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Akash Chowdhury
Department of Zoology, A. P. C. Roy Govt. College, Himachal Vihar, Siliguri, West Bengal, India

Shampa Dutta
Department of Zoology, Katwa College, Katwa, Purba Bardwan, West Bengal, India

Joyanta Karmakar
Department of Microbiology, ESIC Medical College, Joka, Kolkata, West Bengal, India

Madhusudan Das
Department of Zoology, University of Calcutta, Ballygunge Circular Rd, Ballygunge, Kolkata, West Bengal, India

Ardhendu Kumar Maji
Department of Microbiology, Calcutta School of Tropical Medicine, C. R. Avenue, Kolkata, West Bengal, India

Pabitra Saha
Department of Zoology, P. R. Thakur Govt. College, Thakurnagar, North 24, Parganas, West Bengal, India

Corresponding Author:
Pabitra Saha
Department of Zoology, P. R. Thakur Govt. College, Thakurnagar, North 24, Parganas, West Bengal, India

Susceptibility status of *Anopheles culicifacies* and *Anopheles stephensi* to different insecticides in malaria endemic areas of Northern West Bengal, India

Akash Chowdhury, Shampa Dutta, Joyanta Karmakar, Madhusudan Das, Ardhendu Kumar Maji and Pabitra Saha

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Abstract
Informations about insecticide susceptibility status among malaria vectors is helpful for detecting emerging resistance trends, selecting appropriate insecticides and evaluate the impact of vector control interventions. Present study assessed the insecticide susceptibility status of *Anopheles culicifacies* and *Anopheles stephensi* against DDT, deltamethin, malathion and alpha-cypermethrin across five districts of northern West Bengal using WHO bioassay protocol and kit. Both species showed high resistance to DDT, with corrected mortality (CM) rates below 79%. Against pyrethroids, *An. culicifacies* populations from Darjeeling, Uttar Dinajpur, and Malda and *An. stephensi* from Darjeeling and Malda exhibited probable resistance, suggesting emerging tolerance trends. In contrast, malathion remained largely effective in most areas. The spatial variation in resistance levels reflects differing ecological conditions and insecticide exposure histories. Persistent DDT resistance and rising pyrethroid tolerance highlight the need for regular monitoring, insecticide rotation, and integrated vector management strategies to sustain malaria control and support India's malaria elimination program.

Keywords: Malaria vector, *Anopheles stephensi*, *Anopheles culicifacies*, Corrected mortality, Knock down rate, West Bengal

1. Introduction
Malaria continues to be one of the most life-threatening vector-borne diseases affecting human populations across tropical and subtropical regions of the world. Despite substantial progress in its control and elimination in many countries, malaria still poses a significant public health threat, many tropical and subtropical countries, including India [1]. According to the World Health Organization (WHO), malaria control largely depends on the effective management of vector populations by using insecticide-based interventions such as indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) [2]. However, the sustainability of these strategies is increasingly being challenged by the growing problem of insecticide resistance among malaria vector species.

In India, *Anopheles culicifacies* and *Anopheles stephensi* are recognized as two of the most important malaria vectors, each occupying distinct ecological niches [3]. *An. culicifacies*, the predominant vector in rural and peri-urban regions, is responsible for transmitting nearly 60-70% of malaria cases in India [4]. It breeds mainly in clean, sunlit, and stagnant water bodies such as irrigation channels, ponds, and wells. This species complex comprises several sibling species (A, B, C, D, and E) that differ in their vectorial capacity, behavior, and susceptibility to insecticides which makes the vector control efforts more complex [4]. On the other hand, *An. stephensi* is primarily an urban vector that thrives in man-made habitats, particularly overhead tanks, cisterns, and other domestic water storage containers. Its adaptation to urban environments has made it a major concern not only in India but also in neighboring and Middle Eastern countries, where it has expanded its distribution in recent years [5].

The success of malaria control programs has historically been linked to the effective use of chemical insecticides.

In India, vector control depends mainly on four major classes of insecticides i.e., organochlorines (e.g., DDT), organophosphates (e.g., malathion), carbamates (e.g., bendiocarb), and synthetic pyrethroids (e.g., deltamethrin, alpha-cypermethrin, lambda-cyhalothrin) [6]. While these insecticides have played a crucial role in reducing vector densities and disease transmission, but their prolonged and extensive use has exerted intense selection pressure on mosquito populations which leads to the development of resistance. Reports of resistance in *An. culicifacies* and *An. stephensi* to multiple insecticides have reported from various parts of India, raising serious concerns about the continued efficacy of conventional control measures [7-20].

Insecticide resistance is a multifaceted phenomenon that can result from various mechanisms, including target-site mutations, increased detoxification enzyme activity, reduced cuticular penetration, or behavioral changes that reduce contact with insecticides [21]. The knockdown resistance (*kdr*) mutations in the voltage-gated sodium channel (*vgsc*) gene, conferring resistance to DDT and pyrethroids, and acetylcholinesterase (*Ace-1*) mutations linked to organophosphate and carbamate resistance, are among the most well-documented genetic mechanisms [22]. Additionally, metabolic resistance mediated by elevated levels of cytochrome P450 monooxygenases, esterases, and glutathione-S-transferases further complicates the resistance mechanisms [23]. The interplay of these mechanisms often results in cross-resistance, making it challenging to restore vector susceptibility even after switching insecticides.

Understanding the insecticide susceptibility status of *Anopheles* vectors is therefore vital for guiding evidence-based vector control strategies and ensuring the success of malaria elimination programs. Regular monitoring of susceptibility patterns helps detect emerging resistance trends and evaluate the impact of control interventions. It also helps in selecting appropriate insecticides for IRS and LLIN programs and in formulating effective insecticide resistance management strategies, such as rotation, mosaic, or mixture approaches.

The present study was aimed to evaluate the susceptibility status of *An. culicifacies* and *An. stephensi* against four major insecticides such as 4% DDT, 0.05% Deltamethrin, 0.05% Alpha-cypermethrin and 5% Malathion across different ecological zones of northern part of West Bengal, India.

2. Materials and methods

2.1. Study areas: The present study was carried outduring January, 2021 to December, 2022 in five northern districts of West Bengal. The study districts were Darjeeling, Jalpaiguri, Cooch Behar, Uttar Dinajpur and Malda. In each district, one village was selected as study site. The study villages were selected after analysis of last five years malaria cases and in consultation with district health authorities. All the study districts were located on the northern side of the River Ganga. Darjeeling and Jalpaiguri is located at the foot hills of Himalayas whereas Cooch Behar, Uttar Dinajpur and Malda are in the plain alluvial land. The geographical location and demography of the study villages are described in Table 1.

