study sites, malathion was effective against *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* with CM ≥ 98%. In contrast, *Cx. tritaeniorhynchus* was tolerant to malathion in all study areas. Predominant JE vector populations were highly tolerant to all 3 analyzed insecticides, except deltamethrin for *Cx. gelidus* and malathion for *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus*. The results of this study may be useful for better planning and implementing a JE control strategy.

INTRODUCTION

Japanese encephalitis (JE) is a life-threatening mosquito-borne, zoonotic, flaviviral disease in South East Asian countries (1). In the year 2015, the World Health Organization (WHO) estimated that there were 68,000 clinical JE cases with 13,600–20,400 deaths, and 3 billion people at risk globally (2). In India, a total of 1,730 clinical JE cases with 291 deaths were reported by the National Vector Borne Disease Control Programme (NVBDCP) in 2015 (3). In the recent past (in 2014), there was an outbreak of JE infection in northern districts of West Bengal, with 415 cases and 78 deaths (3). The zoonotic cycle of JE virus involves the pig as the major reservoir and amplifying host, water birds as carriers, and mosquitoes as vectors. So far, 16 mosquito species have been identified as JE vectors (4), among which *Culex tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* are the major vectors of JE in India.

Members of the *Cx. vishnui* group commonly breed in paddy fields and are mostly abundant during the rainy season between June and October. Therefore, JE

is mainly studied for its importance in rural areas where rice cultivation and pig farming coexist (5). At present, JE control is mainly based on immunization, case management, and vector control. Insecticides remain the mainstay in most of the vector control programs and are used through indoor residual sprays (IRS), fumigants, space sprays, and long-lasting insecticide-treated bed nets (LLINs). At present, 4 groups of insecticides—organochlorines, organophosphates, carbamates, and synthetic pyrethroids—are in use for various vector control programs. Insecticides are effective if their administration is implemented optimally (6,7); however, factors like inadequate resources, irrational use (8), insecticide resistance (9), and poor-quality insecticides (10) may reduce their efficiency.

Regular monitoring of the susceptibility status of vectors toward various insecticides is the most important element for vector control programs (11). Status of susceptibility of vectors toward different insecticides and changing trends of their resistance are the basic information to guide policy makers for formulating effective vector control measures like selection of insecticides, to ascertain unchanged efficacy of insecticide(s) in use, and to manage insecticide resistance. The insecticide susceptibility bioassay is the primary tool for surveillance and monitoring of insecticide resistance (12).

Despite significant disease burden and vector abundance in India, the susceptibility status of JE vectors against different insecticides has not been monitored on a regular basis. The NVBDCP implemented fogging of malathion against adult JE vectors, but the vector

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Table 1. Demography of study villages

District	Block	Village	Location	Vegetation	Pig rearing (Yes/No)	Nature
Darjeeling	Matigara	Kawakhali	26°41' 23.2" N 88°23' 36.5" E	Paddy field & Tea garden	Yes	Sub-urban
		Nichabasa	26°46' 48.9" N 88°23' 19.3" E	Paddy field & Tea garden	Yes	Rural
	Phansidewa	Helagachh	26°35' 19.6" N 88°21' 12.2" E	Paddy field & Tea garden	Yes	Rural
		Kasiadangi	26°35' 31.8" N 88°17' 14.0" E	Paddy field & Tea garden	Yes	Rural
	Khoribari	Monsajote	26°35' 33.8" N 88°17' 08.6" E	Paddy field & Tea garden	Yes	Rural
		Chekormari	26°35' 30.1" N 88°17' 05.6" E	Paddy field & Tea garden	Yes	Rural
Jalpaiguri	Dhupguri	Mollicksova	26°37' 52.4" N 89°01' 48.4" E	Paddy field	Yes	Rural
	Matiali	Zuranti TG	26°56' 08.4" N 88°46' 22.6" E	Paddy field & Tea garden	Yes	Rural
	Malbazar	Napuchapu	26°47' 21.0" N 88°44' 30.3" E	Paddy field & Tea garden	Yes	Rural
Uttar Dinajpur	Itahar	Mohinagar	25°27' 42.0" N 88°14' 49.4" E	Paddy field	Yes	Rural
		Kokna	25°27' 35.1" N 88°14' 20.0" E	Paddy field	Yes	Rural

Fig. 1. Map showing the study area and study sites.

population is under tremendous pressure from different insecticides used in agricultural fields. Besides, synthetic pyrethroids have been widely used in LLINs, while DDT is used for IRS in many malaria-endemic regions including northern districts of West Bengal. Some recent studies have indicated a considerable level of resistance among different vectors against synthetic pyrethroids and DDT in different states of India (13–21).

Reports on insecticide susceptibility of the JE vector from India are very rare, and no data are available from the JE-endemic region of northern part of West Bengal. The present study was designed to evaluate the susceptibility status of major JE vectors *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* toward 3 major insecticides: 4% DDT, 0.05% deltamethrin, and 5% malathion.

