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Insecticide susceptibility status of Malaria vectors in Mizoram, North East India

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Abstract
Mizoram is one of the hilly forested states in India and has the highest malaria cases based on population wise. Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are malaria vector control measures but the emergence of insecticide resistance presents a significant challenge to the effectiveness of vector control strategies. The survey was conducted in 3 highly malaria endemic districts to generate data on insecticide resistance in malaria vectors. Susceptibility tests were conducted on adult vector species as per the WHO standard technique with wild-caught mosquitoes. Female mosquitoes were exposed in 3 to 4 replicates on each occasion to the impregnated papers with specified discriminating dosages of the insecticides (DDT: 4%, deltamethrin: 0.05%, and alphacypermethrin: 0.05%), for one hour, and mortality was recorded after a 24-hour holding. DDT (4%) resistance was detected among the vector species in all 3 districts with mortality ranging from 64% to 84.3%. Deltamethrin was found susceptible while the possibility of resistance was also observed. Alpha-cypermethrin was found susceptible to all the selected study sites (98.75-100% mortality). Regular monitoring of insecticide susceptibility in malaria vectors is needed for implementing effective vector management strategies.

Keywords: Vector, *Anopheles*, susceptibility, DDT, Delmatherin, Alphacypermethrin

Introduction
Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases have been threatening human and animals. There are 38 genera of mosquitoes worldwide wherein three genera (*Anopheles, Aedes* and *Culex*) were the most important one transmitting dengue fever, yellow fever, malaria, filariasis, chikungunya and encephalitis (Adityaa *et al*., 2006) [1]. In India, 58 *Anopheles* species has been described and six of them were primary vectors of malaria in the country, viz., *Anopheles culicifacies, An. fluviatilis, An. minimus, An. baimaii, An. stephensi* and *An. sundaicus*. In addition, some species of *Anopheles* also play a secondary role in specific geographic areas (Kumar *et al*. 2022) [11].
Vector control is a key component of malaria control strategies and is primarily implemented through indoor residual spraying (IRS) with appropriate insecticides, the use of long-lasting insecticidal nets (LLINs), and larviciding, particularly in urban settings. The National Vector Borne Disease Control Programme (NVBDCP) has approved insecticides from three major classes for use in these interventions: organochlorines (e.g., DDT), organophosphates (e.g., malathion), and synthetic pyrethroids (e.g., deltamethrin, cyfluthrin, lambda-cyhalothrin, bifenthrin, and alpha-cypermethrin) have been approved by The National Vector Borne Disease Control Programme (NVBDCP) for IRS. Prolonged exposure of *Anopheles* vectors to these insecticides has led to the development of resistance in mosquito populations (Das *et al*., 2021) [7]. Consequently, routine monitoring of insecticide susceptibility in local malaria vectors is essential to inform and guide evidence-based vector control strategies.
WHO developed the *Global Technical Strategy for Malaria 2016–2030* which advocates for the rapid intensification of global malaria elimination initiatives. *The Strategy for Malaria Elimination in the Greater Mekong Sub region (2016–2030)* sets 2030 as a target for the six Greater Mekong Sub-region countries. Similarly, the Asia Pacific Leaders Malaria Alliance (APLMA), of which India is a member, has set a target for malaria elimination in all countries of the Asia Pacific region by 2030. National Framework for Malaria Elimination 2016-2030 has divided the states into three categories (I, II, III) based on Annual Parasitic Incidence (I: <1, II: 1-2 and III>2). Mizoram has reported the highest malaria incidence in India, based on the Annual Parasite Incidence (API) over the past three years, and remains

classified as a Category III state, whereas several other states have progressed to the pre-elimination or elimination phase. Mizoram become the problematic state for malaria elimination in India and this can hamper national strategic plan for malaria elimination. We consider these as the 'hot foci', and pose a major challenge for malaria elimination and hence need special attention. Vector control is an integral part of malaria control in the state and introduction of inappropriate insecticides without a proper understanding of the prevailing resistance mechanisms may lead to enhanced vector resistance and disease control failure. Early detection and knowledge on the resistance status and the underlying mechanisms in vector mosquitoes are essential for effective long-term control of the vector (Kumar *et al*. 2022) [11].

However, there is no relevant information regarding the resistant status of insecticides employed against malaria vectors in Mizoram and the present study investigated and identified the efficacy and resistance status of currently using insecticides in Mizoram.