Table 1: Geographical location and demography of the study villages

District	Block	Village	Location	Nature
Darjeeling	Matigara	Kawakhali	$26^0$41’23”N $88^0$23’36”E	Semi Urban
Jalpaiguri	Sadar	Patkata	26^0 54’70”N $88^0$70’91”E	Semi Urban
Coochbehar	Haldibari	Volarhat	$26^0$33’00”N $88^0$77’00”E	Rural
Uttar Dinajpur	Chopra	Lakhipur	$26^0$36’83”N $88^0$31’25”E	Rural
Malda	English Bazer	Doulatpur	$25^0$08’00”N $88^0$14’60”E	Rural

2.2. Collections and identification of mosquitoes: Adult unfed *Anopheles* mosquitoes were collected from human dwellings and cattle sheds of the study villages during morning (from 8.00 to 10.00 am) and dusk (from 6.00 to 8.00 pm) with the help of mouth aspirators (John W. Hock, USA) and torches. After collection, the mosquitoes were released into the cages and supplied with 10% sucrose solution soaked in cotton. The cages were labelled with collection site, date and time and transported to the laboratory for species identification and insecticide susceptibility bio-assays. The mosquitoes were kept for a day in dark place for acclimatization in laboratory conditions (temperature 25 °C ± 2 °C; relativehumidity 70% ± 10%). Laboratory acclimatized female *Anopheles* mosquitoes were used for insecticide susceptibility bioassay. After bioassay, adult mosquitoes were killed by freezing and identified to the species level based on morphological traits, utilizing taxonomic keys and descriptions provided by Nagpal *et al*. (2005) [24] and Tyagi *et al*. (2015) [25].

2.3. Insecticide susceptibility bioassay: The insecticide susceptibility bioassay for adult *Anopheles* mosquitoes was carried out following the WHO (2016) guidelines for malaria vector testing using the standard WHO test kit [26]. Wild-caught, unfed, and laboratory-acclimatized adult female *Anopheles* mosquitoes were exposed to four insecticides, namely 4% DDT, 0.05% deltamethrin, 5% malathion, and 0.05% alpha-cypermethrin. The test kits and insecticide-impregnated papers were obtained from the Vector Control Research Unit (VCRU), Universiti Sains Malaysia, a WHO collaborating center.Each bioassay setup consisted of five holding tubes, where four served as ‘Test’ groups and one as ‘Control’. Approximately 20-25 female mosquitoes were introduced into each tube and allowed to rest for one hour before exposure. Subsequently, ‘Test’ group mosquitoes from the holding tubes were transferred into exposure tubes lined internally with insecticide-treated papers. The ‘Control’ group was placed into tubes lined with risella oil (for DDT), silicone oil (for deltamethrin/alpha-cypermethrin) and olive oil (for malathion) - the corresponding solvents for each insecticide type. The mosquitoes were exposed to used insecticides for one hour.

During exposure, cumulative mortality was recorded at 10, 15, 20, 30, 40, 50, and 60 minutes. After exposure, mosquitoes were transferred back to clean holding tubes and maintained for 24 hours with a 10% sucrose solution provided on cotton pads. After the recovery period, mortality was recorded to assess the susceptibility status of each population, as per WHO criteria (2016) [26]. All mosquitoes i.e., both alive

and dead were morphologically re-identified and stored individually at -20 °C for subsequent use.

2.4. Data analysis: After completion of the adult bioassay, Observed Mortality (OM) was calculated by following formula:

$$\textbf{Observed Mortality [OM]}(\%) = \frac{\text{Total no. of dead mosquitoes X 100}}{\text{Total mosquito exposed}}$$

When mortality in control tube is greater than 5% but lessthan 20%, then the observed mortality was corrected by using the following Abbot's formula to calculate the Corrected Mortality (CM):

$$\textbf{Corrected Mortality [CM]}\ (\%) = \frac{(\%\text{ of observed mortality} - \%\text{ of control mortality}) \text{ X } 100}{(100 - \%\text{ of control mortality})}$$

For adult Insecticide susceptibility bioassays, resistant/susceptible status was classified according to WHO recommendation (WHO, 2016) [26]. Mosquitoes were denoted as Susceptible (S) if the Corrected Mortality (CM) rate was greater than 98.00%, Resistant (R) if CM rate was less than 90.00% and mortality rate between 90-98% as Possible Resistance (PR) for convenience (WHO, 2016) [26]. The cumulative knocked down rates (KDR) were calculated by observing the number of knocked down mosquitoes after 10, 15, 20, 30, 40, 50 and 60 minutes during one hour exposure period. Knockdown time (KDT_{10}, KDT_{50} and KDT_{95}) were determined using Log dose probit (Ldp) Line computer programme according to Finney method (<http://www.ehabsoft.com/ldpline) [27].

2.5 Ethical statement: Prior to the commencement of entomological sampling, an awareness meeting was organized at the village level with the participation of community members, local health personnel and administrative authorities. The aims and significance of the research were explained in detail and residents were encouraged to cooperate with the study team during the study period. Verbal consent was obtained from household owners before entering their premises or surrounding areas for mosquito collection. No endangered or protected species were involved in this research work. The research protocol received ethical clearance from the Institutional Ethics Committee of the Calcutta School of Tropical Medicine, Kolkata. Both adult and immature stages of mosquitoes were gathered during field surveys following standard entomological collection techniques.

3. Results

3.1. Insecticide susceptibility status: Wild caught unfed female *Anopheles culicifacies* and *Anopheles stephensi* mosquitoes were exposed to four major insecticides - 4% DDT, 0.05% deltamethrin, 5% malathion and 0.05% alpha cypermethrin. The results of the insecticide susceptibility bioassay for *An. culicifacies* and *An. stephensi* are presented in Table 2 and Table 3, respectively.

3.1.1. DDT

After a 24-hour recovery period, the overall corrected mortality (CM) rate of *An. culicifacies* exposed to 4% DDT was found to be below 79.00% in all study districts. The highest CM was recorded in Jalpaiguri (78.75%), while the lowest was observed in Malda (48.75%). Detailed results of the susceptibility bioassay are presented in Table 2. The knockdown time (KDT) values obtained for 4% DDT showed a linear relationship in the log-dose-probit analysis, indicating that the knockdown followed a linear regression pattern with time (Table 2). The KDT_{50} values ranged from 48.82 minutes in Cooch Behar (lowest) to 103.58 minutes in Malda (highest). The knockdown rate (KDR) over a 1-hour exposure period is illustrated in Fig.1. Based on both CM and knockdown data, it is evident that *An. culicifacies* populations were highly resistant (R) to DDT in all five study locations.

