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Insecticide resistance status of malaria vectors in endemic states of India (2017−2024): A scoping review

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ABSTRACT

Objective: To collate and summarize phenotypic insecticide susceptibility data of Indian malaria vectors from 2017 to 2024, focusing on insecticides used in adult vector control, dichloro diphenyl trichloroethane, malathion, deltamethrin, alpha-cypermethrin, and permethrin to identify resistance patterns to different classes of insecticides.

Methods: The data included information on vector species, location of the study (state/district), insecticide tested, mortality percentage, and susceptibility classification based on the World Health Organization interpretation criteria. Retrospective data were collected from peer-reviewed publications (2017-2024) and up to June 2025. The data were collated for five major malaria vector species, namely *Anopheles* (*An.*) *culicifacies*, *An. fluviatilis*, *An. stephensi*, *An. baimaii*, and *An. minimus*.

Results: Insecticide susceptibility data were available from 86 districts across 16 Indian states for 40 615 mosquitoes. The majority of the data was on *An. culicifacies* (n=28 308), followed by *An. stephensi* (n=5 611), *An. fluviatilis* (n=5 967), *An. baimaii* (n=365), and *An. minimus* (n=364). Intensity bioassays revealed low to moderate resistance levels in *An. culicifacies* populations from selected districts in 3 states, Odisha, Madhya Pradesh, and Chhattisgarh against deltamethrin and alpha-cypermethrin.

Conclusions: This review highlights spatial and species-level variations in insecticide susceptibility among Indian malaria vectors. The low to moderate intensity suggested that it may not yet be severe enough to cause operational failure with current vector control interventions. Continued monitoring of insecticide resistance, as well as the use of new-generation insecticides and interventions, is suggested to sustain vector control efficacy and manage insecticide resistance in malaria vectors to support India's malaria elimination.

KEYWORDS: Malaria vectors; Insecticide resistance; India

Summary

Question: What is the current status of insecticide susceptibility among primary malaria vectors in India based on retrospective phenotypic data from 2017 to 2024?

Findings: *Anopheles culicifacies* is the major vector of malaria and reported resistance to dichloro diphenyl trichloroethane and malathion and particularly to pyrethroids in many studies. However, the intensity levels remain low to moderate, hence, not yet warranting major changes in current vector control measures but needs caution to avoid or delay the onset of resistance due to their continued use in vector control in indoor residual sprays and insecticide treated nets.

Meaning: To maintain vector control efficacy and the progress toward malaria elimination, India need to adopt proactive resistance management strategies, including broader coverage of existing interventions and introduction of new-generation insecticidal interventions with novel mode of actions.

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1. Introduction

The World Health Organization (WHO) has embarked on the elimination of malaria by 2030, and efforts have been escalated by member countries. Many countries have made significant progress, and India has aligned with this global goal. Several countries have already achieved the goal of elimination, including 22 countries from Europe, 11 from America, 4 from Western Pacific, 3 from Africa and 2 countries each from Eastern Mediterranean and South-East Asia[1]. India has launched a national strategic plan to achieve zero indigenous malaria cases by 2027 and elimination by 2030[2,3]. According to the WHO, 249 million malaria cases were estimated from 85 malaria endemic countries and areas (including the territory of French Guiana), a global increase of 5 million cases in 2023 compared to 2021. The WHO South-East Asia Region accounted for about 2% of malaria cases globally. In 2022, the region reported 5.2 million estimated cases and 8 053 estimated deaths that registered 78% and 79% reduction respectively, compared to the year 2010, the largest decline among the WHO regions[4].

Globally, vector control with chemical insecticide interventions remains a reliable strategy of malaria prevention, mainly indoor residual spraying (IRS), insecticide-treated nets (ITNs) including larviciding, and space spraying. Widespread and prolonged use of insecticides resulted in the emergence and intensification of insecticide resistance in disease vector populations. Globally, vector resistance to the four major insecticide classes used in public health (pyrethroids, organochlorines, carbamates, and organophosphates) has been reported in many malaria-endemic countries, including in India. In India, resistance to pyrethroids and other insecticides has been reported in major malaria vectors, particularly, *Anopheles* (*An.*) *culicifacies* and *An. stephensi*, the primary vectors in many endemic regions of the country. Thus, the precipitation of resistance to insecticides in target disease vectors calls for regular monitoring, reporting and management of the insecticide resistance for disease management. The spread and strength of insecticide resistance depend on the intensity of selection pressure in time and space. To date, four different types of insecticide resistance mechanisms are reported: (Ⅰ) metabolic resistance, (Ⅱ) target-site resistance, (Ⅲ) penetration resistance, and (Ⅳ) behavioral resistance[5]. Insecticide resistance is not only conferred *via* multiple resistance mechanisms but also mediated through the interaction of regulatory genes and resistance genes[6]. The former two mechanisms, target-site resistance and metabolic resistance have been extensively explored at the genetic and molecular level. However, geographical spread of resistance to different insecticides varied between the vectors and regions, and in the vectors within the countries of the region and geographical areas within the countries.

Recent global data on insecticide resistance[4] showed that during 2010-2020, of the 88 malaria endemic countries, 78 have detected resistance to at least one insecticide class in at least one malaria vector and one collection site. Twenty-nine countries have detected resistance to pyrethroid, organochlorine, carbamate and organophosphate insecticide across different sites. Moreover, 19 countries have shown confirmed resistance to all the four classes of insecticides in at least one site and at least one local vector. Globally, resistance to pyrethroids was found in at least one malaria vector in 87% of the countries and 68% of the sites. Resistance to organochlorines was detected in 82% of the countries and 64% of the sites, to carbamates in 69% of the countries and 34% of the sites, and to organophosphates in 60% of the countries and 28% of the sites[4]. Of the 38 countries that reported data on the intensity of pyrethroid resistance (2010-2020), high intensity resistance was detected in 27 countries and 293 sites. Sensing the importance of insecticide resistance management for effective malaria control and elimination, the WHO has drafted Global Plan for Insecticide Resistance Management[7] that can be followed for resistance management at country level, and provide technical advocacy.

In India, the current recommended insecticides for various vector control interventions are: (Ⅰ) Adult vector control: IRS with organochlorine (DDT), organophosphate (malathion), and five synthetic pyrethroid insecticides (alpha-cypermethrin, bifenthrin, cyfluthrin, deltamethrin, lambda-cyhalothrin), along with insecticide-treated nets impregnated with deltamethrin/alpha-cypermethrin/permethrin; (Ⅱ) Larval control: Larviciding with organophosphate (temephos), bacterial pesticide (*Bacillus thuringiensis* var. *israelensis*) and two insect growth regulator compounds (diflubenzuron, pyriproxyfen); and (Ⅲ) Space spray: Malathion (technical), cyphenothrin and natural pyrethrum extract[8]. In addition, household insecticidal products are used extensively by the community as personal protection measures, which are not the part of recommended interventions by the national programme in India. These include smoldering coils, vaporizing mats, liquid vaporizer, *etc*., that contain synthetic pyrethroids transfluthrin, allethrin, bioallethrin *etc*., and are also reportedly responsible for development of pyrethroid-resistance in few vector species[9-11].

India is a country of diverse eco-geography with specific vector systems and variable dynamics of resistance development among the vector species with scattered phenotypic resistance. To suggest appropriate insecticide based vector control strategies, collated data on insecticide resistance is essential for effective vector management. A scoping review approach is appropriate for a broad and inclusive mapping of available evidence on insecticide susceptibiity.

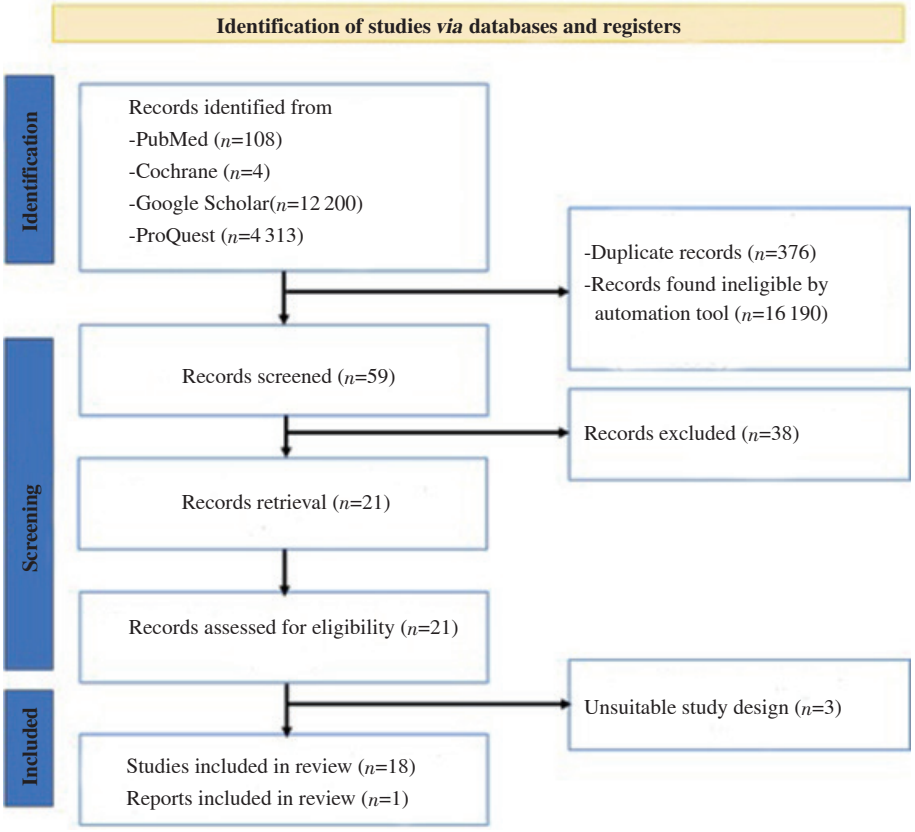

Figure 1. PRISMA flowchart of the selection process.

The present compilation of the retrospective phenotypic insecticide susceptibility data for prevalent adult malaria vectors was for eight years (2017-2024) and upto June, 2025. The data was collated across 86 districts in 16 states. The data were reportedly generated using the WHO method and kit and the results were interpreted following the WHO criterion. This review includes species-wise insecticide susceptibility data for 5 primary malaria vectors, namely *An. culicifacies*, *An. stephensi*, *An. fluviatilis*, *An. minimus* and *An. baimaii*, to the three most commonly used insecticides for adult vector control, DDT, malathion, deltamethrin. Additionally, sparse data were available on other pyrethroid insecticides, such as alpha-cypermethrin and permethrin. This review also present recent intensity bioassay data on *An. culicifacies* in 3 endemic eastern states: Odisha (10 districts), Chhattisgarh (2 districts) and Madhya Pradesh (1 district) to ascertain the operational relevance of ongoing pyrethroid-based interventions for vector control. This scoping review provides an updated descriptive narration of the phenotypic susceptibility data collated from the peer reviewed studies and published reports. The review also provides scope for resistance management as a step to tread that last mile to attain the malaria elimination by date.