Material and method

Collection of mosquito

The study was conducted from 2023 to 2025 across major malaria-endemic districts of Mizoram, including Mamit (23°55'N, 92°29'E), Lunglei (22°52'N, 92°43'E), Lawngtlai (22°18'N, 92°41'E), and Kolasib (23°13'N, 92°40'E), encompassing altitudinal ranges from 54 to 1150 meters (Figure 1). Surveys of water bodies were carried out, followed by sampling for immature mosquito stages using the scoop-net method, as per WHO guidelines (WHO, 1975). Collections were conducted between 08:00 and 17:00 hours using a fine mesh larval net (25 cm diameter) mounted on an iron handle, along with plastic tubs of various sizes, dippers, and droppers. Adult mosquitoes were collected using the hand catch (aspirator) method during dusk and midnight hours (16:00–20:00 and 00:00–02:00), both indoors and outdoors, following WHO protocols (WHO, 1975). Ambient temperatures during collections ranged from 21 °C to 36 °C.

Table 1: PHC-wise malaria incidence indicator of study areas during 2021 to 2024.

District	PHC	2021			2022			2023			2024		
		API	ABER	TPR	API	ABER	TPR	API	ABER	TPR	API	ABER	TPR
Mamit	PDS	30.85	26.77	11.52	66.65	36.67	18.15	143.98	57.86	24.81	112.81	41.78	27.0
	MAR	30.85	54.78	16.9	136.25	60.06	22.68	240	92.6	25.92	226.78	80.10	28.31
Lunglei	TLA	27.6	20.51	13.48	44.3	26.87	16.49	60.73	36.04	16.85	25.20	25.87	18.80
	LUN	49.67	31.53	15.75	54.54	34.87	15.64	75.57	47.27	15.99	53.43	43.76	12.18
Lawngtlai	CHT	11.85	24.9	4.7	20.62	32.9	6.29	41.67	33.15	12.5	38.99	32.14	16.71
	BUN	29.09	31.01	15.5	78.57	50.69	15.5	107.35	52.17	20.57	67.91	23.67	23.34

Identification of mosquito

Morphological identification of adult female mosquitoes was performed using key diagnostic characters, including the color patterns of the wings, palpi, and legs, under a dissecting light microscope and with the aid of a hand lens. Species identification was based on illustrated taxonomic keys provided by Das *et al*. (1990) [5], Reuben *et al*. (1994), Nagpal and Sharma (1995) [15], and Oo *et al*. (2005) [16].

Insecticidal Bioassay: Insecticide susceptibility bioassays were conducted on *Anopheles* specimens using WHO-recommended protocols, involving tarsal-contact exposure to insecticide-impregnated papers. Three insecticides were tested: DDT (4%), malathion (5%), and deltamethrin (0.05%). For each bioassay, three to five replicates of 15–20 mosquitoes per collection site were exposed to each insecticide. Species-specific control groups were maintained to assess natural mortality in unexposed mosquitoes. The field laboratory was maintained under controlled conditions, with a temperature of 27 ± 2 °C and relative humidity between 70% and 85%. Mosquitoes were exposed to insecticides for one hour using WHO test tubes, after which they were transferred to holding tubes supplied with 10% glucose-soaked cotton pads for recovery and subsequent mortality observation. Following insecticide exposure, mosquitoes were held for a 24-hour recovery period, after which mortality was assessed by counting the number of dead individuals. If control mortality ranged between 5% and 20%, Abbott's correction formula was applied to adjust the mortality rates. Insecticide susceptibility status was classified according to WHO criteria: corrected mortality >98% indicated susceptibility (S), mortality between 90–97% suggested possible resistance (PR), and mortality <90% confirmed resistance (R)

$$\text{Abbott's formula: } \frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

Result

During the study period, a total of 3698 mosquito vector species representing six different species were collected across six malaria endemic districts from different study sites. The abundance of vector species (table no. 2) was *An. baimaii* (11.87%), *An. minimus* (16.96%), *An. fluviatilis* (23.17%), *An. annularis* (21.34%) *and An. philippinensis* (23.93%). In the present study, insecticide susceptibility tests were conducted across malaria-endemic districts of Mizoram. The results are summarized in Table 3.