Table 2: Insecticides susceptibility status of *An. culicifacies* against 4% DDT, 0.05% deltamethrin, 5% malathion and 0.05% alpha cypermethrin in northern districts of West Bengal

Insecticides	Dist*	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	JAL	160	20	126	0	78.75	0	78.75	6.90 [3.46-10.08]	57.56 [45.79-83.60]	876.04 [385.17-4130.34]	1.01 (0.96)	1.39±0.22	R
	CBR	144	20	104	0	2.22	0	72.22	11.61 [8.22-14.56]	48.82 [41.90-60.13]	308.13 [194.30-647.49]	0.89 (0.97)	2.05±0.25	R
	DAR	160	20	116	0	72.50	0	72.50	7.872 [4.62-10.81]	49.84 [41.47-65.18]	532.49 [281.66-1607.24]	2.52 (0.77)	1.59±0.22	R
	UD	120	20	80	0	66.67	0	66.67	9.50 [5.69-12.81]	49.61 [41.06-65.71]	413.83 [225.31-1232.65]	0.66 (0.98)	1.78±0.26	R
	MLD	160	20	78	0	48.75	0	48.75	6.35 [2.04-10.42]	103.58 [67.72-68.27]	3727.44 [885.71-18717.61]	2.84 (0.72)	1.05±0.22	R
0.05% DEL	JAL	160	20	157	0	98.13	0	98.13	5.12 [3.38-6.80]	19.23 [16.67-21.69]	105.13 [81.35-152.29]	5.77 (0.32)	2.22±0.22	S
	CBR	176	24	173	0	98.30	0	98.30	3.75 [1.88-5.71]	24.83 [21.09-28.89]	280.74 [172.06-628.99]	1.98 (0.85)	1.56±0.20	S
	DAR	120	20	111	0	92.50	0	92.50	7.07 [5.24-8.74]	18.37 [16.19-20.47]	62.54 [52.05-80.73]	8.48 (0.13)	3.09±0.29	PR
	UD	140	20	129	0	92.14	0	92.14	7.97 [6.29-9.51]	19.92 [17.95-21.85]	64.54 [55.18-79.35]	9.92 (0.07)	3.22±0.26	PR
	MLD	120	20	109	0	90.83	0	90.83	6.90 [4.92-8.73]	20.25 [17.76-22.70]	80.63 [64.94-109.75]	8.33 (0.13)	2.74±0.27	PR
5% MAL	JAL	160	20	157	0	98.13	0	98.13	4.13 [2.60-5.65]	14.79 [12.43-16.93]	75.92 [60.21-106.42]	10.31 (0.06)	2.31±0.24	S
	CBR	160	30	156	0	97.50	0	97.50	4.83 [3.46-6.12]	12.75 [10.98-14.34]	44.32 [38.15-54.22]	2.35 (0.78)	3.04±0.27	PR
	DAR	140	20	140	0	100.0	0	100.0	7.06 [5.66-8.30]	14.20 [12.79-15.52]	34.80 [30.71-41.10]	6.02 (0.30)	4.22±0.37	S
	UD	140	20	136	0	97.14	0	97.14	6.11 [2.67-7.41]	18.41 [13.05-23.11]	75.72 [62.76-157.92]	14.34 (0.01)	2.67±0.26	PR
	MLD	120	20	119	0	99.17	0	99.17	6.85 [4.97-8.55]	17.92 [15.73-20.04]	61.52 [50.52-81.53]	10.13 (0.07)	3.07±0.31	S
0.05% ALPHA	JAL	200	40	196	0	98.00	0	98.00	6.05 [3.83-8.17]	33.24 [29.22-38.43]	295.97 [191.12-580.40]	5.37 (0.37)	1.73±0.19	S
	CBR	200	20	197	0	98.50	0	98.50	3.84 [1.794-6.01]	33.89 [28.82-41.11]	553.58 [287.86-1712.50]	3.38 (0.64)	1.35±0.18	S
	DAR	140	20	128	0	91.43	0	91.43	7.34 [5.58-8.97]	19.66 [17.55-21.74]	69.54 [57.99-89.12]	6.39 (0.26)	2.99±0.26	PR
	UD	160	20	146	0	91.25	0	91.25	7.47 [5.83-8.99]	19.75 [17.80-21.67]	68.75 [58.14-85.92]	4.783 (0.44)	3.03±0.24	PR
	MLD	160	20	145	0	90.63	0	90.63	9.40 [7.56-11.09]	24.82 [22.63-27.11]	86.25 [72.21-109.24]	5.80 (0.32)	3.04±0.24	PR

*JAL = Jalpaiguri, CBR = Cooch Behar, DAR = Darjeeling, UD = Uttar Dinajpur, MLD = Malda; T = Test, C = Control, CM = Corrected Mortality #S = Susceptible (CM ≥98%); R = Confirmed Resistance (CM <90%); PR = Possible Resistance (CM = 90 - 97%)

Table 3: Insecticides susceptibility status of *An. stephensi* against 4% DDT, 0.05% deltamethrin, 5% malathion and 0.05% alpha cypermethrin in northern districts of West Bengal