Our previous review reported 25-year insecticide susceptibility data (1991-2016) in Indian malaria vectors[12]. This scoping review provides an update on insecticide susceptibility data from important malaria vectors beyond 2016 based on published studies in peer-reviewed journals from 2017 to 2024 and up to June 2025 available in public domain using relevant keywords and including publications in reports. District was the unit for reporting the data.

No formal protocol was registered for this scoping review. However, the review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines[13]. Data were compiled from peer-reviewed publications in the public domain, mainly journal articles and published reports. The last search was conducted on June 30, 2025. A systematic search was conducted using combinations of relevant keywords: “malaria vectors,” “India”, insecticide”, “insecticide susceptibility,” “WHO bioassay,” “*Anopheles*”, “*Anopheles culicifacies*,” “*An. stephensi*,” and “pyrethroid resistance”. Review included data generated following WHO protocol and exposure to WHO-specified discriminatory concentrations. Eligible data sources were identified for inclusion through title and abstract screening, followed by full-text review. Data were collated using a

structured format. The documented data included vector species, year and location (state and district) of testing, insecticide tested and WHO discriminatory concentration, number of mosquitoes tested, percent mortality, resistance interpretation (susceptible, possible resistance, confirmed resistance) and intensity bioassay results where available (1×, 5×, and 10× concentrations of the 1× discriminatory concentration). District was the unit for analysis and mortality response to insecticide exposure was the criterion for assessing the susceptibility status.

A total of 16 625 records were identified through literature searches between 2017 and 2025. After screening based on inclusion criteria (adherence to WHO protocols, reporting of adult susceptibility or intensity bioassays for Indian malaria vectors), 19 studies were included in the final synthesis. A PRISMA flow diagram (Figure 1) illustrates the screening and selection process. The included sources reported phenotypic insecticide susceptibility data for the five malaria vector species-*An. culicifacies*, *An. stephensi*, *An. fluviatilis*, *An. minimus*, and *An. baimaii* across 86 districts in 16 Indian states. The compiled data is presented species-wise and insecticide-wise and susceptibility status in each district of the state. The findings are summarized.

2. Vector prevalence

In India, malaria is reportedly transmitted by six primary vector species in specific ecotypes *viz*., *An. culicifacies* (rural plains), *An. fluviatilis* (foothill and forested areas), *An. stephensi* (urban areas), *An. baimaii* (forested north east), *An. minimus sensu stricto* (forested north east and eastern regions) and *An. sundaicus* (species D) (coastal areas, mainly in Andaman and Nicobar islands)[14,15].

3. Susceptibility tests, intensity bioassays and interpretation

The reported data from the studies adhered to the standard WHO protocols. Adult susceptibility tests were conducted to assess the susceptibility of commonly used insecticides and WHO kit, impregnated papers with prescribed discriminatory concentrations (DC) and methods. The insecticides tested were DDT-4.0% (organochlorine), malathion-5.0% (organophosphate), and pyrethroids, alpha-cypermethrin-0.05%, deltamethrin-0.05%, permethrin-0.75%[16]. The general protocol for adult susceptibility tests involves exposing wild-caught/laboratory-reared mosquitoes to the WHO-prescribed discriminating concentrations of insecticides. Typically, 4-5 replicates of 20-25 mosquitoes each (n=100) were exposed to the insecticide, and in parallel, two replicates of 25 mosquitoes each (n=50) were exposed to insecticide controls. The data tables indicated data sets where the mosquitoes were fewer and sample was insufficient. The exposures were for one hour, followed by a 24-h holding period. After 24 h, the mosquitoes were scored for alive and dead mosquitoes, and the results were expressed as the percentage mortality. Based on the mortality rates, the population is designated as susceptible/resistant following the WHO criterion: >98% mortality-susceptible; between 90% to 97% mortality-possible resistance; <90% mortality-confirmed resistance[16-19].

Intensity bioassays were conducted on vector species with confirmed reported resistance to the DC of insecticide (<90% mortality by testing 100 mosquitoes). The level/intensity of resistance, *i.e.*, "strength" of a resistance phenotype, was determined by exposing the mosquito population to higher concentrations, 5 times (5×) and 10 times (10×) the DC (1×) following the testing procedure described above for adult susceptibility tests. Results of the exposures at higher concentrations (resistance intensity) will help assessing the operational usability of the insecticide being used in the intervention[17,19]. Results of intensity bioassays are interpreted as follows:

-Mortality in the range of 98%-100% at 5× concentration indicates a low resistance intensity and further testing at 10× concentration is not necessary-suggests to continue the existing insecticide for vector control intervention.

-Mortality of <98% at the 5× concentration indicates a moderate resistance intensity. It is recommended to assay further at the 10×concentration.

-Mortality between 98% and 100% at the 10× concentration confirms a moderate resistance intensity.

-Mortality of <98% at the 10× concentration indicates a high resistance intensity.

If the resistance is confirmed at 5× and especially at 10× concentrations, operational failure is likely and change of insecticide is preferred.

4. Results

4.1. Insecticide susceptibility data in malaria vectors

Insecticide susceptibility data were available from 86 districts across 16 Indian states for 40 615 mosquitoes. This review showed *An. culicifacies* (n=28 308) was the most prevalent species and was extensively tested, followed by *An. stephensi* (n=5 611),

Table 1. Insecticide susceptibility status of *Anopheles culicifacies* in different states of India.

State	District (Place)	Year	DDT 4.0%			Malathion 5.0 %			Alpha-cypermethrin 0.05%			Deltamethrin 0.05%			Permethrin 0.75%			Reference
			No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	
Assam	Kamrup	2018	80[a]	67.5	CR	40[a]	80.0	CR	-	-	-	-	-	-	-	-	-	[8]
	Udalguri	2018	105	65.7	CR	102	91.8	PR	-	-	-	115	99.1	S	-	-	-	
Chhattisgarh	Balod	2017	-	-	-	-	-	-	-	-	-	-	-	-	102	69.6	CR	Raghavendra unpublished
	Kanker	2017	-	-	-	-	-	-	-	-	-	-	-	-	107	81.3	CR	
	Bastar	2019-2020							180	92.2	PR	150	89.3	CR				[20]
	Bastar (Jagdalpur)	2022	80[a]	16.3	CR	80[a]	75.0	CR	80[a]	82.5	CR	80[a]	91.3	PR	-	-	-	[21]
	Kanker	2022	80[a]	10.0	CR	80[a]	68.8	CR	100	77.0	CR	100	86.0	CR	-	-	-	
Gujarat	Anand	2017	-	-	-	-	-	-	-	-	-	236	30.0	CR	-	-	-	Raghavendra unpublished
	Kheda	2019	-	-	-	160	70.0	CR	160	95.0	PR	160	75.0	CR	160	85	CR	[8]
	Panchmahal	2019	-	-	-	160	75.0	CR	160	95.0	PR	160	85.0	CR	160	90	PR	
Haryana	Nuh	2019	120	38.0	CR	129	78.0	CR	-	-	-	155	86.0	CR	-	-	-	[8]
		2021-2022	428	43.7	CR	431	59.4	CR	-	-	-	427	78.6	CR	-	-	-	[22]
	Palwal	2019	165	29.0	CR	86[a]	90.0	PR	-	-	-	150	83.0	CR	-	-	-	[8]
Jharkhand	Chatra	2019	200	4.5	CR	200	87.0	CR	-	-	-	200	58.5	CR	200	65.5	CR	[8]
	Dhanbad	2019	200	12.0	CR	200	89.5	CR	-	-	-	200	70.5	CR	100	94	PR	
	Giridih	2019	200	17.5	CR	200	89.0	CR	-	-	-	200	86.5	CR	200	93.5	PR	
	Godda	2018	200	10.0	CR	-	-	-	-	-	-	180	76.0	CR	180	93.4	PR	
	Gumla	2018	157	8.3	CR	165	100.0	S	-	-	-	160	100.0	S	162	100	S	
	Khunti	2018	180	7.5	CR	160	100.0	S	-	-	-	200	85.0	CR	155	100	S	
	Koderma	2019	200	9.5	CR	200	93.0	PR	-	-	-	200	85.0	CR	200	89	CR	
	Latehar	2019	200	15.5	CR	200	88.5	CR	-	-	-	200	93.5	PR	200	93.5	PR	
	Palamu	2019	200	11.5	CR	200	83.5	CR	-	-	-	200	87.5	CR	200	89	CR	
	Sahibganj	2018	200	23.5	CR	100	98.0	S	-	-	-	145	77.7	CR	175	95.5	PR	
	Simdega	2017	180	25.3	CR	180	99.0	S	-	-	-	200	85.0	CR	155	79.3	CR	
	West Singhbhum	2018	160	47.0	CR	200	69.0	CR	-	-	-	200	84.0	CR	160	85.6	CR	
Karnataka	Kalaburgi	2019	50[a]	2.0	CR	51[a]	49.0	CR	50[a]	16.0	CR	52[a]	86.5	CR	-	-	-	[8]
	Bagalkot (Almatti Dam)	2017	-	-	-	-	-	-	-	-	-	100	86.0	CR	-	-	-	[23]
		2020-2021	80[a]	27.5	CR	-	-	-	-	-	-	100	86.0	CR	-	-	-	[24]
Madhya Pradesh	Alirajpur	2018-2019	105	7.6	CR	105	58.1	CR	105	87.6	CR	105	96.2	PR	-	-	-	[25]
	Anuppur	2017	105	16.2	CR	105	64.8	CR	105	98.1	S	105	98.1	S	-	-	-	
	Bhind	2019	105	16.2	CR	105	63.8	CR	105	95.2	PR	105	97.1	PR	-	-	-	
	Datia	2019	100	60.0	CR	100	93.0	PR	100	97.0	PR	100	100.0	S	-	-	-	
	Dhar	2018-2019	120	17.5	CR	120	65.0	CR	120	84.2	CR	120	93.3	PR	-	-	-	
	Hoshangabad	2018	105	7.6	CR	105	72.4	CR	105	96.2	PR	105	95.2	PR	-	-	-	
	Khargone	2018	120	12.5	CR	120	65.0	CR	120	93.3	PR	120	96.7	PR	-	-	-	
	Mandla	2017	75[a]	28.0	CR	75[a]	84.0	CR	120	95.0	PR	120	98.3	S	-	-	-	[26]
		2019	105	24.8	CR	105	50.5	CR	135	82.2	CR	135	95.6	PR	-	-	-	
	Panna	2017	105	12.4	CR	105	73.3	CR	105	99.0	S	105	99.0	S	-	-	-	[25]
	Shivpuri	2019	100	41.0	CR	100	87.0	CR	100	93.0	PR	100	97.0	PR	-	-	-	
	Singrauli	2017	105	18.1	CR	105	70.5	CR	105	90.5	PR	105	93.3	PR	-	-	-	
	Tikamgarh	2017	105	16.2	CR	105	84.8	CR	105	100.0	S	105	100.0	S	-	-	-	
	Umaria	2017	105	14.3	CR	105	76.2	CR	105	95.2	PR	105	96.2	PR	-	-	-	
Maharashtra	Gadchiroli	2016-2017	80[a]	37.0	CR	80[a]	74.0	CR	-	-	-	125	83.8	CR	-	-	-	[27]
		2012-2018	200	14.0	CR	200	89.5	CR	-	-	-	200	95.5	PR	-	-	-	[28]
	Gadchiroli (18 PHC, 147 villages)	2015-2019	2 415	18.0-58.0	CR	3 963	60.0-95.0	PR	-	-	-	12 025	77.0-100.0	PR	660	97.5-100.0	S	[29]
	Gadchiroli	2019	-	-	-	-	-	-	-	-	-	125	83.8	CR	90	91.3	PR	[8]