MATERIALS AND METHODS

Study area: The present study was carried out in 11 villages of 7 blocks of 3 districts of the northern part of West Bengal from March to September, 2016. The study blocks were Matigara, Phansidewa, and Khoribari of the Darjeeling district; Malbazar, Matiali, and Dhupguri of the Jalpaiguri district; and the Itahar block of Uttar Dinajpur. The geographical locations of study sites are given in Table 1 and Fig. 1. Approximately 150 JE cases were reported in 3 study districts in 2014 (3). The study

sites were selected by analyzing the line list of the last 5 years of JE cases, in consultation with the respective district health authorities.

Mosquito collection, rearing, and identification:

The mosquito larvae and pupae were collected from the natural breeding sites like paddy fields, irrigation canals, cesspools, rain water pools, ponds, and slow-flowing streams. Dippers of different sizes were used for the collection of larvae and pupae, which included soup ladles holding ~100–150 ml of water or a larger 500 ml dipper. The larvae were collected by either skimming the dipper through water at an angle or by lowering the dipper slowly into the water. The collected larvae and pupae from the water bodies were transferred to small plastic containers (500 ml) and transported to the laboratory on the same day. Then, the collected larvae and pupae were transferred into the larvae rearing tray along with water collected from the field and supplied with artificial food such as yeast and fish food (used for ornamental fish) obtained at a local market. The temperature and relative humidity of the laboratory were maintained between 25–30°C and 80–90%, respectively. The immature stages in the tray were monitored at regular intervals, and once larvae reached the pupal stage, they were removed from the tray and placed into a mosquito cage in a glass petri dish, which contained a small volume of water (approximately 7 mm in depth). After emergence, the adult mosquitoes were anesthetized with ethyl ether and identified morphologically according to the keys of Reuben et al., 1994 (22) and Tyagi et al., 2012 (4). The identified major JE vectors of the study area were used for an insecticide susceptibility bioassay.

Insecticide susceptibility bioassay: Two- to three-day-old, laboratory hatched, unfed female mosquitoes were used for the insecticide susceptibility assay. Adult insecticide susceptibility bioassays were carried out using a WHO test kit (23). The insecticide-impregnated pieces of paper were obtained from the Vector Control Research Unit, Universiti Sains Malaysia, Malaysia. Bioassays were conducted against 4% DDT, 0.05% deltamethrin, or 5% malathion. A total of 20 to 25 adult female mosquitoes were kept in each holding tube for 1 h. Subsequently, 5 such tubes were taken during each set of experiments: 4 as a test and 1 as a control. After 1 h of holding, mosquitoes in 4 tubes marked as a test were exposed to insecticide-impregnated paper. The control tests were performed using silicone oil, olive oil, and risella oil preimpregnated paper for deltamethrin, mala-

thion, and DDT, respectively. Mosquitoes were exposed to the tube for 1 h and cumulative knockdown was recorded at an interval of 10 minutes. After 60 minutes, the mosquitoes were transferred to holding tubes and fed a 5% sucrose solution for the next 24 h. Mortality was scored after 24 h to determine the susceptibility status as per WHO recommendations (23). After completion of each test, mosquitoes were reidentified morphologically to avoid any error.

Ethical statement: Before initiation, the purpose of the study was explained to the village population. The mosquito larvae and pupae were collected from the natural breeding sources (such as paddy fields, rain water pools, mud pools, irrigation canals, river bed pools, cess-pools, and ponds) present in the surroundings of a study population but not from any private land or residence. Therefore, no specific permission was required for collection of immature stages of mosquitoes from breeding sites. The study did not involve any endangered and protected species. Mosquitoes were maintained under optimal conditions such as temperature, humidity, and adequate food supply in the laboratory. The study protocol was approved by the Institutional Ethics Committee of Calcutta School of Tropical Medicine, Kolkata.

Data analysis: After completion of the adult bioassay, the observed mortality was calculated via the formula: observed mortality (%) = (Total No. of dead mosquitoes × 100) ÷ Total No. of mosquitoes exposed. When mortality in the control tube was greater than 5% but less

than 20%, the observed mortality was corrected via the Abbott's formula: Corrected Mortality (CM) (%) = (% of observed mortality – % of control mortality) × 100 ÷ (100 – % of control mortality). For adult bioassays, resistant/susceptible status was defined according to WHO recommendations (23). Mosquitoes were denoted as susceptible if the CM rate was greater than 98%, and resistant if the mortality rate was less than 90%; the mortality rate between 90 and 98% was labeled as possible resistance for convenience (23). The cumulative knockdown 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) was determined using the Log dose probit (Ldp) computer program following the Finney method (24).