Insecticides	Dist*	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	JAL	160	40	112	0	70.00	0	70.00	7.86 [4.33-11.05]	57.69 [46.41-81.53]	744.62 [350.81-2943.27]	1.04 (0.95)	1.48±0.22	R
	CBR	160	20	100	0	62.50	0	62.50	6.97 [3.17-10.47]	70.23 [52.93-117.21]	1360.04 [504.94-10084.61]	1.26 (0.93)	1.27±0.22	R
	DAR	160	20	126	0	78.75	0	78.75	6.49 [2.65-10.09]	75.58 [55.30-137.34]	1764.67 [585.42-18273.31]	1.40 (0.92)	1.20±0.22	R
	UD	120	20	83	0	69.17	0	69.17	8.72 [5.95-11.22]	32.27 [28.31-37.24]	172.93 [121.49-299.62]	1.17 (0.94)	2.25±0.26	R
	MLD	160	20	82	0	51.25	0	51.25	7.32 [2.73-11.47]	105.05 [69.35-259.20]	3204.16 [824.41-75726.78]	1.10 (0.95)	1.10±0.22	R
0.05% DEL	JAL	160	20	158	0	98.75	0	98.75	4.85 [3.09-6.57]	19.52 [16.82-22.12]	116.61 [88.15-176.03]	2.89 (0.71)	2.11±0.22	S
	CBR	160	20	157	0	98.13	0	98.13	6.23 [4.47-7.89]	20.42 [18.07-22.74]	93.71 [75.10-127.63]	2.94 (0.70)	2.48±0.22	S
	DAR	120	20	108	0	90.00	0	90.00	7.57 [5.84-9.15]	18.28 [16.28-20.21]	56.66 [48.34-70.16]	5.97 (0.30)	3.34±0.29	PR
	UD	120	20	118	0	98.33	0	98.33	7.24 [2.72-7.98]	20.10 [13.13-26.43]	74.49 [66.66-181.98]	21.5 (0.001)	2.89±0.27	S
	MLD	140	20	128	0	91.43	0	91.43	6.75 [4.11-8.24]	15.77 [12.32-18.71]	46.85 [39.28-68.74]	11.64 (0.04)	3.48±0.30	PR
5% MAL	JAL	160	20	116	0	100.0	0	100.0	5.92 [4.59-7.17]	14.16 [12.56-15.63]	43.29 [38.12-50.95]	8.68 (0.12)	3.38±0.27	S
	CBR	160	20	160	0	100.0	0	100.0	6.85 [5.25-8.34]	18.70 [16.74-20.60]	67.86 [57.29-85.07]	3.08 (0.68)	2.93±0.24	S
	DAR	160	20	160	0	100.0	0	100.0	7.01 [4.38-8.62]	18.40 [14.72-21.76]	63.47 [52.58-94.47]	11.10 (0.04)	3.05±0.24	S
	UD	120	20	118	0	98.33	0	98.33	7.19 [5.32-8.91]	19.26 [17.00-21.46]	68.19 [56.46-88.64]	10.43 (0.06)	2.99±0.28	S
	MLD	160	20	154	0	96.25	0	96.25	8.67 [5.95-10.29]	20.06 [16.38-23.57]	58.80 [49.98-82.15]	13.50 (0.01)	3.52±0.24	PR
0.05% ALPHA	JAL	200	40	196	0	98.00	0	98.00	7.49 [5.32-9.52]	32.42 [28.99-36.63]	212.49 [151.17-348.83]	4.21 (0.51)	2.01±0.19	S
	CBR	200	30	197	0	98.50	0	98.50	5.14 [3.24-7.00]	26.34 [23.17-29.84]	214.48 [147.78-375.27]	1.79 (0.87)	1.80±0.19	S
	DAR	200	40	185	0	92.50	0	92.50	8.59 [7.12-9.95]	21.39 [19.68-23.10]	69.01 [59.96-82.48]	7.83 (0.16)	3.23±0.22	PR
	UD	120	20	118	0	98.33	0	98.33	7.31 [5.41-9.06]	19.78 [17.48-22.03]	70.86 [58.45-92.65]	6.38 (0.27)	2.96±0.28	S
	MLD	160	20	147	0	91.88	0	91.88	5.91 [3.17-8.54]	39.81 [33.69-49.60]	459.89 [249.17-1329.17]	0.97 (0.96)	1.54±0.21	PR

*JAL = Jalpaiguri, CBR = Cooch Behar, DAR = Darjeeling, UD = Uttar Dinajpur, MLD = Malda; T = Test, C = Control, CM = Corrected Mortality #S = Susceptible (CM ≥98%); R = Confirmed Resistance (CM <90%); PR = Possible Resistance (CM = 90 - 97%)

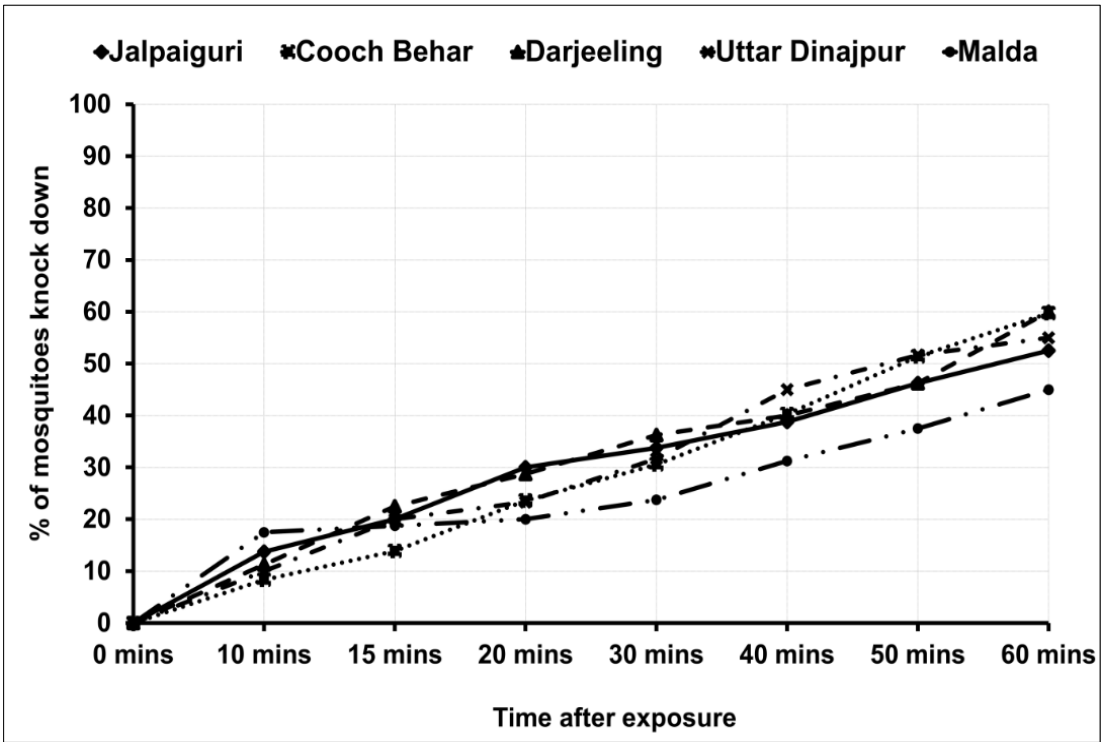

Fig 1: Knock down rate of *An. culicifacies* against 4% DDT in northern districts of West Bengal

Similarly, the overall CM rate of *An. stephensi* against 4% DDT after 24 hours of recovery was below 79.00% across all districts. The maximum CM was observed in Darjeeling (78.75%), while the minimum was recorded in Malda (51.25%). The detailed susceptibility bioassay data for *An. stephensi* are presented in Table 3. The KDT values also exhibited a linear trend in the log-dose-probit analysis, fitting a linear regression model for knockdown progression over time (Table 3). The KDT_{50} values varied from 32.27 minutes in Uttar Dinajpur (lowest) to 105.05 minutes in Malda (highest). The 1-hour knockdown rate is shown in Fig. 2. Analysis of CM and knockdown parameters clearly indicates that *An. stephensi* populations were highly resistant (R) to DDT in all five study districts.