Table 1 continues in the next page.

Table 1. Continued.

State	District (Place)	Year	DDT 4.0%			Malathion 5.0%			Alpha-cypermethrin 0.05%			Deltamethrin 0.05%			Permethrin 0.75%			Reference
			No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	
Odisha	Angul	2020	120	33.0	CR	120	71.0	CR	-	-	-	120	92.5	PR	-	-	-	
	Balangir	2019	100	70.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Bhadrak	2017-2018	30[a]	60.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Cuttack	2017-2018	120	28.0	CR	120	98.0	S	-	-	-	120	95.2	PR	-	-	-	
	Gajapati	2019	100	74.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Ganjam	2019	100	76.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Kandhamal	2019	100	74.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[30]
		2017	-	-	-	-	-	-	-	-	-	75[a]	74.6	CR	-	-	-	[31]
	Kalahandi	2019	100	78.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[30]
		2020	227	37.0	CR	196	71.9	CR	110	88.2	CR	255	87.8	CR	90[a]	92.2	PR	[8]
	Khurda	2017-2018	30[a]	60.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Koraput	2017	-	-	-	-	-	-	-	-	-	75[a]	73.3	CR	-	-	-	[31]
		2019	30[a]	40.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[30]
	Jagatsinghpur	2017-2018	30[a]	50.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[8]
	Jajpur	2017-2018	30[a]	46.6	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Jharsuguda	2022	60[a]	25.0	CR	-	-	-	-	-	-	60[a]	95	PR	-	-	-	[32]
	Malkangiri	2017	-	-	-	-	-	-	-	-	-	75[a]	84	CR	-	-	-	[31]
		2019	100	71.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[30]
	Mayurbhanj	2017-2018	30[a]	60.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[8]
	Nabarangpur	2017	-	-	-	-	-	-	-	-	-	75[a]	85.3	CR	-	-	-	[31]
		2019	100	73.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[30]
	Nuapada	2019	100	80.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Puri	2017-2018	30[a]	56.6	CR	-	-	-	-	-	-	-	-	-	-	-	-	[8]
	Raygada	2017	-	-	-	-	-	-	-	-	-	75[a]	80.0	CR	-	-	-	[31]
		2019	100	72.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	[30]
Uttar Pradesh	Badaun	2017	130	6.9	CR	130	93.1	PR	-	-	-	130	91.5	PR	-	-	-	[8]
	Banda	2018	120	2.5	CR	120	94.2	PR	-	-	-	120	92.5	PR	-	-	-	
	Gautam Budh Nagar	2018	120	0	CR	120	95.0	PR	-	-	-	120	96.7	PR	-	-	-	
	Hathras	2018	180	3.9	CR	180	92.2	PR	-	-	-	180	91.7	PR	-	-	-	
	Jhansi	2018	60[a]	3.3	CR	60[a]	80.0	CR	-	-	-	60[a]	91.7	PR	-	-	-	
	Kanpur Dehat	2018	120	4.2	CR	120	95.8	PR	-	-	-	120	86.7	CR	-	-	-	
	Mizapur	2019	160	0	CR	160	93.3	PR	-	-	-	160	91.9	PR	-	-	-	
	Prayag Raj	2019	100	80.0	CR	-	-	-	-	-	-	-	-	-	-	-	-	
	Saharanpur	2017	120	5.8	CR	120	96.7	PR	-	-	-	120	93.3	PR	-	-	-	
	Sonebhadra	2019	160	4.4	CR	160	94.4	PR	-	-	-	160	96.3	PR	-	-	-	

n[a]: <100; DDT-dichloro diphenyl trichloroethane; No. Exp.: Number of mosquitoes exposed; % Mort.: Percent mortality; Status: S-Susceptible, ⩾98% mortality; PR: Possible resistance, 90%-97% mortality; CR: Confirmed resistance, <90% mortality[16-19].

An. fluviatilis (*n*=5 967), *An. baimaii* (*n*=365) and *An. minimus* (*n*=364). Susceptibility status data on *An. sundaicus* (species D) was not available during this period of reporting. Adult susceptibility tests results to the commonly used insecticides in vector control, DDT, malathion, alpha-cypermethrin, deltamethrin, and in some states for permethrin were compiled (Tables 1-5, Supplementary Figures 1 and 2). Representation of susceptibility status for data sets from longitudinal studies for more than one year, the status of resistance in the most recent year is considered. Intensity bioassays on pyrethroid-resistant *An. culicifacies* in the states of Odisha, Chhattisgarh and Madhya Pradesh are reported (Tables 6 and 7) .

4.1.1. Anopheles culicifacies

Results of adult susceptibility data to different insecticides in districts are given in Table 1 and Supplementary Figure 1.

DDT-Results of the studies undertaken in 67 districts in 10 states, namely Assam (2 districts), Chhattisgarh (3), Gujarat (3),

Table 2. Insecticide susceptibility status of *Anopheles stephensi* in different states of India.

State	District (Place)	Year	DDT 4.0%			Malathion 5.0%			Alpha -cypermethrin 0.05%			Deltamethrin 0.05%			Permethrin 0.75%			Reference
			No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	
Haryana	Nuh	2019	100	31.0	CR	80[a]	69.0	CR	-	-	-	166	85.0	CR	-	-	-	[8]
		2021-2022	406	38.7	CR	403	46.5	CR				404	80.9	CR				[22]
	Palwal	2019	110	48.0	CR	120	81.0	CR	-	-	-	219	72.0	CR	-	-	-	[8]
Rajasthan	Alwar (Thanagazi)	2019	105	89.9	CR	-	-	-	95[a]	100.0	S	97[a]	100.0	S	-	-	-	[33]
	Churu (Rajgarh)	2019	105	86.9	CR	-	-	-	98[a]	100.0	S	99[a]	100.0	S	-	-	-	
	Dausa (Mandawar)	2019	95[a]	87.3	CR	-	-	-	97[a]	100.0	S	103	100.0	S	-	-	-	
	Dungarpur	2019	787	76.5	CR	-	-	-	772	99.9	S	93[a]	98.8	S	775	99.4	S	[34]
	Sikar (Ramgarh)	2019	83[a]	85.7	CR	-	-	-	97[a]	100.0	S	102	100.0	S	-	-	-	[33]

n[a]: <100; DDT-dichloro diphenyl trichloroethane; No. Exp.: Number of mosquitoes exposed; % Mort.: Percent mortality; Status: S-Susceptible, ⩾98% mortality; PR: Possible resistance, 90%-97% mortality; CR: Confirmed resistance, <90% mortality[16-19].

Haryana (2), Jharkhand (12), Karnataka (2), Madhya Pradesh (13), Maharashtra (1), Odisha (19), and Uttar Pradesh (10 districts). A total of 8 112 mosquitoes were exposed and the species registered mortality in the range of 0% to 80% and reported confirmed resistance in all the districts (67/67).

Malathion-Susceptibility data from 45 districts of 10 states, Assam (2 districts), Chhattisgarh (2), Gujarat (2), Haryana (2), Jharkhand (11), Karnataka (1), Madhya Pradesh (13), Maharashtra (1), Odisha (3), and Uttar Pradesh (9 districts) were reported. For the tests, 6 670 mosquitoes were exposed and the species registered mortality in the range of 49% to 100% and the species reported resistance in most of the districts, confirmed resistance in 28 districts, possible resistance in 12 but were reportedly susceptible in 5 districts.

Alpha–cypermethrin-Data of susceptibility status in 5 states in 19 districts, Chhattisgarh (2 districts), Gujarat (2), Karnataka (1), Madhya Pradesh (13) and Odisha (1 district) were reported. For susceptibility tests, 2 375 mosquitoes were exposed. The species registered mortality in the range of 16% to 100%. The species reported resistance, mostly confirmed resistance (7 districts), and in few districts possible resistance (9), while 3 districts in Madhya Pradesh (Annupur, Panna and Tikamgarh) reported complete susceptibility.

Deltamethrin-Study results from 53 districts of 10 states, Assam (1 district), Chhattisgarh (2), Gujarat (3), Haryana (2), Jharkhand (12), Karnataka (2), Madhya Pradesh (13), Maharashtra (1) Odisha (8) and Uttar Pradesh (9 districts) were reported. A total of 8 355 mosquitoes were exposed. Mortality in susceptibility tests was in the range of 30% to 100% and the species was mostly resistant, confirmed resistance in 26 districts and possible resistance in 21 districts while it was reportedly susceptible to deltamethrin in 6 districts.

Permethrin-Susceptibility data from insecticide exposures in 18 districts of 5 states, namely Chhattisgarh (2 districts), Gujarat (2), Jharkhand (12), Maharashtra (1) and Odisha (1 district) were presented. A total of 2 796 mosquitoes were exposed and the species registered mortality in the range of 65.5% to 100% and the species reported resistance equally, 8 each in confirmed resistance and possible resistance category while 2 districts (Gumla and Khunti) in Jharkhand state reported complete susceptibility.