RESULTS

Demography of the study area: The study was conducted in 7 different blocks of 3 districts of the northern part of West Bengal from March to September, 2016. The study sites of Dhupguri and Itahar blocks were surrounded by paddy fields, whereas the presence of both paddy fields and tea gardens were characteristic of the remaining 5 study sites. At all study sites, pig rearing was a common practice. Study sites of Matigara were suburban, whereas all other sites were rural (Table 1). Most of the people of the study villages were either

Table 2. Insecticides susceptibility status of JE vectors against 4% DDT in West Bengal

JE vectors	Districts	Blocks	Mosquito exposed		Mosquito died		Observed mortality (%)		CM* (%)	KDT ₁₀ (95% CI)	KDT ₅₀ (95% CI)	KDT ₉₅ (95% CI)	χ^2 (<i>p</i>)	Slope	Status#
			T*	C*	T*	C*	T*	C*							
<i>Cx. tritaeniorhynchus</i>	Darjeeling	Phansidewa	304	96	174	10	57.2	10.4	52.3	17.5 (14.4-20.3)	57.6 (50.7-68.3)	65 (187-443)	0.78 (0.98)	2.5 ± 0.2	R
		Matigara	160	40	62	2	38.8	5.0	35.5	14.3 (11.1-16.9)	56.7 (49.1-68.8)	333 (220-622)	1.72 (0.89)	2.1 ± 0.2	R
		Khoribari	80	20	27	0	33.8	0	33.8	8.2 (4.7-11.3)	67.7 (53.5-98.8)	1020 (461-4202)	5.43 (0.36)	1.4 ± 0.2	R
	Jalpaiguri	Dhupguri	80	20	41	1	51.3	5.0	48.7	15.6 (13.1-17.9)	44.5 (40.6-49.7)	171 (133-240)	0.41 (0.99)	2.8 ± 0.2	R
		Malbazar	80	20	37	2	46.3	10.0	40.3	18.1 (15.5-20.5)	47.7 (43.5-53.2)	165 (130-229)	0.42 (0.99)	3.1 ± 0.3	R
		Matiali	80	20	28	1	35.0	5.0	31.6	20.4 (17.5-22.9)	52.9 (47.9-59.8)	179 (139-257)	1.74 (0.88)	3.1 ± 0.3	R
U. Dinajpur	Itahar	80	20	16	1	20.0	5.0	15.8	19.7 (15.3-23.5)	98.3 (75.8-150.7)	773 (394-2470)	2.95 (0.71)	1.8 ± 0.3	R	
<i>Cx. vishnui</i>	Darjeeling	Phansidewa	80	20	10	0	12.5	0	12.5	20.6 (14.7-25.4)	168 (107-416)	2493 (804-25500)	8.4 (0.14)	1.4 ± 0.2	R
		Matigara	80	20	11	0	13.8	0	13.8	13.0 (8.7-16.6)	92.9 (70.0-150)	1158 (510-5050)	3.7 (0.6)	1.5 ± 0.2	R
		Khoribari	80	20	13	1	16.3	5.0	11.8	24.7 (19.0-29.7)	160 (105-359)	1764 (649-12900)	4.8 (0.4)	1.6 ± 0.3	R
	Jalpaiguri	Dhupguri	160	40	33	0	20.6	0	20.6	17.6 (13.9-20.9)	75.1 (62.1-99.8)	482 (287-1100)	2.5 (0.8)	2.0 ± 0.2	R
		Malbazar	80	20	21	1	26.3	5.0	22.4	18.2 (14.1-21.7)	86.1 (68.6-123)	633 (346-1730)	2.0 (0.8)	1.9 ± 0.2	R
		Matiali	80	20	23	1	28.8	5.0	25.0	19.9 (15.8-23.4)	86.9 (69.7-123)	575 (323-1480)	2.3 (0.8)	2.0 ± 0.3	R
U. Dinajpur	Itahar	160	40	29	0	18.1	0	18.1	10.3 (6.8-13.4)	68.5 (55.2-96.0)	778 (391-2500)	3.1 (0.7)	1.6 ± 0.2	R	
<i>Cx. pseudovishnui</i>	Darjeeling	Phansidewa	80	20	27	0	33.8	0	33.8	17.2 (11.7-19.9)	44.3 (37.3-57.6)	150 (127-295)	16.4 (0.006)	3.1 ± 0.3	R
		Matigara	80	20	20	0	25.0	0	25.0	12.7 (9.6-15.4)	54.6 (47.2-66.4)	355 (230-689)	4.1 (0.5)	2.0 ± 0.2	R
		Khoribari	80	20	24	1	30.0	5.0	26.3	16.2 (11.2-18.9)	41.7 (35.2-53.0)	140 (118-259)	15.8 (0.007)	3.1 ± 0.2	R
	Jalpaiguri	Dhupguri	80	20	25	0	31.3	0	31.3	22.3 (13.0-30.1)	509 (191-10446)	28100 (2730-41600000)	1.1 (0.96)	0.9 ± 0.2	R
		Malbazar	80	20	60	1	75.0	5.0	73.7	16.4 (13.8-18.6)	44.7 (40.9-49.8)	163 (128-225)	10.5 (0.06)	2.9 ± 0.2	R
		Matiali	80	20	42	0	52.5	0	52.5	14.3 (10.9-17.3)	65.5 (54.9-84.4)	460 (277-1024)	10.9 (0.05)	1.9 ± 0.2	R
U. Dinajpur	Itahar	80	20	29	1	36.3	5.0	32.9	70.3 (54.6-142)	265 (135-2749)	1448 (405-130000)	1.3 (0.94)	2.2 ± 0.6	R	
<i>Cx. gelidus</i>	Darjeeling	Phansidewa	80	20	49	0	61.3	0	61.3	11.5 (9.5-13.3)	31.7 (29.2-34.5)	116 (95.9-150)	8.3 (0.1)	2.9 ± 0.2	R
		Matigara	80	20	64	0	80.0	0	80.0	18.9 (12.6-21.6)	43.7 (36.5-57.4)	128 (115-249)	21.8 (0.0006)	3.5 ± 0.3	R
		Khoribari	80	20	39	1	48.8	5.0	46.0	5.0 (1.0-4.8)	24.2 (15.2-34.8)	183 (196-1090)	23.4 (0.0003)	1.8 ± 0.2	R
	Jalpaiguri	Dhupguri	160	40	35	2	21.9	5.0	17.8	9.4 (5.2-11.4)	34.5 (27.8-45.3)	183 (145-439)	13.9 (0.01)	2.3 ± 0.2	R
		Malbazar	80	20	31	0	38.8	0	38.8	8.3 (6.2-10.3)	32.4 (29.1-36.2)	185 (137-285)	6.3 (0.27)	2.2 ± 0.2	R
		Matiali	80	20	29	0	36.3	0	36.3	7.1 (3.3-10.6)	89.1 (64.1-165)	2291 (753-21400)	4.8 (0.44)	1.2 ± 0.2	R
U. Dinajpur	Itahar	160	40	48	0	30.0	0	30.0	5.7 (1.4-9.9)	162 (91.0-685)	11891 (1822-1620000)	1.0 (0.9)	0.9 ± 0.2	R	