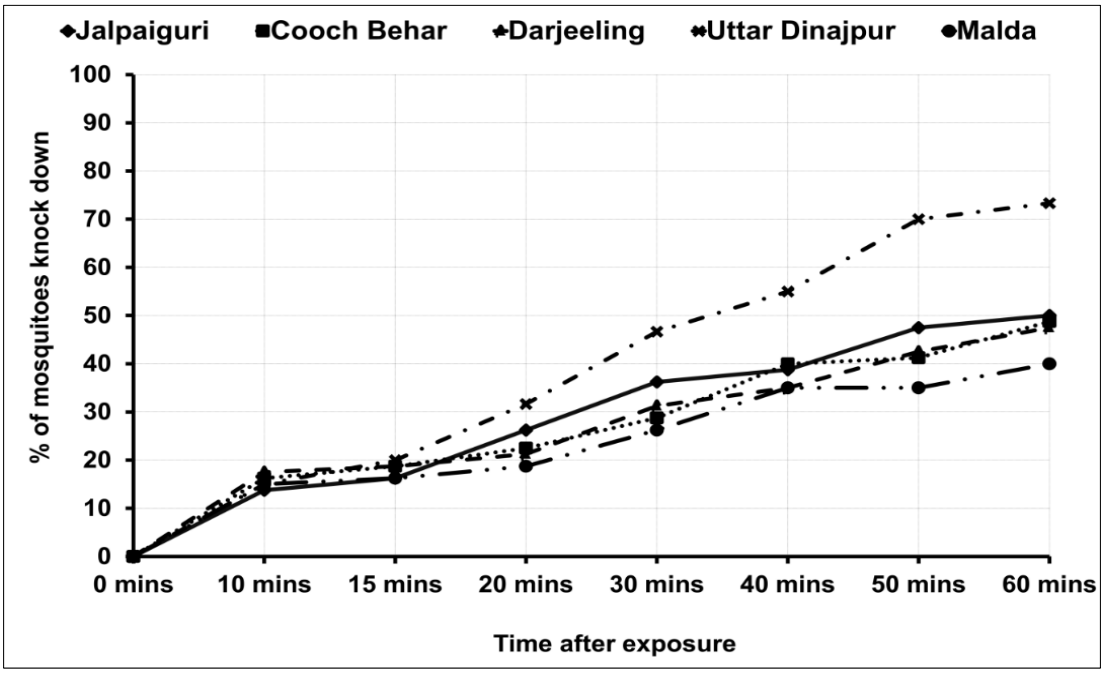

Fig 2: Knock down rate of *An. stephensi* against 4% DDT in northern districts of West Bengal

3.1.2. Deltamethrin

The results of the susceptibility bioassay of *An. culicifacies* against 0.05% deltamethrin are presented in Table 2. The overall corrected mortality (CM) of *An. culicifacies* ranged between 90.83% and 98.30%. The highest CM was recorded in Cooch Behar (98.30%), while the lowest occurred in Malda (90.83%). The knockdown time (KDT) data for 0.05% deltamethrin exhibited a linear trend in the log-dose-probit analysis, indicating a good fit to the linear regression model for knockdown over time (Table 2). The KDT_{50} values varied among districts, with the maximum observed in Cooch Behar (24.83 minutes) and the minimum in Darjeeling (18.37 minutes). The knock down rate (KDR) after 1 hour of exposure is illustrated in Fig. 3. The *An. culicifacies* populations from Darjeeling, Uttar Dinajpur, and Malda showed probable resistance (PR) to 0.05% deltamethrin, whereas those from Jalpaiguri and Cooch Behar were found to be susceptible (S).

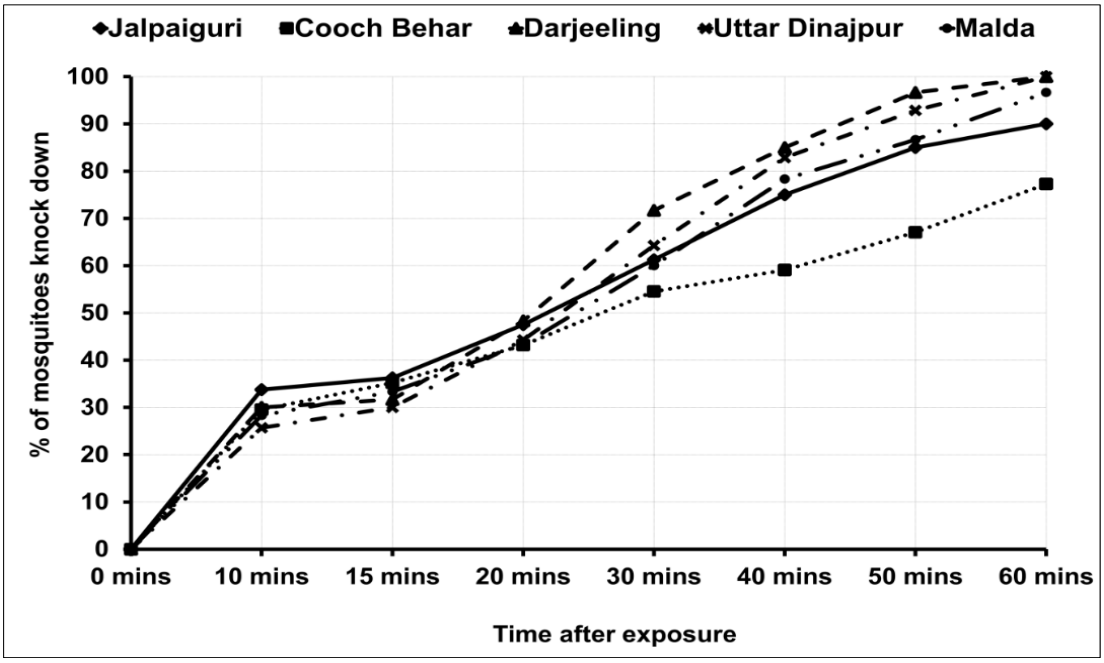

Fig 3: Knock down rate of *An. culicifacies* against 0.05% deltamethrin in northern districts of West Bengal

Similarly, the detailed susceptibility bioassay results of *An. stephensi* against 0.05% deltamethrin across study districts are provided in Table 3. After 24 hours of recovery, the CM values ranged from 90.00% to 98.75%, with Jalpaiguri showing the highest CM (98.75%) and Darjeeling the lowest (90.00%). The KDT values followed a linear regression pattern, confirming a consistent knockdown trend with time (Table 3). The KDT_{50} ranged from 18.28 minutes in Darjeeling (lowest) to 20.42 minutes in Cooch Behar (highest). The KDR over the 1-hour exposure period is shown

in Fig.4. From the analysis of CM and knockdown data, it is evident that *An. stephensi* populations from Darjeeling and Malda exhibited probable resistance (PR) to deltamethrin, whereas those from Jalpaiguri, Cooch Behar, and Uttar Dinajpur remained susceptible (S).