In the present review (2017-2025), *An. culicifacies* registered confirmed resistance to at least one insecticide, DDT, in all the 67 districts. While the species reported resistance to malathion in 28 of the 45 districts, alpha-cypermethrin in 7 of 19 districts; deltamethrin in 26 of 53 districts and permethrin in 8 of 18 districts. Double resistance to DDT and malathion was reported from 18 districts, DDT and deltamethrin from 8 districts and triple resistance to DDT, malathion and deltamethrin including possible resistance status in 33 districts.

4.1.2. Anopheles stephensi

Adult susceptibility tests were conducted in few districts in 2 states, Haryana and Rajasthan, against DDT, malathion, alpha-cypermethrin, deltamethrin and permethrin[33.34]. Results are given in Table 2 and Supplementary Figure 1.

DDT-Exposures in 2 states and 7 districts, Haryana (2 districts) and Rajasthan (5 districts) reported confirmed resistance in all the districts. A total of 1 791 mosquitoes were exposed, the species

Table 3. Insecticide susceptibility status of *Anopheles fluviatilis* in different states of India.

State	District	Year	DDT 4.0%			Malathion 5.0%			Deltamethrin 0.05%			Permethrin 0.75%			Reference
			No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	
Jharkhand	Giridih	2019	40[a]	70.0	CR	-	-	-	-	-	-	-	-	-	[8]
	Godda	2018	130	38.2	CR	135	100.0	S	140	100.0	S	125	100.0	S	
	Gumla	2018	130	40.2	CR	120	98.4	S	120	100.0	S	120	100.0	S	
	Khunti	2018	120	45.0	CR	100	100.0	S	115	100.0	S	105	100.0	S	
	Latehar	2019	20[a]	80.0	CR	-	-	-	-	-	-	-	-	-	
	Palamu	2019	40[a]	75.0	CR	-	-	-	-	-	-	-	-	-	
	Sahibganj	2018	115	18.5	CR	110	100.0	S	110	100.0	S	115	100.0	S	
	Simdega	2018	160	29.5	CR	140	100.0	S	120	100.0	S	110	100.0	S	
	West Singhbhum	2018	175	55.9	CR	155	100.0	S	145	99.3	S	140	100.0	S	
Odisha	Angul	2019	100	91.0	PR	100	100.0	S	100	100.0	S	-	-	-	
	Kalahnadi	2019	100	90.0	PR	100	98.5	S	100	99.0	S	-	-	-	
	Jharsuguda	2022	60[a]	98.3	S	-	-	-	-	-	-	60[a]	100.0	S	[32]
Maharashtra	Gadchiroli	2012-2018	150	36.0	CR	150	88.0	CR	-	-	-	150	98.6	S	[28]
	Gadchiroli (9 PHC, 22 villages)	2015-2019	195	43.0-51.0	CR	239	74.0-80.0	CR	1 208	98.0-100.0	S				[29]

n^{a}: <100; DDT-dichloro diphenyl trichloroethane; No. Exp.: Number of mosquitoes exposed; % Mort.: Percent mortality; Status: S-Susceptible, ⩾98% mortality; PR: Possible resistance, 90%-97% mortality; CR: Confirmed resistance, <90% mortality[16-19].

registered mortality in the range of 31% to 90%.

Malathion-In studies undertaken in Haryana (2 districts), the species reported confirmed resistance, 603 mosquitoes were exposed and the species registered mortality of 46.5 % and 81% in districts Nuh and Palwal, respectively.

Alpha-cypermethrin-Studies were reported from Rajasthan (5 districts). A total of 1 159 mosquitoes were exposed and the species was reported susceptible in all the districts and the observed mortality was in the range of 99% to 100%.

Deltamethrin-Results of susceptibility tests undertaken in 2 states and 8 districts, Haryana (2 districts) and Rajasthan (6 districts) were reported. A total of 1 283 mosquitoes were exposed. The species registered 85% mortality (Nuh) and 72% mortality (Palwal) to deltamethrin, thus the species reported confirmed resistance to deltamethrin in Haryana. While in Rajasthan, species registered susceptibility to deltamethrin in all the 5 districts.

Permethrin-In Rajasthan (Dungarpur districts), a total of 775 mosquitoes were exposed and the species was reported susceptible.

The present compilation reported limited data for this species, showed resistance to DDT in all the 7 districts across the 2 states. Resistance to deltamethrin was reported in Haryana, while in Rajasthan, the species was susceptible to the pyrethroids-deltamethrin, alpha-cypermethrin, and permethrin.

4.1.3. Anopheles fluviatilis

Susceptibility tests were conducted in few districts in 3 states, Jharkhand, Maharashtra and Odisha, against DDT, malathion, deltamethrin and permethrin[8]. Insecticide- and state-wise results are given in Table 3 and Supplementary Figure 1.

DDT-Studies were reported from 3 states, Jharkhand (9 districts), Maharashtra (1), and Odisha (3 districts). A total of 1 535 mosquitoes were exposed and the species registered mortality in the range of 18.5% to 98%. The species was reported resistant in Jharkhand and Maharashtra, while in Odisha the species reported possible resistance in 2 districts, Kalahandi (90% mortality) and Angul (91% mortality) but was reported susceptible in Jharsuguda.

Malathion-Reported results were from 3 states, namely Jharkhand (6 districts), Maharashtra (1), and Odisha (2 districts). A total of 1 349 mosquitoes were exposed and the species was reportedly susceptible in both the states with mortality in the range of 98% to 100% but reported resistant in Gadchiroli (88%).

Deltamethrin-Studies reported from the states Jharkhand (6 districts), and Odisha (2 districts) indicated *An. fluviatilis* completely susceptible. A total of 2 158 mosquitoes were exposed and the species registered mortality in the range of 98.4% to 100%.

Permethrin-Reported results were from 3 states, namely Jharkhand (6 districts), Maharashtra (1), and Odisha (1 districts). A total of 925 mosquitoes were exposed and the species registered complete susceptibility in all the 8 districts.

In the present compilation (2017-2025), the species was reported mostly resistant to DDT in all the 13 districts of 3 states, while it was mostly susceptible to malathion, deltamethrin and permethrin. In

Table 4. Insecticide susceptibility status of *Anopheles minimus* in different states of India.

State	District	Year	DDT 4.0%			Malathion 5.0%			Alpha -cypermethrin 0.05%			Deltamethrin 0.05%			Permethrin 0.75%			Reference
			No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	
Arunachal Pradesh	Changlang	2019	40[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	[8]
Assam	Dibrugarh	2019	60[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Golaghat	2019	20[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Kamrup (M)	2017	12[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Karbi Anglong	2019	37[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Kokrajhar	2018	15[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Udalguri	2019	10[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
Jharkhand	West Singhbhum	2018	30[a]	96.7	PR	30[a]	100	S	-	-	-	30[a]	100	S	30[a]	100	S	[35]
Meghalaya	East Garo Hills	2017	10[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	[8]
	South Garo Hills	2017-2019	b	100	S	b	100	S	-	-	-	-	-	-	-	-	-	[36]
		2019	10[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	[8]
Mizoram	Kolasib	2019	10[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Mamit	2019	20[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
Tripura	Dhalai	2017-2019	b	100	S	b	100	S	-	-	-	-	-	-	-	-	-	[36]

n[a]: <100; b: Number not mentioned; DDT-dichloro diphenyl trichloroethane; No. Exp.: Number of mosquitoes exposed; % Mort.: Percent mortality; Status: S-Susceptible, ⩾98% mortality; PR: Possible resistance, 90%-97% mortality; CR: Confirmed resistance, <90% mortality[16-19].

the earlier review (1991-2016) by Raghavendra *et al.*[12], the species reported complete susceptibility to malathion and deltamethrin. However, widespread resistance to DDT was reported in the states of Jharkhand, Karnataka, Tamil Nadu, Maharashtra, Chhattisgarh and Himachal Pradesh in the surveyed districts, while it was reported susceptible in the state of Odisha. In the present review, the status of DDT resistance in Jharkhand was similar to earlier observation; in contrast, the species registered possible resistance to DDT in Odisha. The species was still reported susceptible to malathion and deltamethrin in both the states.

4.1.4. Anopheles minimus

Adult susceptibility tests were conducted in 12 districts in 5 northeastern states, Arunachal Pradesh (1 district), Assam (6), Meghalaya (2) and Mizoram (2), Tripura (1 district) and 1 district in eastern state Jharkhand against DDT. Additionally in the West Singhbhum district of Jharkhand state, susceptibility tests were reported against malathion, deltamethrin and permethrin[8].

A total of 274 mosquitoes were exposed to DDT, and the species reported 100% mortality in all the northeastern states, indicating complete susceptibility (Table 4). In Jharkhand, however, the species exhibited 96.7% mortality, placing it in the possible resistance category. In Jharkhand, the species reported 100% susceptible to malathion, deltamethrin and permethrin (Table 4 and Supplementary Figure 2).

Similarly, in the earlier review (1991-2016)[12], *An. minimus*, which is prevalent in the northeast region, was reported susceptible to DDT, malathion and deltamethrin, with only one instance of DDT resistance reported in Odisha, an eastern state of India[35].

4.1.5. Anopheles baimaii

Adult susceptibility tests data was available for this species in 13 districts in 6 northeastern states, Arunachal Pradesh (2 district), Assam (4), Meghalaya (2), Mizoram (2), Nagaland (1) and Tripura (2 districts) against DDT, and against malathion in one district each in Meghalaya (South Garo hills) and Tripura (South Tripura)[8]. A total of 345 mosquitoes were exposed to DDT and the species registered complete susceptibility (100% mortality) in all the states. While in the 3 districts surveyed, one in Meghalaya and 2 districts in Tripura states, the species registered complete susceptibility to malathion (Table 5 and Supplementary Figure 2). In earlier review (1991-2016)[12], this species in Northeastern region also reported susceptibility to all the three insecticides, namely DDT, malathion and to deltamethrin.

Table 5. Insecticide susceptibility status of *Anopheles baimaii* in different states of India.