*: T, test; C, control; CM, corrected mortality.

#: S, susceptible (CM ≥ 98%); R, confirmed resistance (CM < 90%); PR, possible resistance (CM = 90–97%).

working in paddy fields or in tea gardens. Ponds, irrigation canals, riverbed pools, and unused wells were the mosquito breeding sites throughout the year, whereas paddy fields, rainwater pools, cement tanks, and culverts were the seasonal breeding sites. The climatic conditions of all study sites were humid and subtropical in nature, and the temperature varied from 8°C in winter to 40°C in summer.

Vector population: During the study period, 14 different species of mosquitoes were identified in the collected samples. The percentages of identified mosquitoes were as follows: *Cx. quinquefasciatus* (13.06%), *Cx. tritaeniorhynchus* (16.10%), *Cx. pseudovishnui* (7.31%), *Cx. vishnui* (2.96%), *Cx. gelidus* (14.82%), *Cx. sitiens* (0.51%), *Cx. whitmorei* (0.46%), *Anopheles stephensi* (13.71%), *An. dirus* (3.43%), *An. minimus* (2.01%), *Armigeres subalbatus* (11.18%), *Mansonia uniformis* (5.62%), *Ma. annulifera* (2.30%), and *Aedes albopictus* (6.54%). Among them, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* were the major JE vectors in the study areas.

Insecticide susceptibility status: Two- to three-day-old, laboratory hatched, unfed female mosquitoes of 4 different species were used for the insecticide susceptibility bioassay against 3 insecticides: 4% DDT, 0.05% deltamethrin, and 5% malathion.

DDT: The results of the adult susceptibility bioassay of *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* toward 4% DDT are given in Table 2. After 24 h of holding, the overall CM rate was below 90% in all 4 species throughout the study areas. The KDT values determined for DDT for all 4 vector species showed a straight line in Ldp analysis and fol-

lowed a linear regression model for knockdown with time (Table 2). The knockdown effect of DDT toward different species of JE vector during a 1 h exposure is given in Fig. 2 (A–D). By analyzing the values of CM and knockdown effects, we found that all the 4 JE vector species were highly tolerant to DDT at all study sites.

Deltamethrin: Table 3 shows the results of the adult susceptibility bioassay of *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus* toward 0.05% deltamethrin. It is evident from Table 3 that the *Cx. tritaeniorhynchus*, *Cx. vishnui*, and *Cx. pseudovishnui* populations at all the study sites were tolerant to 0.05% deltamethrin. In contrast, *Cx. gelidus* populations of Phansidewa, Matigara, and Khoribari were susceptible to deltamethrin, with CM > 98%. *Cx. gelidus* populations of Dhupguri, Matiali, and Itahar were tolerant to deltamethrin with CM < 90%, whereas CM was 91.3% in Malbazar, showing probable resistance. The KDT values for all 4 vector species showed a linear relation in Ldp analysis and followed a linear regression model for knockdown with time (Table 3). The knockdown effect of deltamethrin against 4 different species of JE vectors during a 1 h exposure is given in Fig. 3 (A–D).