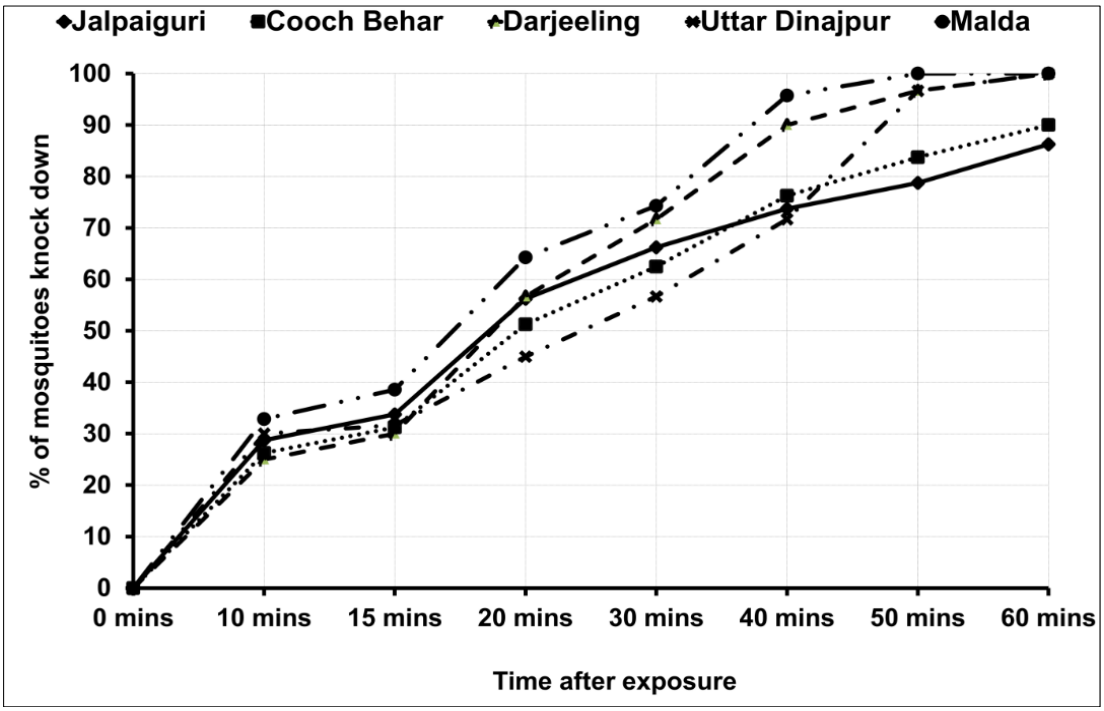

Fig 4: Knock down rate of *An. stephensi* against 0.05% deltamethrin in northern districts of West Bengal

3.1.3. Malathion

The corrected mortality (CM) rate of *An. culicifacies* exposed to 5% malathion varied from 97.14% to 100.00% across all study districts. The highest CM was observed in Darjeeling (100.00%), while the lowest occurred in Uttar Dinajpur (97.14%). Detailed results of the susceptibility bioassay are presented in Table 2. The knockdown time (KDT) data for 5% malathion revealed a linear relationship between probit and log-time values, confirming a good fit to the regression model (Table 2). The KDT_{50} values ranged from 12.75 minutes in Cooch Behar (lowest) to 18.41 minutes in Uttar Dinajpur (highest). The knockdown rate (KDR) over the 1-hour exposure period is illustrated in Figure 5. Based on CM and KDT analyses, *An. culicifacies* populations from Jalpaiguri, Darjeeling, and Malda were found to be susceptible to 5% malathion, whereas those from Cooch Behar and Uttar Dinajpur exhibited probable resistance (PR).

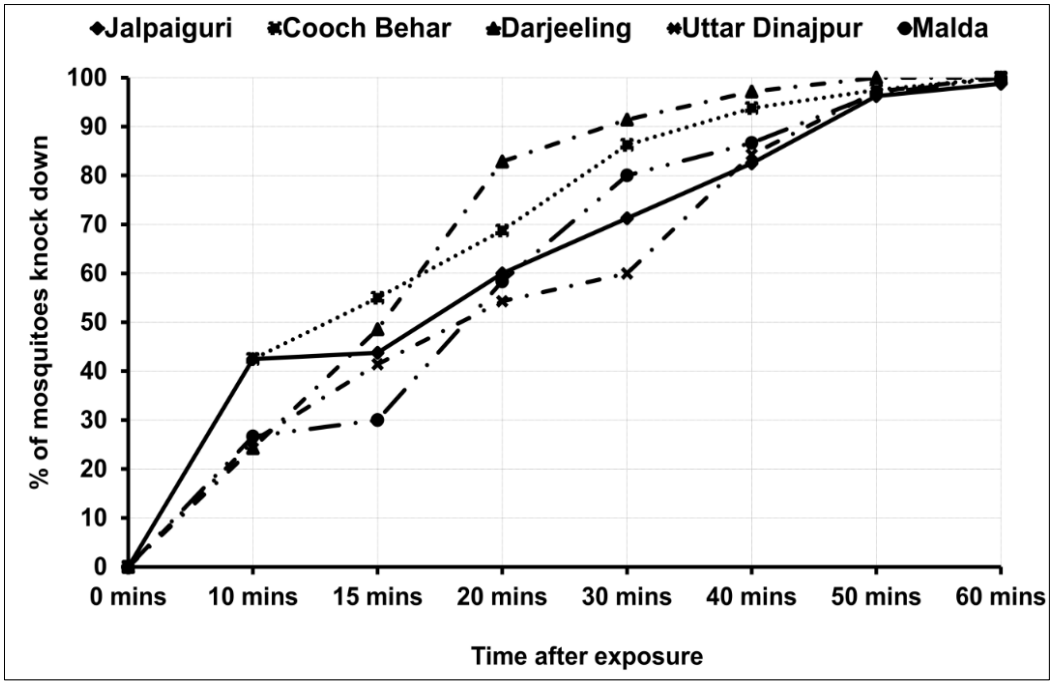

Fig 5: Knock down rate of *An. culicifacies* against 5% malathion in northern districts of West Bengal

In the case of *An. stephensi*, the CM rate after exposure to 5% malathion ranged from 96.25% to 100.00% across study districts. The maximum CM was recorded in Jalpaiguri, Cooch Behar, and Darjeeling (100.00%), while Malda showed the lowest CM (96.25%). The detailed district-wise susceptibility results are presented in Table 3. The KDT values for *An. stephensi* displayed a straight-line relationship in log-dose-probit analysis, consistent with a linear regression model (Table 3). The KDT_{50} values ranged from 14.16 minutes in Jalpaiguri (lowest) to 20.06 minutes in Malda (highest). The knockdown rate over a 1-hour exposure period is shown in Fig. 6. Overall, *An. stephensi* populations from all study districts were susceptible to malathion, except for the population from Malda, which showed probable resistance (PR).