State	District	Year	DDT 4.0%			Malathion 5.0%			Alpha-cypermethrin 0.05%			Deltamethrin 0.05%			Permethrin 0.75%			References
			No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	No. Exp.	% Mort.	Status	
Assam	Dibrugarh	2019	40[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	[8]
	Golaghat	2019	20[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Karbianglong	2019	40[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Udalguri	2018	10[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
Arunachal Pradesh	Changlang	2019	40[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Namsai	2019	30[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
Meghalaya	East Garo Hills	2017	20[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	South Garo Hills	2017-2019	b	100	S	b	100	S	-	-	-	-	-	-	-	-	-	[36]
		2019	10[a]	100	S	10	100	S	-	-	-	-	-	-	-	-	-	[8]
Mizoram	Kolasib	2019	30[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
	Mamit	2019	50[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
Nagaland	Mokokchung	2019	20[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	
Tripura	Dhalai	2017-2019	b	100	S	b	100	S	-	-	-	-	-	-	-	-	-	[36]
		2019	20[a]	100	S	-	-	-	-	-	-	-	-	-	-	-	-	[8]
	South Tripura	2019	15[a]	100	S	10	100	S	-	-	-	-	-	-	-	-	-	

n^a: <100; b: Number not mentioned; DDT-dichloro diphenyl trichloroethane; No. Exp.: Number of mosquitoes exposed; % Mort.: Percent mortality; Status: S-Susceptible, ⩾98% mortality; PR: Possible resistance, 90%-97% mortality; CR: Confirmed resistance, <90% mortality[16-19].

4.2. Intensity bioassays

Intensity bioassay studies are reported for the first time in India from 3 states, Odisha[37], Madhya Pradesh, and Chhattisgarh[21].

In Odisha, intensity bioassays were performed on deltamethrin-resistant-*An. culicifacies*, the reported major vector of malaria in 10 southern districts, namely Bolangir, Gajapati, Ganjam, Kalahandi, Kandhamal, Koraput, Malkangiri, Nabarangpur, Nuapada and Rayagada from June to September, 2019[30]. In the last 5 years since 2017, these districts received insecticide treated nets (ITNs) (deltamethrin/alpha-cypermethrin ITNs) from the Global Fund to Fight AIDS, Tuberculosis and Malaria[37]. The DDT-IRS was withdrawn from ~80% areas of study districts since 2017 and yearly one round of IRS was being carried out selectively in areas showing annual parasite incidence>10[37]. The last round of IRS with DDT was conducted in selected areas of these districts during September-October 2018. In a study by Sahu *et al*[30] on DDT-susceptibility in *An. culicifacies* in the 10 malaria hyper-endemic southern districts in Odisha before and after the ITN distribution in 2017 and 2018 registered DDT-resistance. The frequency of resistant phenotype ranged from 63% to 89% with an average of 79.8% during 2017 and from 72% to 90% with an average of 82.0% during 2018.

An. culicifacies registered confirmed resistance to 1×DC of deltamethrin (0.05%) in all the 10 southern districts with mortality in the range of 70% to 80% (*i.e.*⩽90% mortality). While with intensity bioassays the deltamethrin-resistant-*An. culicifacies* registered mortality in the range of 92% to 97% against 5×DC of deltamethrin (0.25%) indicating moderate resistance but reported 100% mortality against 10× DC of deltamethrin (0.50%) that confirmed presence of moderate resistance[37] (Table 6). Thus, *An. culicifacies* was reportedly moderate resistant in intensity bioassays and indicated absence of high resistance, hence, operational failure of vector control with the use of deltamethrin/alpha-cypermethrin- ITNs was unlikely. Thus, use of synthetic pyrethroid- ITNs for malaria vector control in these districts can be continued until change in intensity of resistance is evidenced.

Intensity bioassays data was available from district Dindori in the state of Madhya Pradesh on deltamethrin-and alpha-cypermethrin-resistant *An. culicifacies*[21]. Long lasting insecticidal nets were distributed in years 2012, 2013 and 2017, and 2019. The species registered resistance to deltamethrin (84.8% mortality) and alpha-cypermethrin (77.1% mortality) to 1× DC of deltamethrin (0.05%) and alpha-cypermethrin (0.05%), respectively. However, the species registered 100% and 99% mortality in intensity bioassays with 5× DC of deltamethrin (0.25%) and alpha-cypermethrin (0.25%) and reported low resistance (Table 6). As the species registered 98%-100% susceptibility to 5× DC, that indicated low resistance, and as suggested, susceptibility tests with 10× DC of deltamethrin (0.50%) and 10× DC of alpha-cypermethrin (0.50%) were not conducted. These results prompted no change in the current pyrethroid interventions in this district and can be continued, as operational failure is unlikely.

Intensity bioassays conducted against deltamethrin and alpha-cypermethrin against *An. culicifacies* in two districts, Kanker

Table 6. Percent mortality of *Anopheles culicifacies s.l.* to discriminating concentrations (DC) of deltamethrin, 0.05% (1×) and associated resistance intensity to 0.25% (5×DC) and 0.5% (10×DC) in 10 southern districts of Odisha.

Districts of Odisha State	Exposures	Concentration			Resistance intensity	Reference
		0.05% (1×)	0.25% (5×)	0.5% (10×)		
Balangir	Number exposed	100	100	100	Moderate	[37]
	24-h % mortality	80	96	100		
Gajapati	Number exposed	100	100	100	Moderate	
	24-h % mortality	78	94	100		
Ganjam	Number exposed	100	100	100	Moderate	
	24-h % mortality	80	97	100		
Kalahandi	Number exposed	100	100	100	Moderate	
	24-h % mortality	70	96	100		
Kandhamal	Number exposed	100	100	100	Moderate	
	24-h % mortality	74	94	100		
Koraput	Number exposed	100	100	100	Moderate	
	24-h % mortality	71	96	100		
Malkangiri	Number exposed	100	100	100	Moderate	
	24-h % mortality	72	97	100		
Nabarangpur	Number exposed	100	100	100	Moderate	
	24-h % mortality	74	94	100		
Nuapada	Number exposed	100	100	100	Moderate	
	24-h % mortality	76	95	100		
Rayagada	Number exposed	100	100	100	Moderate	
	24-h % mortality	73	92	100		

Resistance intensity status based on % mortality at different concentrations: Low intensity–DC5×, ⩾98% mortality; Moderate intensity–DC5×, <98% mortality and DC10×, ⩾98% mortality; High intensity–DC10×, <98% mortality[19].

Table 7. Percent mortality of *Anopheles culicifacies s.l.* to discriminating concentrations (DC) of deltamethrin and alpha-cypermethrin, 0.05% (1×) and associated resistance intensity to 0.25% (5×DC) and 0.5% (10×DC) in one district of Madhya Pradesh and two districts of Chhattisgarh.

State	District(s)	Insecticide	Exposures	Concentration			Resistance intensity	Reference
				0.05% (1×)	0.25% (5×)	0.5% (10×)		
Madhya Pradesh	Dindori	Deltamethrin	Number exposed	105.0	100.0	-	Low	[21]
			24-h % mortality	84.8	100.0	-		
		Alpha-cypermethrin	Number exposed	105.0	100.0	-	Low	
			24-h % mortality	77.1	99.0	-		
Chhattisgarh	Bastar	Deltamethrin	Number exposed	80.0	80.0	-	Low	
			24-h % mortality	91.3	100.0	-		
		Alpha-cypermethrin	Number exposed	80.0	80.0	-	Low	
			24-h % mortality	82.5	98.8	-		
	Kanker	Deltamethrin	Number exposed	100.0	40.0	20.0	Moderate	
			24-h % mortality	86.0	95.0	100.0		
		Alpha-cypermethrin	Number exposed	100.0	40.0	20.0	Moderate	
			24-h % mortality	77.0	92.5	100.0		

Resistance intensity status based on % mortality at different concentrations: Low intensity–DC5×, ⩾98% mortality; Moderate intensity–DC5×, <98% mortality and DC10×, ⩾98% mortality; High intensity–DC10×, <98% mortality[19].

and Bastar in Chhattisgarh indicated variable intensity in the resistance[21]. In district Kanker, to 1× deltamethrin DC (0.05%), the species registered 86% mortality (confirmed resistance), while to 5× DC of deltamethrin (0.25%), species recorded 95% mortality registering moderate resistance and 100% mortality to 10× DC of deltamethrin that further confirmed the presence of moderate resistance. Similarly, the species registered moderate resistance in intensity bioassays with alpha-cypermethrin. In susceptibility tests with 1× DC alpha-cypermethrin (0.05%), the species registered 77% mortality (confirmed resistance). While to 5× DC of alpha-cypermethrin (0.25%), species recorded 92.5% mortality, indicating moderate resistance and 100% mortality to 10× DC of alpha-cypermethrin that again confirmed the presence of moderate resistance. Thus, as observed in district Dindori in Madhya Pradesh, the intensity bioassay results in Kanker prompted no change in current insecticide intervention as operational failure with the use of these insecticide interventions is unlikely (Table 7).

In contrast, in district Bastar of Chhattisgarh, the species registered low resistance in intensity bioassays to deltamethrin and alpha-cypermethrin. In Bastar, the species recorded 91.3% mortality to 1× DC of deltamethrin (0.05%) and 82.5% mortality to alpha-cypermethrin (0.05%), while the species recorded 100% and 98.8%

mortality in bioassays to 5× DC of deltamethrin (0.25%) and alpha-cypermethrin (0.25%) (Table 7). Further, exposure to 10× DC of deltamethrin (0.50%) and alpha-cypermethrin (0.50%) was not needed as species registered susceptibility between 98% and 100% mortality indicating susceptibility to 5× DC for both the insecticides. These results prompted no change of current pyrethroid intervention in this district, as operational failure is unlikely.

5. Discussion

This compilation of insecticide susceptibility data for the last eight years (2017-2024), and period upto June, 2025, on different malaria vectors in 16 states showed *An. culicifacies* as a species of concern in India, still bearing most of the burden of insecticide resistance among the prevalent vectors similar to earlier reports of Raghavendra *et al*., in 2017 and 2022[8,12]. This species has reported wide spread multiple-resistance to the three main insecticide classes in use for vector control in India, DDT (organochlorine), malathion (organophosphate) and deltamethrin and other pyrethroid insecticides.

An. culicifacies, the primary malaria vector in India, is reportedly responsible for 60%-70% of annual malaria cases[12]. *An. culicifacies* epitomize the burden of insecticide resistance in malaria vectors and its management need to be prioritized for disease control. Actually, management of this species is *per se* control of malaria in India[38]. A review by Kumari *et al.*[39] stated wide-spread resistance of *An. culicifacies* to different insecticides in use in vector control prior to 1990s. A review by Raghavendra *et al.*[12] on insecticide resistance data from 145 districts reported during the years 1991-2016, showed that the major vector *An. culicifacies* reported resistance to at least one insecticide in 101 districts *i.e.* in 70% of the districts. In another multi-centric study on insecticide susceptibility status of the Indian malaria vectors during 2017-2019 in 328 villages in 79 districts from 15 states with varied ecotypes showed *An. culicifacies* resistant to at least one insecticide in 52 districts *i.e.* 66% of the districts[8]. In the present review (2017-2025), *An. culicifacies* registered resistance to at least one insecticide in 67 districts (78%) of the 86 districts. Intensity bioassays on this spesies with deltamethrin- and alpha-cypermethrin-resistant *An. culicifacies* in some districts in three malaria endemic states have shown low to moderate resistance, indicating unlikely control failure and no change in the ongoing pyrethroid intervention for the present[21,37].