Malathion: The results of adult susceptibility bioassay of 4 major JE vectors against 5% malathion are given in Table 4. *Cx. tritaeniorhynchus* of all the sites was tolerant to 5% malathion but other 3 species were found to be susceptible to it. The KDT values determined toward malathion for all 4 vector species showed a linear effect in Ldp analysis and followed a linear regression model for knockdown with time (Table 4). The knockdown effect of malathion against 4 different species of JE vector during a 1 h exposure is given in Fig. 4 (A–D).

Fig. 2. Knock down rate (KDR) of JE vectors against 4% DDT. (A) *Cx. tritaeniorhynchus*, (B) *Cx. vishnui*, (C) *Cx. pseudovishnui*, (D) *Cx. gelidus*.

Resistant Status of JE Vectors in India

Table 3. Insecticides susceptibility status of JE vectors against 0.05% deltamethrin in West Bengal

JE vectors	Districts	Blocks	Mosquito exposed		Mosquito died		Observed mortality (%)		CM* (%)	KDT ₁₀ (95% CI)	KDT ₅₀ (95% CI)	KDT ₉₅ (95% CI)	χ^2 (<i>p</i>)	Slope	Status#
			T*	C*	T*	C*	T*	C*							
<i>Cx. tritaeniorhynchus</i>	Darjeeling	Phansidewa	285	70	111	8	38.9	11.4	31.1	17.5 (9.9-23.3)	291(142-1778)	10700 (1760-1120000)	3.3 (0.6)	1.1 ± 0.2	R
		Matigara	240	60	145	3	60.4	5.0	58.3	8.8 (6.2-11.2)	42.4 (37.2-49.9)	319 (208-610)	2.4 (0.7)	1.9 ± 0.2	R
		Khoribari	80	20	16	0	20.0	0	20.0	3.4 (0.5-6.8)	119 (71.8-418)	11500 (1720-1860000)	3.5 (0.6)	0.8 ± 0.2	R
	Jalpaiguri	Dhupguri	80	20	20	1	25.0	5.0	21.1	8.7 (5.3-11.7)	64.5 (51.9-90.4)	843 (408-2950)	1.4 (0.9)	1.5 ± 0.2	R
		Malbazar	80	20	18	1	22.5	5.0	18.4	11.7 (8.1-14.8)	68.1 (55.6-92.7)	651 (350-1800)	1.1 (0.9)	1.7 ± 0.2	R
		Matiali	80	20	19	2	23.8	10.0	15.3	9.4 (6.1-12.3)	60.3 (49.7-80.5)	657 (347-1900)	1.1 (0.9)	1.6 ± 0.2	R
U. Dinajpur	Itahar	80	20	16	1	20.0	5.0	15.8	7.0 (4.2-9.6)	47.7 (40.3-60.1)	556 (304-1500)	1.3 (0.9)	1.5 ± 0.2	R	
<i>Cx. vishnui</i>	Darjeeling	Phansidewa	80	20	31	0	38.8	0	38.8	8.6 (5.4-11.4)	56.7 (47.0-74.6)	639 (339-1800)	0.6 (0.9)	1.6 ± 0.2	R
		Matigara	80	20	27	0	33.8	0	33.8	15.4 (6.9-17.8)	64.9 (55.4-138)	413 (407-809)	17.9 (0.003)	2.0 ± 0.2	R