DDT (4%)

All vector species tested exhibited resistance to DDT across the study sites, with mortality rates ranging from 64.0% to 84.3%. The highest level of resistance was observed in Mamit district, where *An. annularis* showed the lowest mortality at 60.2%. In contrast, the highest DDT-induced mortality (84.3%) was recorded in *An. baimaii* from Lawngtlai district.

Deltamethrin (0.05%)

Deltamethrin susceptibility varied among vector species and districts. While most species remained susceptible, possible resistance was detected in certain populations. In Lawngtlai district, *An. minimus* and *An. philippinensis* showed 95% and 96% mortality, respectively, indicating suspected resistance. In Lunglei district, *An. annularis* showed 96.8% mortality, also suggesting possible resistance, while other species exhibited full susceptibility (98–99% mortality). In Mamit district, reduced susceptibility was more widespread. *An. annularis* showed 94% mortality, falling within the possible resistance category, whereas *An. fluviatilis* exhibited 99% mortality, indicating susceptibility.

Table 2: Total collection of mosquito vector across three malaria endemic districts (six study sites). Study sites ie. Sil-silsuri, Pds-phuldungsei, Lun-lungsen, Tui-tuichawng, Sai-sawizawh, Cht-chawnge.

Vector Species	**Mamit**		**Lungei**		**Lawngtlai**		**Total (%)**
	Sil	**Pds**	**Lun**	**Tui**	**Sai**	**Cht**	
An. baimaii	64	86	57	88	62	82	439 (11.87)
An. minimus	102	115	134	155	134	88	728 (19.69)
An. fluviatilis	124	134	156	176	143	124	857 (23.17)
An. annularis	112	102	165	175	211	234	789 (21.34)
An. philippinensis	145	165	210	189	152	155	885 (23.93)

Alpha-cypermethrin (0.05%)

All tested vector species were susceptible to alphacypermethrin. In Lunglei district, 100% mortality was observed for all species tested. In Lawngtlai, mortality ranged from 98% to 100%, indicating full susceptibility with slight variability. In Mamit district, 100% mortality was recorded for all species except *An. philippinensis*, which showed 99% mortality.

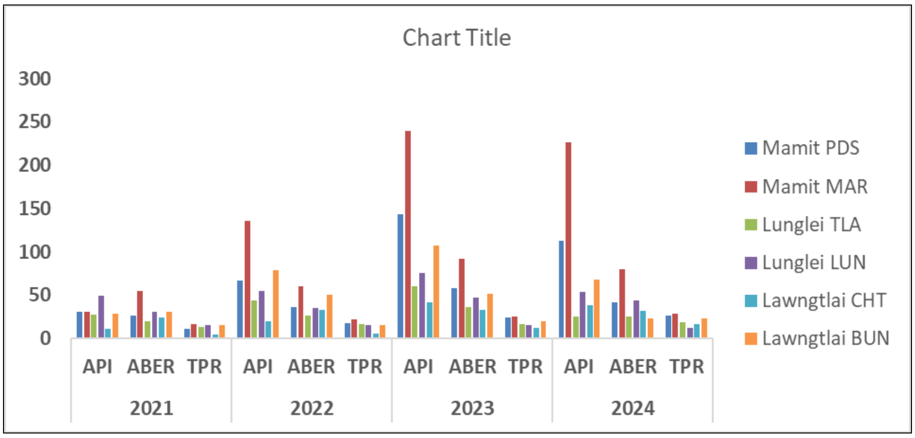

Fig 1: Bar chart showing malaria indicator in 3 malaria endemic study districts.

Table 3: Insecticide susceptibility test conducted in study sites. Anb-*An. baimaii*, Anm- *An. minimus*, Anf- *An. fluviatilis*, Ana- *An. annularis*, Anp- *An. philippinensis.*

Study sites	**Species**	**DDT (4%)**			**Deltamethrin (0.05%)**			**Alphacypermethrin (0.05%)**		
		Sample (n)	**% mor**	**Sus. status**	**Sample (n)**	**% mor**	**Sus. status**	**Sample (n)**	**% mor**	**Sus. status**
Lawngtlai (W. Saizawh,Chawngte areas)	*Anb*	32	84.3	R	31	100	S	62	100	S
	Anm	100	73	R	80	95	PR	80	98.75	S
	Anf	100	84	R	100	98	S	100	100	S
	Ana	66	81.2	R	60	98.3	S	98	100	S
	Anp	105	76.2	R	100	96	PR	102	99.01	S
Lunglei (Lunseng, Tuichawng)	*Anb*	54	77.8	R	52	98	S	83	100	S
	Anm	98	73.46	R	100	98	S	94	100	S
	Anf	100	81	R	100	99	S	100	100	S
	Ana	92	65.2	R	96	96.8	PR	100	100	S
	Anp	100	71	R	100	99	S	100	100	S
Mamit (Silsuri, Phuldungsei)	*Anb*	42	77	R	64	98	S	60	99	S
	Anm	100	65	R	92	96.7	PR	96	100	S
	Anf	100	74	R	96	98.9	S	100	100	S
	Ana	88	60.2	R	100	94	PR	98	100	S
	Anp	100	64	R	100	97	PR	100	99	S