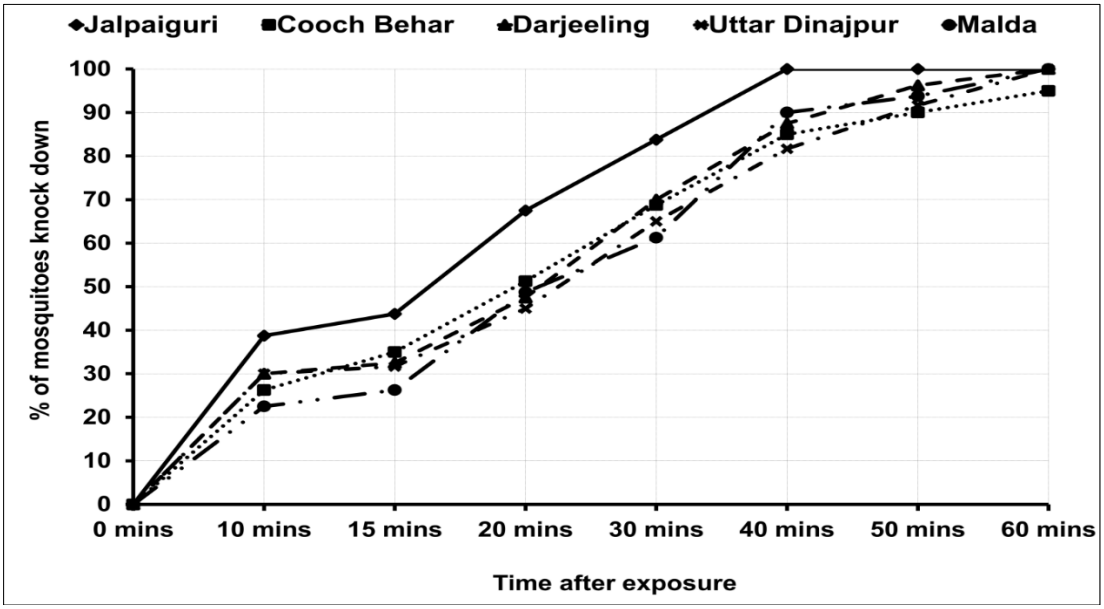

Fig 6: Knock down rate of *An. stephensi* against 5% malathion in northern districts of West Bengal

3.1.4. Alpha-cypermethrin

The susceptibility test results of *An. culicifacies* against 0.05% alpha-cypermethrin across different study districts are summarized in Table 2. The corrected mortality (CM) rate of *An. culicifacies* ranged from 90.63% to 98.50%. The highest mortality was noted in Cooch Behar (98.50%), while the lowest was recorded in Malda (90.63%). The KDT values for 0.05% alpha-cypermethrin showed a linear relationship between log-dose and probit, indicating a good fit to the linear regression model of knockdown with time (Table 2). The KDT_{50} values varied across districts, with the highest observed in Cooch Behar (33.89 mins) and the lowest in Darjeeling (19.66 mins). The knockdown rate (KDR) over the 1-hour exposure period is depicted in Fig.7. The*An. culicifacies* populations from Darjeeling, Uttar Dinajpur, and Malda showed probable resistance (PR) to 0.05% alpha-cypermethrin, while populations from Jalpaiguri and Cooch Behar were susceptible (S).

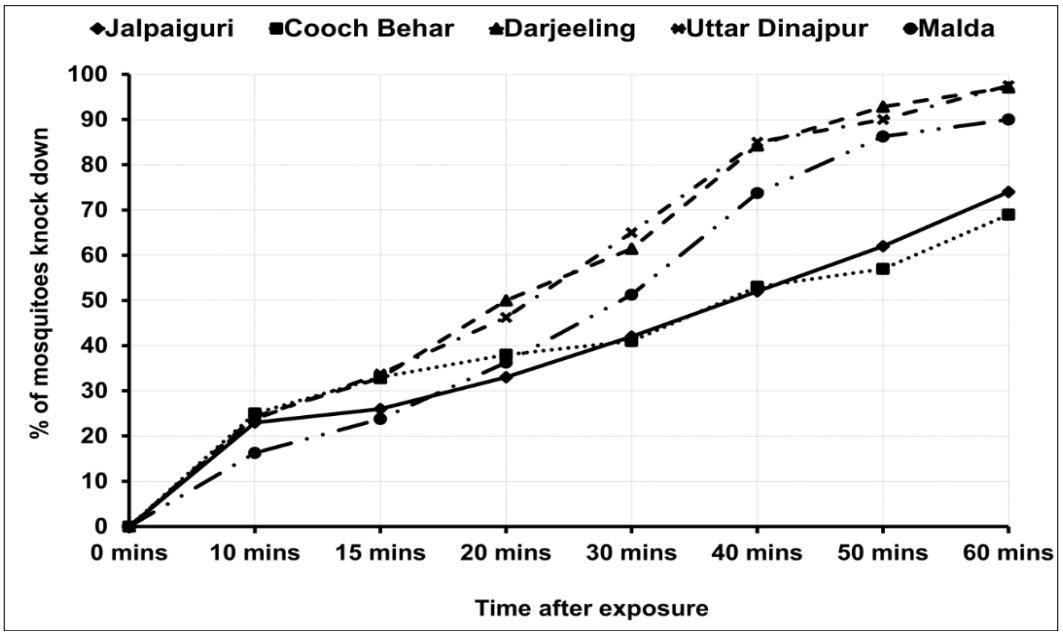

Fig 7: Knock down rate of *An. culicifacies* against 0.05% alpha-cypermethrin in northern districts of West Bengal

Similarly, the district-wise susceptibility results of *An. stephensi* against 0.05% alpha-cypermethrin are presented in Table 3. After 24 hours of recovery, the CM values ranged between 91.88% and 98.50%, with the highest mortality recorded in Cooch Behar (98.50%) and the lowest in Malda (91.88%). The KDT values followed a linear regression pattern, confirming a consistent knockdown trend with exposure time (Table 3). The KDT_{50} varied from 19.78 mins in Uttar Dinajpur (lowest) to 39.81 mins in Malda (highest). The 1-hour KDR profile is shown in Fig. 8. Analysis of CM and knockdown data revealed that *An. stephensi* populations from Darjeeling and Malda exhibited probable resistance (PR) to alpha-cypermethrin, whereas those from Jalpaiguri, Cooch Behar, and Uttar Dinajpur were found to be susceptible (S).