		Khoribari	80	20	30	1	37.5	5.0	34.2	11.1 (8.2-13.7)	49.4 (43.1-59.2)	335 (219-640)	1.0 (0.9)	1.9 ± 0.2	R
	Jalpaiguri	Dhupguri	160	40	99	1	61.9	2.5	60.9	5.7 (3.5-7.8)	31.9 (28.0-36.8)	292 (188-576)	5.3 (0.3)	1.7 ± 0.1	R
		Malbazar	80	20	47	1	58.8	5.0	56.6	7.3 (5.4-9.1)	26.5 (23.9-29.3)	138 (107-197)	6.7 (0.24)	2.3 ± 0.2	R
		Matiali	80	20	44	1	55.0	5.0	52.6	7.7 (5.7-9.6)	29.5 (26.6-32.8)	165 (124-245)	7.9 (0.1)	2.2 ± 0.2	R
U. Dinajpur	Itahar	160	40	47	1	29.4	2.5	27.6	10.6 (7.7-13.2)	49.9 (43.2-60.3)	362 (231-723)	0.6 (0.9)	1.9 ± 0.2	R	
<i>Cx. pseudovishnui</i>	Darjeeling	Phansidewa	80	20	50	0	62.5	0	62.5	6.5 (4.8-8.0)	21.7 (19.5-23.8)	102 (83-136)	7.3 (0.2)	2.4 ± 0.2	R
		Matigara	80	20	55	0	68.8	0	68.8	5.9 (4.2-7.5)	22.2 (19.8-24.7)	123 (95.9-174)	7.1 (0.2)	2.2 ± 0.1	R
		Khoribari	80	20	58	2	72.5	10.0	69.4	6.4 (4.7-8.0)	22.9 (20.5-25.3)	118 (93.1-163)	5.0 (0.4)	2.3 ± 0.2	R
	Jalpaiguri	Dhupguri	80	20	49	0	61.3	0	61.3	6.2 (4.4-8.0)	25.7 (22.9-28.7)	159 (119-241)	6.9 (0.2)	2.1 ± 0.1	R
		Malbazar	240	60	126	1	52.5	1.67	51.7	10.6 (7.9-12.9)	43.6 (38.6-50.6)	268 (185-463)	1.3 (0.9)	2.1 ± 0.2	R
		Matiali	80	20	44	0	55.0	0	55.0	6.9 (4.9-8.8)	28.5 (25.6-31.9)	175 (129-269)	5.1 (0.4)	2.1 ± 0.1	R
U. Dinajpur	Itahar	80	20	23	0	28.8	0	28.8	7.9 (4.4-9.4)	25.1 (19.5-31.5)	110 (92.5-212)	17.4 (0.004)	2.6 ± 0.2	R	
<i>Cx. gelidus</i>	Darjeeling	Phansidewa	80	20	79	1	98.8	5.0	98.7	3.9 (2.5-5.2)	14.8 (12.6-16.8)	83.2 (66.9-113)	8.4 (0.1)	2.2 ± 0.2	S
		Matigara	80	20	79	0	98.8	0	98.8	1.7 (0.7-2.9)	9.3 (6.7-11.5)	79.8 (60.5-123)	3.5 (0.6)	1.8 ± 0.2	S
		Khoribari	80	20	80	1	100	5.0	100	2.9 (0.6-3.2)	12.4 (6.6-15.1)	79.4 (69.3-209)	18.5 (0.002)	2.0 ± 0.2	S
	Jalpaiguri	Dhupguri	160	40	122	0	76.3	0	76.3	2.0 (0.9-3.3)	11.7 (8.9-14.2)	110 (81.1-180)	3.1 (0.6)	1.7 ± 0.1	R
		Malbazar	80	20	73	0	91.3	0	91.3	2.3 (1.2-3.5)	10.7 (8.3-12.8)	77.0 (60.4-110)	1.8 (0.8)	1.9 ± 0.2	PR
		Matiali	160	40	133	0	83.1	0	83.1	3.4 (2.1-4.6)	12.8 (10.6-14.7)	71.1 (57.7-95.3)	0.7 (0.9)	2.2 ± 0.2	R
U. Dinajpur	Itahar	160	40	82	0	51.3	0	51.3	4.4 (2.8-6.0)	19.6 (17.1-22.1)	133 (99.8-200)	6.7 (0.2)	1.9 ± 0.1	R	