Discussion and Conclusion

Chemical insecticides play a major role in vector control. However, the continuous and indiscriminate use of insecticide in a population will lead to the development of physiological resistance in the insects. In India, the introduction of DDT in the early 1960s as a residual insecticide in public health sprays into the mosquito control program had a major impact on malaria. Bansal and Singh (1996) [3] studied the susceptibility levels of some anophelines, such as *An. culicifacies*, *An. annularis, An. stephensi* and *An. subpictus* from Rajasthan, India and found that all these species were resistant to DDT and dieldrin, but were susceptible to fenitrothion and permethrin. Until 1977, DDT was the principal insecticide used in malaria vector control efforts in Sri Lanka.

The emergence of DDT resistance in *Anopheles culicifacies* and *Anopheles subpictus* was initially identified in Sri Lanka in 1969 (Perera *et al*., 2008) [17]. *An. culicifacies* was resistant to DDT (30%–60% mortality) in all nine districts of Odhisha, *An. culicifacies* and *An. fluviatilis* was found resistant to DDT in the surveyed 12 districts Jharkhand. The present study also found resistance to DDT in all three malaria-endemic districts of Mizoram, which may be due to long-term usage of DDT for vector control in Mizoram and necessitating its replacement to maintain the efficacy of vector control interventions. DDT resistance in the mosquito species *An. culicifacies* and *An. subpictus* was initially detected in Sri Lanka in 1969 (Perera *et al*., 2008) [17]. Following the discontinuation of DDT use, resistance levels gradually declined; however, a resurgence was observed after 1983, attributed to a glutathione S-transferase (GST)-mediated resistance mechanism that was originally selected through prior exposure to DDT (Perera *et al*., 2008) [17]. *An. culicifacies* in the Northeastern region was also resistant to DDT in Assam (Raghavendra *et al*. 2022) [2].

Resistance to DDT and susceptibility to deltamethrin were observed in *An. culicifacies* in all six districts as per the study done by Kumar *et al*. 2022 while *An. culicifacies* was reported to have resistance in ten districts of Himachal Predesh. *An. fluviatilis* collected Kangra and Mandi of Himachal Pradesh found resistant to DDT (mortality range 23–36.5%) but susceptible to malathion (mortality range 98–100%) and deltamethrin (mortality range 99–100%). Our findings also rely on these observations; deltamethrin is also susceptible to malaria vectors in majority of the study sites in the districts but some vectors showed the possibility of resistance. Decreased mortality in deltamethrin found in study areas was due to engagement of deltamethrin for bed net impregnation in the last 10–15 years and the effectiveness may have declined.

Studies in districts like Dungarpur and other areas of Rajasthan have shown that *An. stephensi* and other *Anopheles* species are susceptible to alpha-cypermethrin with mortality rates between 99% and 100%. Similarly, reports from Jharkhand and Odisha have indicated that *An. fluviatilis* is completely susceptible to deltamethrin and other pyrethroid. Susceptibility to malathion, deltamethrin, and permethrin was observed in *An. minimus*, the predominant vector in the North East region (Das, 2021) [7]. In support of the earlier findings, insecticide susceptibility screening in the present study on alphacypermethrin suggested that this insecticide is susceptible (99-100% mortality).

The current study confirms the development of multiple resistance to DDT in most important vectors and highlights the need for effective strategies to manage insecticides. Deltamethrin and alphacypermethrin are still susceptible and will be useful for vector control measures in case of IRS or bed net impregnation. Regular and intensive resistance monitoring should be the guidance for effective vector control. Insecticide resistance in malaria vectors has been a major challenge to vector control programs and updated information to the commonly used insecticides is essential for planning appropriate vector control measures.

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