The data in the present review on *An. stephensi* from 7 districts in 2 states reported resistance to DDT in all the districts. The species exhibited triple resistance to DDT, malathion and deltamethrin in both the districcts of Haryana. While in Rajasthan, the species was susceptible to pyrethroids, alpha-cypermethrin, deltamrethrin and permethrin. Presently in India, larviciding is the main intervention measure for this species and indoor residual spray is not targeted for the control of *An. stephensi* except in Rajasthan where this is the reported primary vector of malaria including in rural areas[14].

Though the 25-year data (1991-2016)[12] is not comparable to the data presented in the present compilation (2017-2025) in time and space, an upward trend in resistance is observed, with a proportional increase in districts reporting resistance to different insecticides. The continuous and widespread use of public health insecticides of different classes in public health has resulted in intensive insecticidal selection pressure on malaria vector population. This has resulted in the development of insecticide resistance in disease vectors, leading to reduced efficacy of insecticidal interventions and consequently, lower disease control[40].

Major resistance mechanism/s include behavioural resistance/adaptation, target-site resistance, metabolic resistance, cuticular penetration resistance. Behavioral resistance/adaptation is an adaptation by mosquitoes to avoid exposure to insecticides resulting in decreased mortalities affecting the disease control. Target-site resistance occurs when the site of action that an insecticide acts on (within the nervous system) is modified in resistant strains such that the insecticide no longer binds effectively and the insect is therefore unaffected, or less affected, by the insecticide. The four main types of target site insensitivity mechanisms in resistant insects are: (i) knockdown resistance (*kdr*-type), pyrethroids and DDT; (ii) altered AChEs, to OPs and carbamates; (iii) insensitive GABA receptors (also known as *rdl*-mutation), to cyclodienes and phenylpyrazoles; and (iv) altered nAChRs, to neonicotinoids[41]. Metabolic resistance is related to the enzyme systems, which all insects possess to detoxify foreign materials or xenobiotics, including insecticides. It occurs when increased levels or modified activities of an enzyme system prevent the insecticide from reaching its intended site of action. There are three main enzyme systems: Esterases, mono-oxygenases (or MFOs), and glutathione *S*-transferases, while metabolic resistance is important across all the four insecticide classes, there are different enzymes affecting different classes. For pyrethroids, MFOs are most important, followed by esterases. For DDT, glutathione *S*-transferases are important, followed by MFOs. For OPs and carbamates, esterases and MFOs are of utmost importance[42]. The strategic approach for malaria control require proactive approaches, especially with the goal of elimination that is round the corner by the end of this decade. For effective management, a robust database on insecticide resistance is crucial to track the status and trends of resistance in malaria vectors. The best-known strategy to abate insecticide resistance in insects is to avoid or delay the onset of resistance and is possible only by minimizing the insecticide selection pressure from the interventions in use in public health. A reduction in insecticide selection pressure can also augment the reversal of existing resistance and extend the effective lifespan of insecticides. Studies are needed to assess the resulting

variations in susceptibility levels. Such studies are rare but a study conducted in Surat, Gujarat (India) on the stability of insecticide resistance in *An. culicifacies* has provided some insights, wherein DDT- and malathion-resistance did not reverse completely even after long-term withdrawal of DDT for >30 years and malathion for 9 years in IRS, while reversal of resistance to susceptibility was observed for deltamethrin within 2-3 years of its withdrawal[7,43]. Further, the reversion of resistance depends on intrinsic fitness ratios of homozygotes and heterozygotes and frequency of resistance gene and the nature of inheritance of the gene.

In principle resistance management tactics[44] are divided into three categories namely management by moderation (lower the mortality caused by any given chemical), by saturation (saturation of the resistance mechanism of the pest by higher dosage or synergists), or by multiple attacks (mixing or alternating pesticides)[41]. Major strategies for management include, (1) management by rotating two or more insecticide classes with differerent mode of actions. This approach assumes that if resistance to each insecticide is rare, then multiple resistances will be extremely rare, (2) mosaic application is application of different compounds against same insect, like using two insecticides in different houses within the same village. This results in decreased selection pressure of insecticides and preservation of susceptible genes in the population and (3) mixture application of two or more insecticides of unrelated mode of actions simultaneously[44,45].

Management by interventions is a strategy for management of insecticide resistance in disease vectors. These strategies include combination, in which two vector control tools, ITN and IRS with different mode of actions can be used simultaneously or alternatively with IRS insecticides, having different mode of actions. This strategy is not for resistance management but for eliminating resistant mosquito by using insecticide of alternative class of insecticide to which it is susceptible. The combination should not contain insecticides with the same mode of action, as this would increase selection pressure rather than reduce it.

Another strategy for insecticide resistance management in mosquitoes is the usage of co-formulated interventions, IRS insecticides and ITNs, with insecticides of different mode of actions or synergists. Synergists are the natural or synthetic chemicals, those are considered nontoxic by themselves but enhance the potency of an insecticide. For these reasons, insecticide/synergists co-formulations are considered straightforward tools for overcoming resistance in disease vectors, and can also delay the manifestation of resistance.

Studies on biochemical resistance mechanisms indicated involvement of carboxylesterases for malathion resistance[46], GST for DDT resistance[47], monooxygenases for pyrethroid resistance[48]. Biochemical assays showed significantly elevated monooxygenases (P<0.05), while esterases were found significantly elevated in Chhattisgarh and both enzymes were involved in conferring pyrethroid resistance, not discounting the feeble involvement of *kdr* (L1014L/S) gene that was heterozygous and at low frequency (4%-5%)[48].

An. culicifacies, *An. fluviatilis*, *An. minimus* and *An. baimaii* exist as species complexes, exhibiting variations in various biological characteristics including insecticide resistance, which makes their control more complex[14]. Such strategies based on sibling species distribution are not being implemented, as it is technically intensive and require regular monitoring for sibling species composition and insecticide susceptibility in the species in field.

To effectively manage the existing resistance in malaria vectors, there is a need for novel insecticide intervention tools. Presently available tools for resistance management to mitigate the existing resistance in vectors especially to pyrethroids, include the use of interventions with insecticides having novel modes of action: IRS [clothianidin (neo-nicotinoid)]; insecticide co-formulations [clothianidin+deltamethrin]; mosaic or co-formulated ITNs-insecticide+insecticide [chlorfenapyr (pyrrole)+alpha-cypermethrin] or insecticide+synergist [alpha-cypermethrin / deltamethrin + piperonyl butoxide (synergist)].

Several limitations were identified. The major ones are gaps in geographical data on susceptibility, non-availability of longitudinal and recent susceptibility data, incomplete or variable sample sizes, limited or no information on molecular and biochemical resistance characterization including information on sibling species complexes of important vectors, *An. culicifacies* and *An. fluviatilis*. Additionally, historical comparisons with data from earlier reviews and studies were constrained due to non-availability of recent data from the respective geographical locations limiting the strategizing the management tactics. The limitation for data gaps in insecticide resistance has been the non-availability of insecticide impregnated papers for testing that are presently being imported.

6. Conclusions

As a way forward for the management of vectors, especially multiple-insecticide resistant *An. culicifacies*, there is a need for good coverage of ongoing insecticide interventions, periodic surveillance of insecticide resistance and intensity bioassays, especially where the species is reportedly resistant to pyrethroids. Besides, there is a need for proactive and effective resistance management strategies based on the available information on phenotypic insecticide resistance and insecticide resistance mechanisms. It is prudent to make efforts to establish laboratories to identify the insecticide resistance mechanisms in malaria vectors using phenotypic, biochemical and genotype studies. Information on resistance mechanisms will aid in formulating appropriate, alternative chemical insecticide based intervention strategies. Many such interventions are available

globally and national malaria control program can adapt these strategized interventions in public health for vector management, especially when malaria elimination date is fast approaching. Lastly, there is a need for networking by national control program, state health departments, and research institutes for generating quality data for insecticide resistance database. These efforts facilitate implementation of appropriate situation specific insecticide based intervention strategies for vector management and disease control to achieve elimination by date.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Protocol statement

This protocol was not registered in a formal repository such as PROSPERO or OSF at the time of conduct, as PROSPERO does not accept scoping reviews unless they directly inform health policy or clinical decision-making. Nevertheless, we have closely adhered to the PRISMA-ScR guidelines to maintain methodological transparency and rigor.

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Authors' contributions

The manuscript is conceptualized by RK and drafted by KR and RK. Data was compiled and analyzed by VS and PSV and illustrations were provided by PSV. All authors have reviewed and approved the draft.