*: T, test; C, control; CM, corrected mortality.

#: S, susceptible (CM ≥ 98%); R, confirmed resistance (CM < 90%); PR, possible resistance (CM = 90–97%).

Fig. 3. Knock down rate (KDR) of JE vectors against 0.05% deltamethrin. (A) *Cx. tritaeniorhynchus*, (B) *Cx. vishnui*, (C) *Cx. pseudovishnui*, (D) *Cx. gelidus*.

Table 4. Insecticides susceptibility status of JE vectors against 5% malathion in West Bengal

JE vectors	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*							
Cx. tritaeniorhynchus	Darjeeling	Phansidewa	180	45	49	8	27.2	17.8	11.5	5.6 (2.7-8.6)	60.4 (47.7-88.9)	1260 (514-6700)	0.4 (0.9)	1.2 ± 0.1	R
		Matigara	160	40	92	3	57.5	7.5	54.1	6.9 (4.6-9.1)	36.5 (32.1-42.5)	308 (199-601)	4.5 (0.4)	1.8 ± 0.2	R
		Khoribari	80	20	6	1	7.50	5.0	2.6	9.4 (4.1-13.9)	162 (96.2—524)	6300 (1340-230000)	5.5 (0.3)	1.0 ± 0.2	R
	Jalpaiguri	Dhupguri	80	20	22	1	27.5	5.0	23.7	14.9 (10.5-18.6)	95.3 (72.2-152)	1030 (470-4040)	3.9 (0.5)	1.6 ± 0.2	R
		Malbazar	80	20	26	1	32.5	5.0	29	15.0 (10.8-18.5)	88.3 (68.6-133)	857 (422-2900)	3.9 (0.5)	1.7 ± 0.2	R
		Matiali	80	20	19	1	23.8	5.0	19.7	11.9 (7.8-15.3)	84.1 (64.8-130)	1039 (475-4100)	3.1 (0.6)	1.5 ± 0.2	R
U.Dinajpur	Itahar	80	20	13	1	16.3	5.0	11.8	8.3 (4.0-12.2)	109 (74.3-228)	2900 (874-35000)	0.5 (0.9)	1.1 ± 0.2	R	
Cx. vishnui	Darjeeling	Phansidewa	80	20	79	1	98.8	5.0	98.7	13.8 (10.6-15.9)	30.1 (26.1-34.8)	81.8 (70.1-112)	12.8 (0.02)	3.8 ± 0.2	S
		Matigara	80	20	80	0	100	0	100	13.2 (8.5-14.8)	27.5 (21.8-34.3)	70.6 (63.4-115)	27.8 (< 0.0001)	4.0 ± 0.2	S
		Khoribari	80	20	78	1	97.5	5.0	97.4	14.1 (12.2-15.8)	32.9 (30.6-35.4)	97.1 (83.4-118)	5.1 (0.4)	3.5 ± 0.2	PR
	Jalpaiguri	Dhupguri	160	40	160	1	100	2.5	100	17.5 (15.7-19.1)	31.0 (29.3-32.8)	64.6 (58.6-72.9)	8.6 (0.1)	5.1 ± 0.3	S
		Malbazar	80	20	79	1	98.8	5.0	98.7	17.5 (11.1-19.5)	35.9 (28.6-46.4)	89.9 (84.2-162)	33.6 (< 0.0001)	4.1 ± 0.2	S
		Matiali	80	20	80	0	100	0	100	20.4 (11.7-21.6)	36.2 (27.6-47.6)	75.4 (75.4-146)	50.9 (< 0.0001)	5.2 ± 0.3	S
U.Dinajpur	Itahar	80	20	80	0	100	0	100	12.3 (9.3-14.1)	25.1 (21.1-29.4)	62.6 (54.7-83.7)	19.2 (0.002)	4.2 ± 0.2	S	
Cx. pseudovishnui	Darjeeling	Phansidewa	80	20	80	0	100	0	100	15.7 (9.1-17.3)	33.7 (26.0-45.0)	90.2 (86.2-178)	38.4 (< 0.0001)	3.8 ± 0.3	S
		Matigara	80	20	80	0	100	0	100	14.7 (8.6-16.3)	32.3 (24.8-42.9)	88.6 (83.7-172)	37.4 (< 0.0001)	3.8 ± 0.3	S
		Khoribari	80	20	79	0	98.8	0	98.8	13.3 (11.3-15.2)	33.9 (31.5-36.8)	113 (94.2-142)	6.7 (0.2)	3.2 ± 0.2	S
	Jalpaiguri	Dhupguri	80	20	79	0	98.8	0	98.8	16.1 (8.9-17.1)	37.1 (28.8-51.9)	109 (108-250)	36.6 (< 0.0001)	3.5 ± 0.3	S
		Malbazar	160	40	159	1	99.4	2.5	99.4	13.2 (11.2-14.9)	33.2 (30.8-35.9)	109 (91.4-136)	5.0 (0.4)	3.2 ± 0.2	S
		Matiali	80	20	79	1	98.8	5.0	98.7	14.1 (9.8-16.0)	29.8 (24.5-36.3)	78.5 (69.1-118)	23.2 (0.0003)	3.9 ± 0.3	S
U.Dinajpur	Itahar	160	40	119	0	74.4	0	74.4	22.3 (19.4-24.7)	51.8 (47.5-57.6)	153 (123-208)	5.5 (0.3)	3.5 ± 0.3	R	
Cx. gelidus	Darjeeling	Phansidewa	80	20	77	2	96.3	10.0	95.8	12.6 (8.2-14.5)	31.0 (25.1-38.8)	99.0 (86.3-168)	21.3 (0.0007)	3.3 ± 0.2	PR
		Matigara	80	20	77	0	96.3	0	96.3	12.1 (8.3-13.8)	26.0 (21.1-31.5)	69.6 (60.9-103)	23.2 (0.0003)	3.8 ± 0.2	PR
		Khoribari	160	40	159	2	99.4	5.0	99.3	11.5 (5.2-12.3)	32.8 (24.2-47.6)	126 (129-364)	34.1 (< 0.00001)	2.8 ± 0.2	S
	Jalpaiguri	Dhupguri	160	40	157	1	98.1	2.5	98.1	9.7 (6.2-11.5)	27.8 (22.6-34.1)	107 (89.4-184)	15.3 (0.009)	2.8 ± 0.2	S
		Malbazar	80	20	80	0	100	0	100	6.7 (3.6-7.9)	20.7 (15.6-25.5)	88.4 (74.6-160)	17.4 (0.003)	2.6 ± 0.2	S
		Matiali	80	20	80	0	100	0	100	9.1 (2.8-8.4)	27.9 (18.3-42.6)	119 (142-493)	42.6 (< 0.0001)	2.6 ± 0.2	S
U.Dinajpur	Itahar	160	40	157	1	98.1	2.50	98.1	12.3 (5.6-13.6)	41.2 (32.3-64.8)	194 (197-753)	25.9 (0.0001)	2.4 ± 0.2	S	

*: T, test; C, control; CM, corrected mortality.