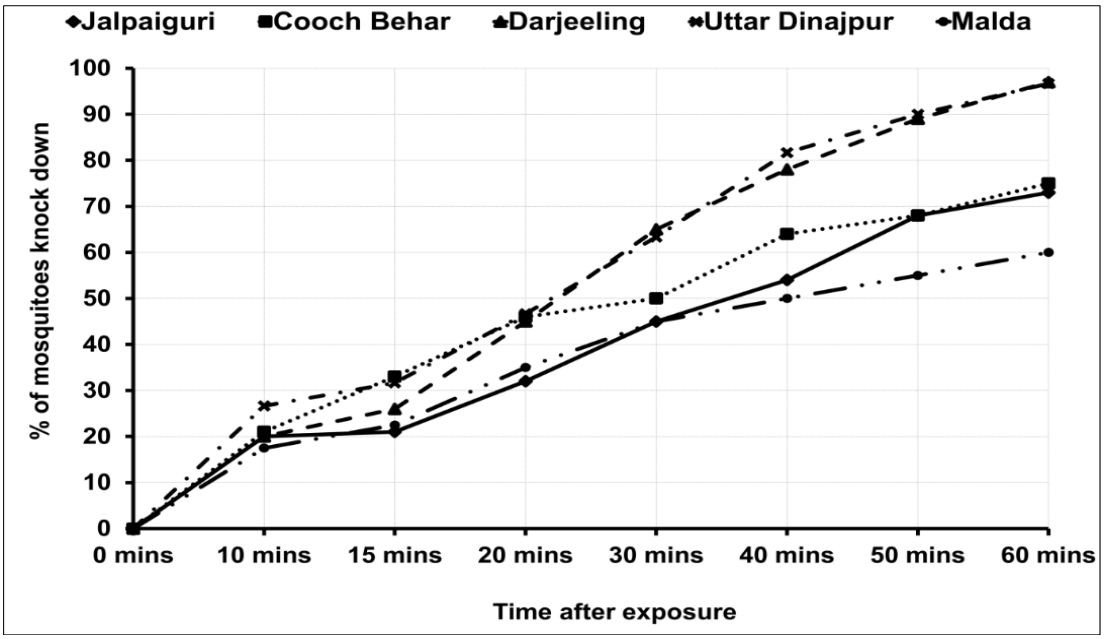

Fig 8: Knock down rate of *An. stephensi* against 0.05% alpha-cypermethrin in northern districts of West Bengal

4. Discussion

The present study investigated the insecticide susceptibility status of two major malaria vectors i.e., *An. culicifacies* and *An. stephensi* across diverse ecological zones of northern part of West Bengal. The findings revealed variable susceptibility patterns against four major insecticides such as DDT, deltamethrin, malathion, and alpha-cypermethrin, reflecting a complex resistance scenario that aligns with national and global trends of increasing insecticide resistance in malaria vectors.

Both *An. culicifacies* and *An. stephensi* exhibited high levels of resistance to 4% DDT, with CM rates consistently below 79% across all study districts. These results are consistent with earlier reports from several parts of India, including Madhya Pradesh, Odisha, Gujarat, and Assam, where *An. culicifacies* populations have demonstrated long-standing resistance to DDT [8, 9, 11, 13]. Similarly, widespread DDT resistance in *An. stephensi* has been reported from Rajasthan, Tamil Nadu, and Delhi [14, 16, 19]. The persistence of DDT resistance even after decades of limited use indicates the stability of resistance mechanisms, likely due to*kdr* mutations in the voltage-gated sodium channel gene. This genetic factor is known to confer cross-resistance to both DDT and pyrethroids [21, 22]. The prolonged KDT_{50} values observed in both species further support this interpretation, suggesting reduced sensitivity of the nervous system to DDT-induced knockdown.

Pyrethroids remain a cornerstone of vector control, particularly through LLINs and IRS. However, reduced efficacy due to emerging resistance is increasingly reported in India. In this study, *An. culicifacies* populations from Darjeeling, Uttar Dinajpur, and Malda exhibited probable resistance (CM between 90-98%) to both deltamethrin and alpha-cypermethrin, while populations from Jalpaiguri and Cooch Behar remained susceptible. Similarly, *An. stephensi* from Darjeeling and Malda showed probable resistance to both pyrethroids. Comparable findings have been reported from southern and western India, where *An. culicifacies* showed partial resistance to deltamethrin and alpha-cypermethrin [10, 15, 18]. A study byRaghavendra *et al*. (2022) also noted declining mortality rates in *An. culicifacies* from urban areas of Gujarat, suggesting selection pressure due to continuous exposure to pyrethroids through domestic and agricultural usage [6].

The spatial heterogeneity in resistance levels observed across districts may be attributed to local variations in insecticide exposure history, ecological conditions, and species composition of *An. culicifacies* sibling species complex. Elevated KDT_{50} values in some districts, particularly for alpha-cypermethrin, further indicate the onset of resistance development. These results emphasize the need for close monitoring, as pyrethroid resistance directly threatens the operational success of LLINs, which form the backbone of India's malaria elimination program.

Both *An. culicifacies* and *An. stephensi* populations showed high susceptibility to 5% malathion, with CM values between 96-100%, except for slight reductions in Uttar Dinajpur and Malda. These findings are in line with reports from central and eastern India, where most *An. culicifacies* populations remain susceptible or show only early signs of reduced susceptibility to malathion [6, 17]. However, the detection of probable resistance in certain sites is concerning, as organophosphate resistance, once established, can spread rapidly due to the Ace-1 mutation and elevated esterase

activity [23]. The consistent susceptibility in Darjeeling and Jalpaiguri suggests limited exposure to malathion in these regions, possibly due to the predominance of IRS with pyrethroids rather than organophosphates.

Comparatively, *An. stephensi* demonstrated slightly higher corrected mortality values and faster KDT_{50}s than *An. culicifacies* for most insecticides, suggesting greater susceptibility overall. This may be due to ecological differences - *An. stephensi* being more urban and exposed to intermittent vector control interventions, whereas *An. culicifacies* populations in rural areas face continuous selection pressure due to agricultural insecticide use. The observed differences highlight the necessity of tailoring vector control strategies according to species distribution and local ecological conditions.

The persistence of DDT resistance and the emergence of probable resistance to pyrethroids underscore the urgent need for resistance management strategies. Continued reliance on pyrethroids alone may undermine malaria control efforts in northern West Bengal. Rotational use of insecticides with different modes of action, integration of non-chemical control measures, and periodic susceptibility monitoring are essential steps toward sustaining control efficacy. Furthermore, molecular investigations targeting *kdr* and metabolic resistance markers should be prioritized to elucidate underlying resistance mechanisms in local vector populations.As per WHO guidelines (2016), insecticide rotation, mixture use, and integrated vector management should be prioritized to delay the spread of resistance[26]. Introducing alternative insecticide classes such as neonicotinoids or clothianidin-based formulations could also be considered in pilot IRS programs. Moreover, the inclusion of molecular monitoring for *kdr* and metabolic resistance markers in surveillance programs would provide early warning of resistance trends.

Conclusions

This study provides updated evidence of the insecticide resistance status of *An. culicifacies* and *An. stephensi* in northern West Bengal. Both species exhibited widespread DDT resistance and emerging pyrethroid resistance, while malathion remained largely effective. The findings highlight the necessity for adaptive insecticide resistance management, informed decision-making for IRS and LLIN deployment, and continuous entomological surveillance to support India's malaria elimination goals.

Conflicts of interest

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

Acknowledgement

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