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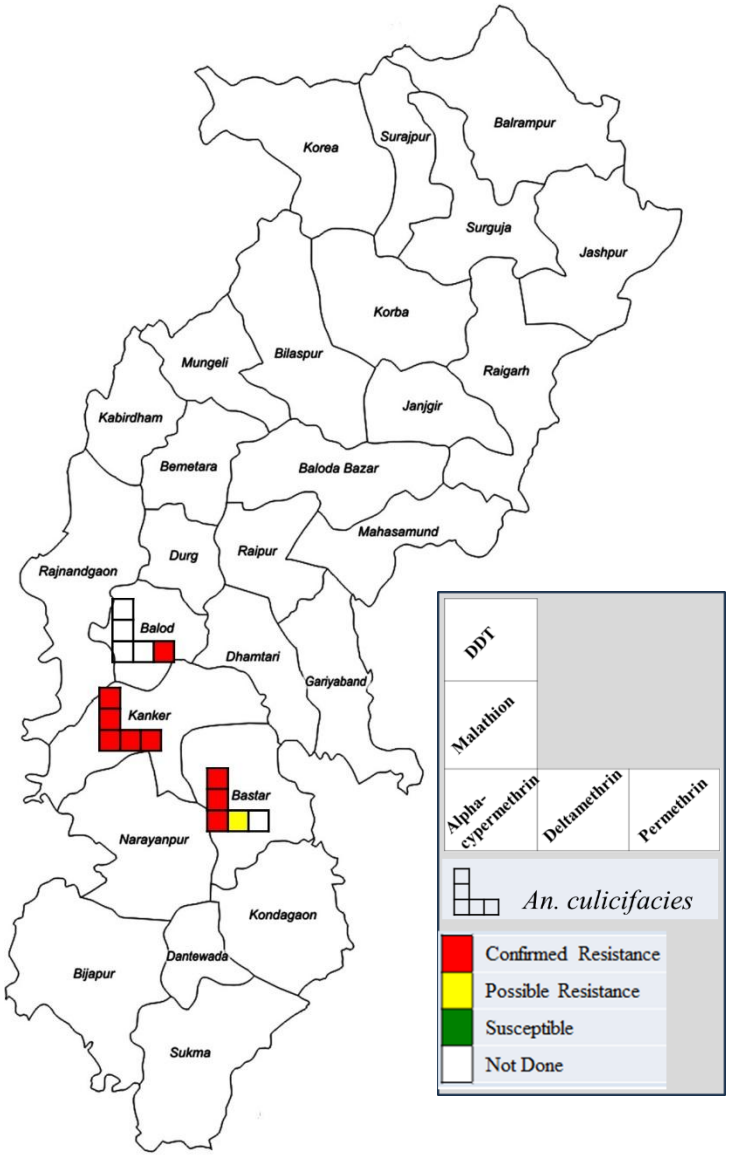
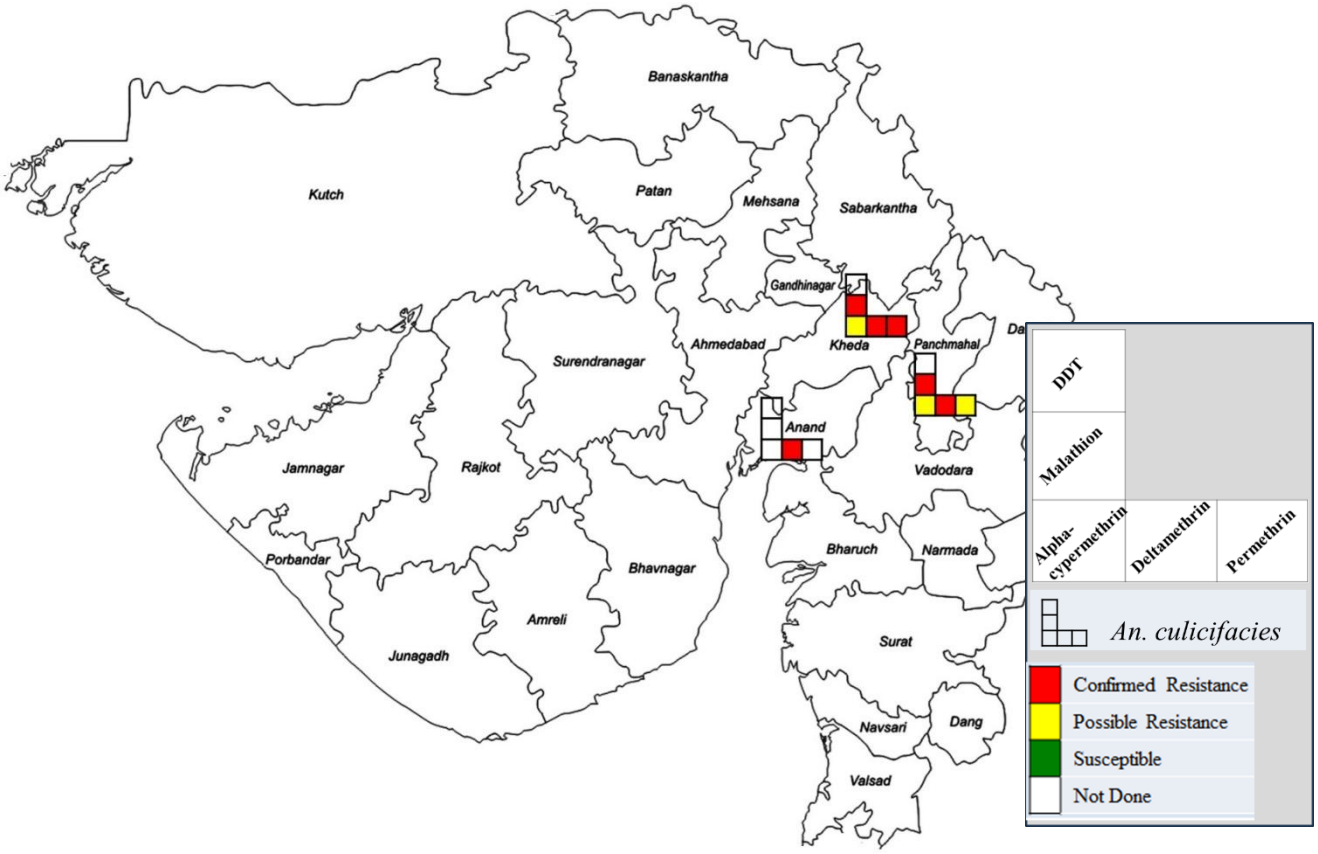
Map-based spatial visualizations of insecticide resistance in Anopheles mosquitoes across India from 2017 to 2024 and upto June 2025, as presented in this article:

Supplementary Figure 1. Insecticide resistance status of anophelines in different states of India (2017-2024) and up to June 2025: (a) Chhattisgarh, (b) Gujarat, (c) Haryana, (d) Jharkhand, (e) Karnataka, (f) Madhya Pradesh, (g) Maharashtra, (h) Odisha, (i) Rajasthan, and (j) Uttar Pradesh.



(a) Chhattisgarh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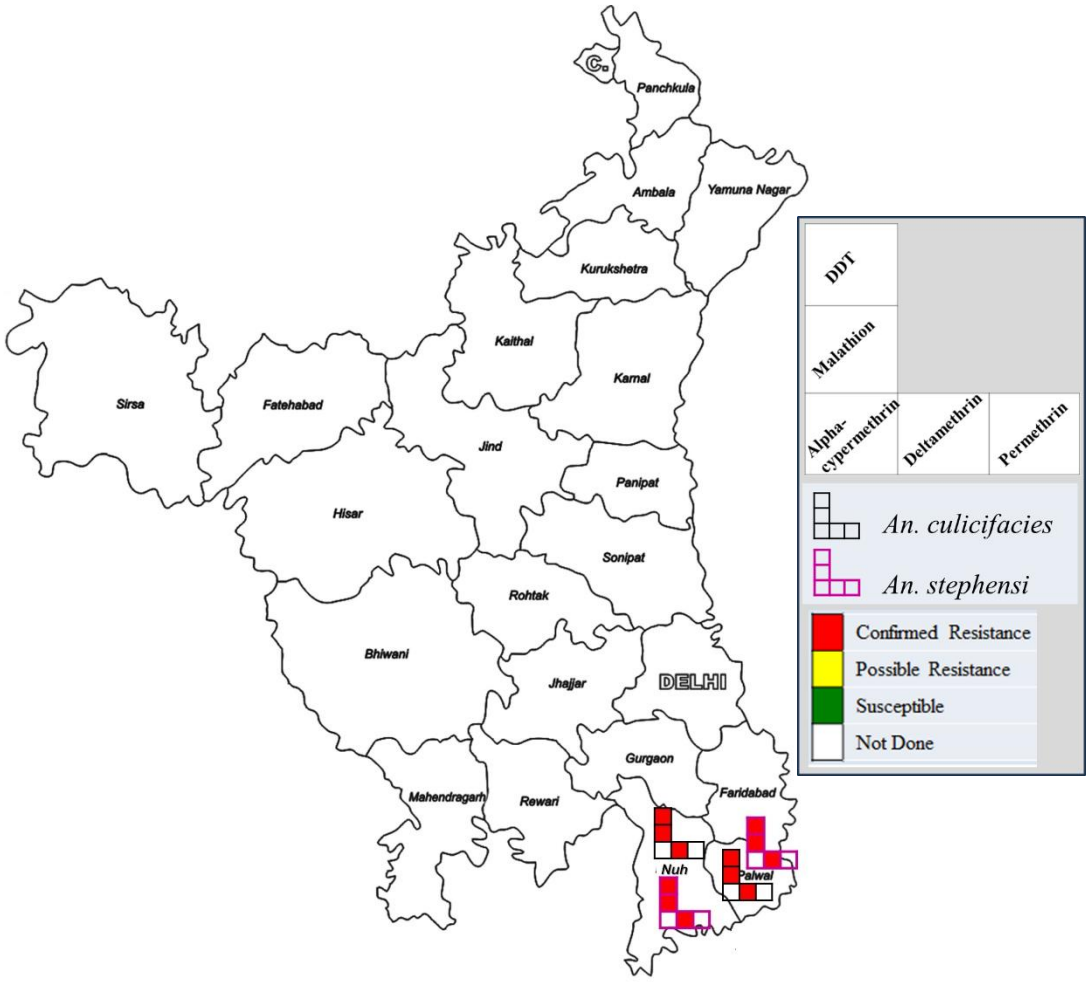
Vadodara
Porbandar
Bhavnagar
Bharuch
Narmada
Amreli
Junagadh
Surat
Navsari
Dang
Valsad
DDT
Malathion
Alpha-cypermethrin
Deltamethrin
Permethrin
An. culicifacies
Confirmed Resistance
Possible Resistance
Susceptible
Not Done