#: S, susceptible (CM ≥ 98%); R, confirmed resistance (CM < 90%); PR, possible resistance (CM = 90–97%).

Fig. 4. Knock down rate (KDR) of JE vectors against 5% Malathion. (A) *Cx. tritaeniorhynchus*, (B) *Cx. vishnui*, (C) *Cx. pseudovishnui*, (D) *Cx. gelidus*.

DISCUSSION

Susceptibility status of 4 major JE vectors toward 3 insecticides was assessed in 7 blocks of 3 districts of northern West Bengal. All the study blocks were endemic for JE, malaria, and 2 blocks—Khoribari and Phansidewa—were endemic for kala-azar too. In all study districts, DDT was used for indoor residual spraying, deltamethrin was employed for the LLINs, and malathion served for fogging occasionally, when any outbreak of JE occurred. Therefore, these 3 insecticides were chosen for the susceptibility tests. Out of 7 study blocks, 5 were characterized by paddy fields and tea gardens, and 2 blocks were surrounded by paddy fields only. Because the JE vector mosquitoes breed in paddy fields, they were under tremendous pressure from insecticides, which were used to control agricultural pests. DDT was first introduced for mosquito control in 1946; unfortunately, the first case of DDT resistance occurred in *Aedes* sp. in 1947, just after 1 year of implementation of DDT treatment. Since then, several species of mosquitoes have been found to be resistant to DDT (25). In India, DDT resistance in *An. culicifacies* was first reported in 1959 in the Panch Mahal district of Gujarat (26), and then spread throughout the country. It is evident from our study that all 4 major JE vector species were highly tolerant to DDT with a significantly lower CM value (< 90%), higher KDTs, and lower KDRs. Similar results have also been reported in other parts of the country (19,20). Other vector mosquitoes for malaria and dengue were also found to be resistant to DDT throughout the country (14–16, 27–30). Therefore, the use of DDT as an insecticide to control JE vectors is questionable, particularly in the 7 study blocks.

Both pyrethroid and DDT target the voltage-gated sodium channel and they share the same mechanism of resistance. At present, pyrethroids are widely used in agricultural fields in the study areas, and pyrethroid-treated bed nets have been introduced by NVBDCP to reduce the transmission of malaria. A significant proportion of pyrethroid-resistant mosquitoes was found to be associated with agricultural activity, indicating that the 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. Accordingly, the vector mosquitoes were under tremendous pressure from pyrethroid for a long period, which is reflected by the higher level of tolerance to it. Other vector mosquito species were found to be tolerant to pyrethroid, which were reported from other parts of the country (14–16,18,21).

Thermal fogging of malathion is usually done in India to reduce the counts of JE vectors (31). During the last few years, malathion fogging was conducted sporadically to manage JE outbreaks in the villages under study. Malathion was used widely in paddy fields and also in tea gardens for pest control. JE vectors breed in paddy fields; therefore, the JE vector population was under strong pressure from malathion, which might be a cause of malathion tolerance in the analyzed areas. In the present study, *Cx. tritaeniorhynchus* showed higher degree of tolerance to malathion in all study blocks but

the other 3 species were susceptible to malathion. It is hardly possible to explain the species-specific difference in malathion susceptibility. Similarly, malathion resistance was reported from different parts of the country in different species of *Anopheles* (15,16,21), *Aedes* (14,18), and *Culex* (19). We used an adult bioassay to evaluate the susceptibility to different insecticides as it is considered to be the steadiest method. Two other methods—an enzyme bioassay and molecular marker analyses—have been developed to quickly test the involvement of major resistance mechanisms; these have not been used in the present study.

In this study, it was observed that all 4 major JE vector species were highly tolerant to DDT. Thus, the policy makers have to think about the replacement of the use of DDT as a vector control measure. In Darjeeling, deltamethrin still is effective against *Cx. gelidus* only. Malathion resistance was detected in the *Cx. tritaeniorhynchus* population of study areas but 3 other species—*Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. gelidus*—were susceptible to malathion. Among the 4 JE vectors, *Cx. tritaeniorhynchus* was the predominant species followed by *Cx. gelidus*. Given that 3 JE vector species, other than *Cx. tritaeniorhynchus*, were susceptible to malathion, the use of malathion may be continued. Such a study in other parts of the state, along with an enzyme bioassay and molecular marker detection, will be helpful for better understanding the insecticide susceptibility status of the JE vectors.

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Conflict of interest None to declare.

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