(b) Gujarat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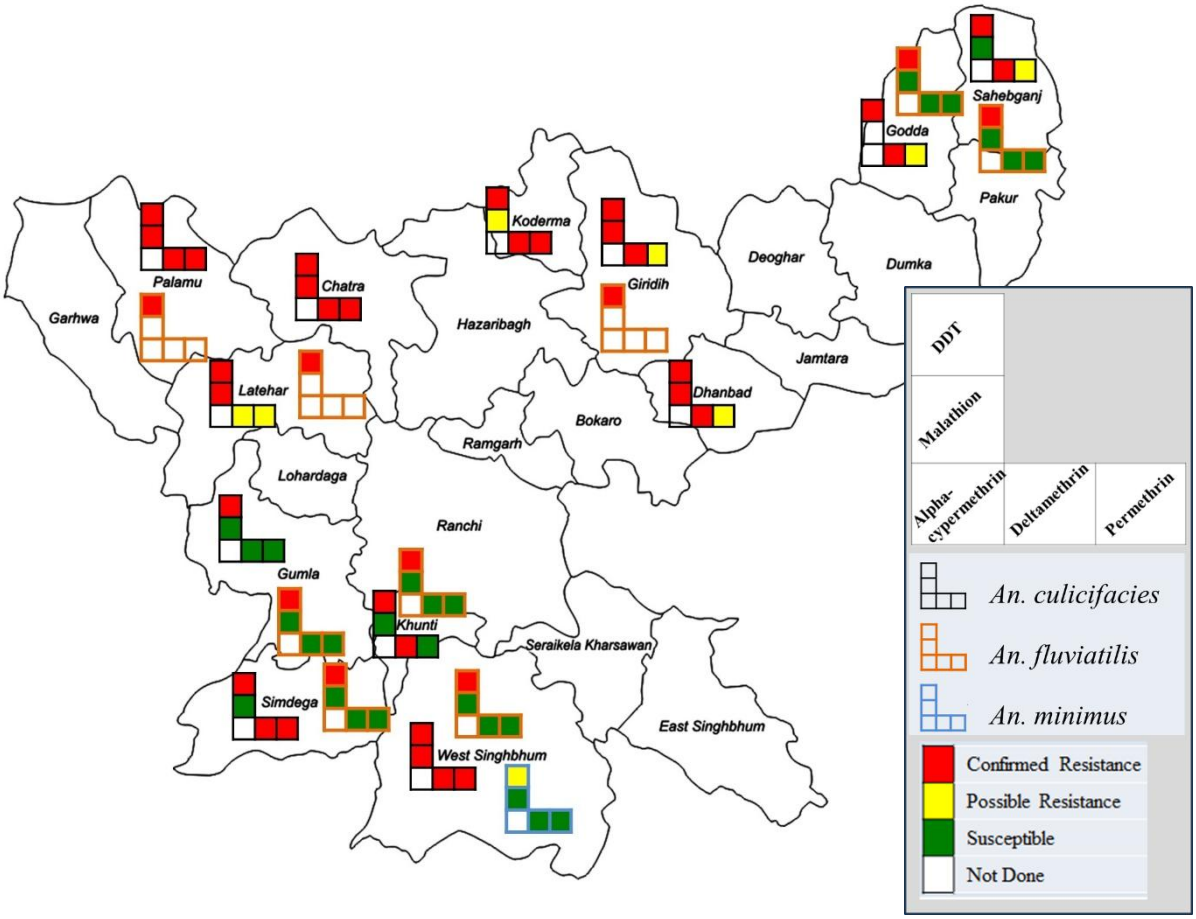
Rohtak
Bhiwani
Jhajjar
DELHI
Gurgaon
Mahendragarh
Rewari
Faridabad
Nuh
Palwal
DDT
Malathion
Alpha-cypermethrin
Deltamethrin
Permethrin
An. culicifacies
An. stephensi
Confirmed Resistance
Possible Resistance
Susceptible
Not Done

(c) Haryana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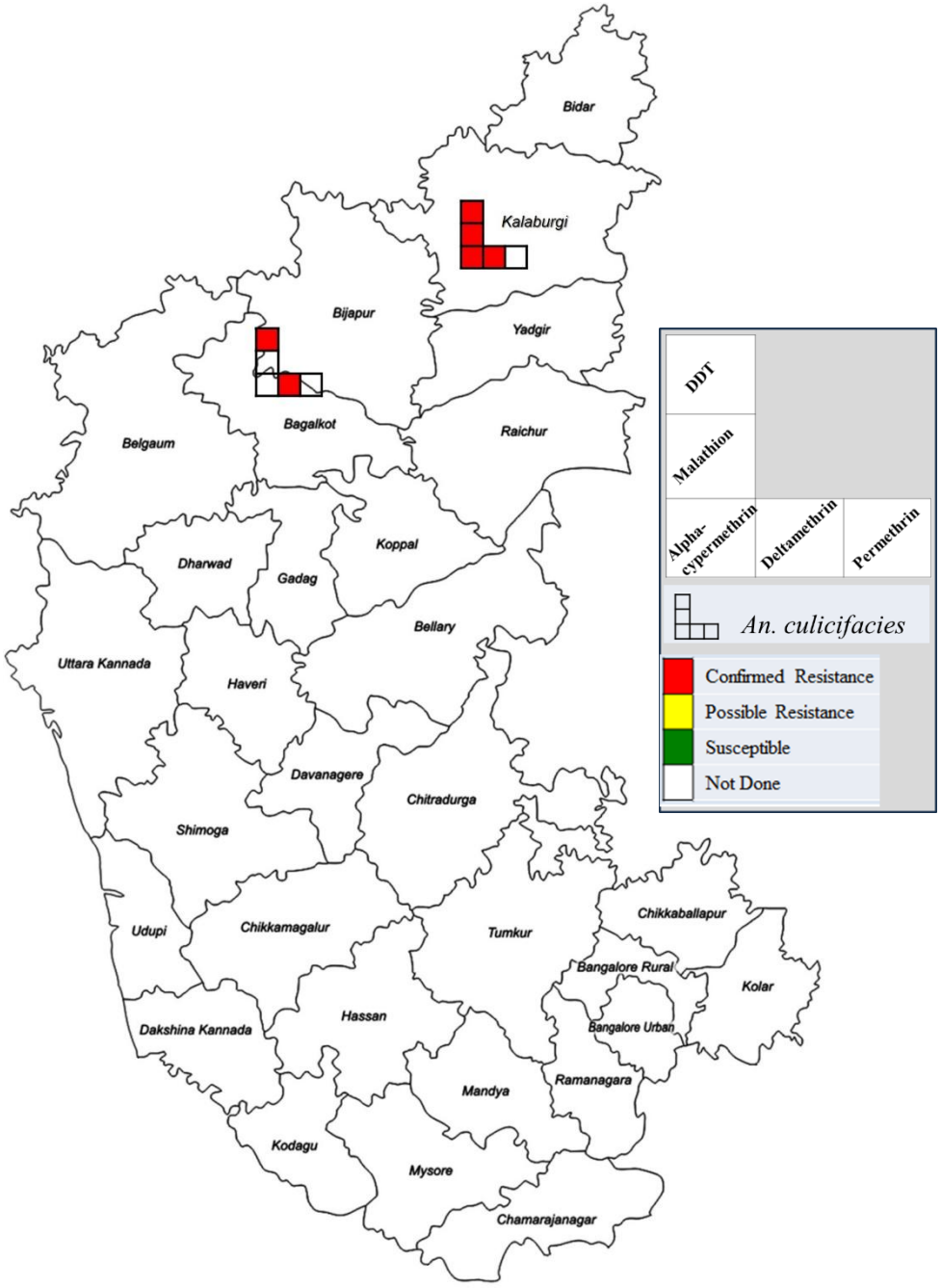
Dhanbad
Bokaro
Ramgarh
Lohardaga
Ranchi
Gumla
Khunti
Seraikela Kharsawan
Simdega
East Singhbhum
West Singhbhum
DDT
Malathion
Alpha-cypermethrin
Deltamethrin
Permethrin
An. culicifacies
An. fluviatilis
An. minimus
Confirmed Resistance
Possible Resistance
Susceptible
Not Done

(d) Jharkhand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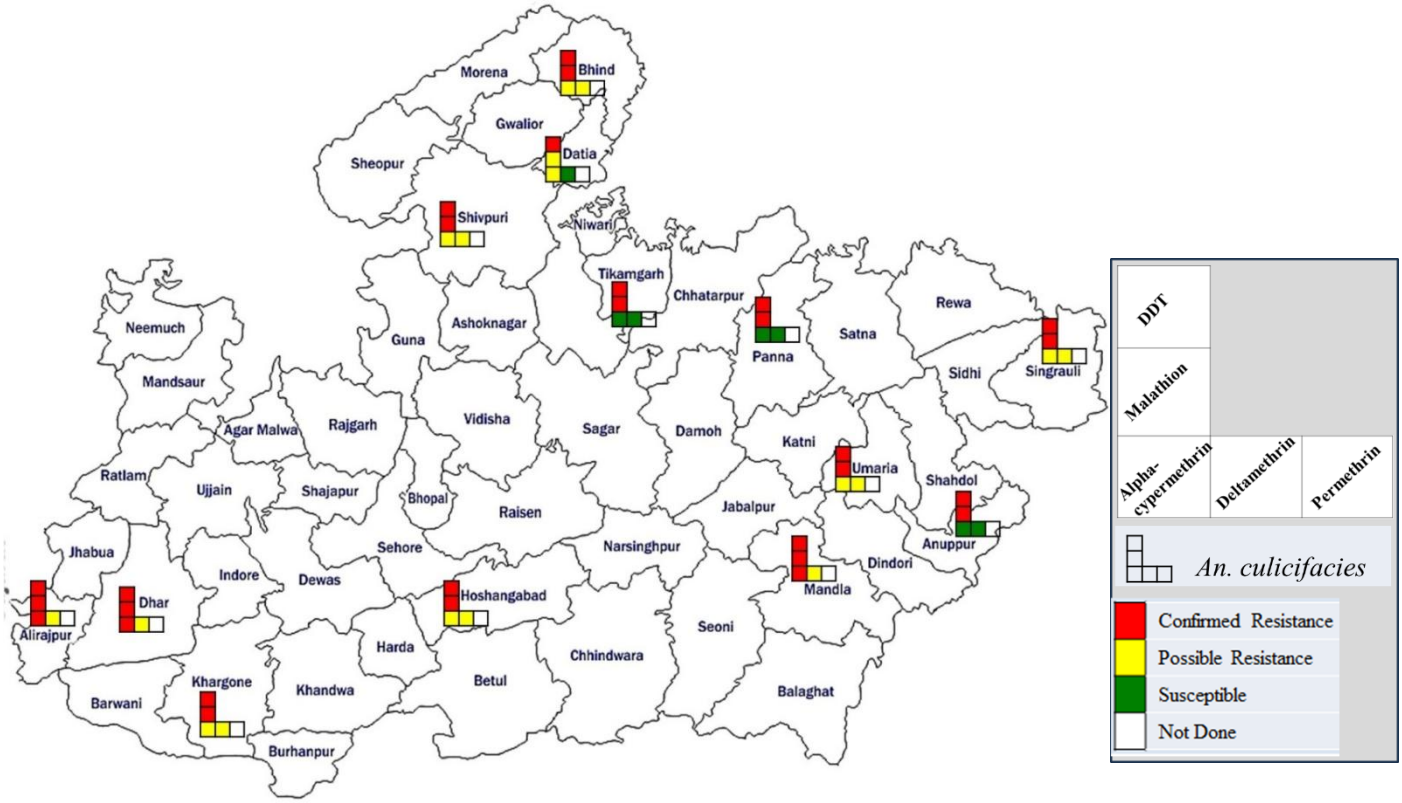
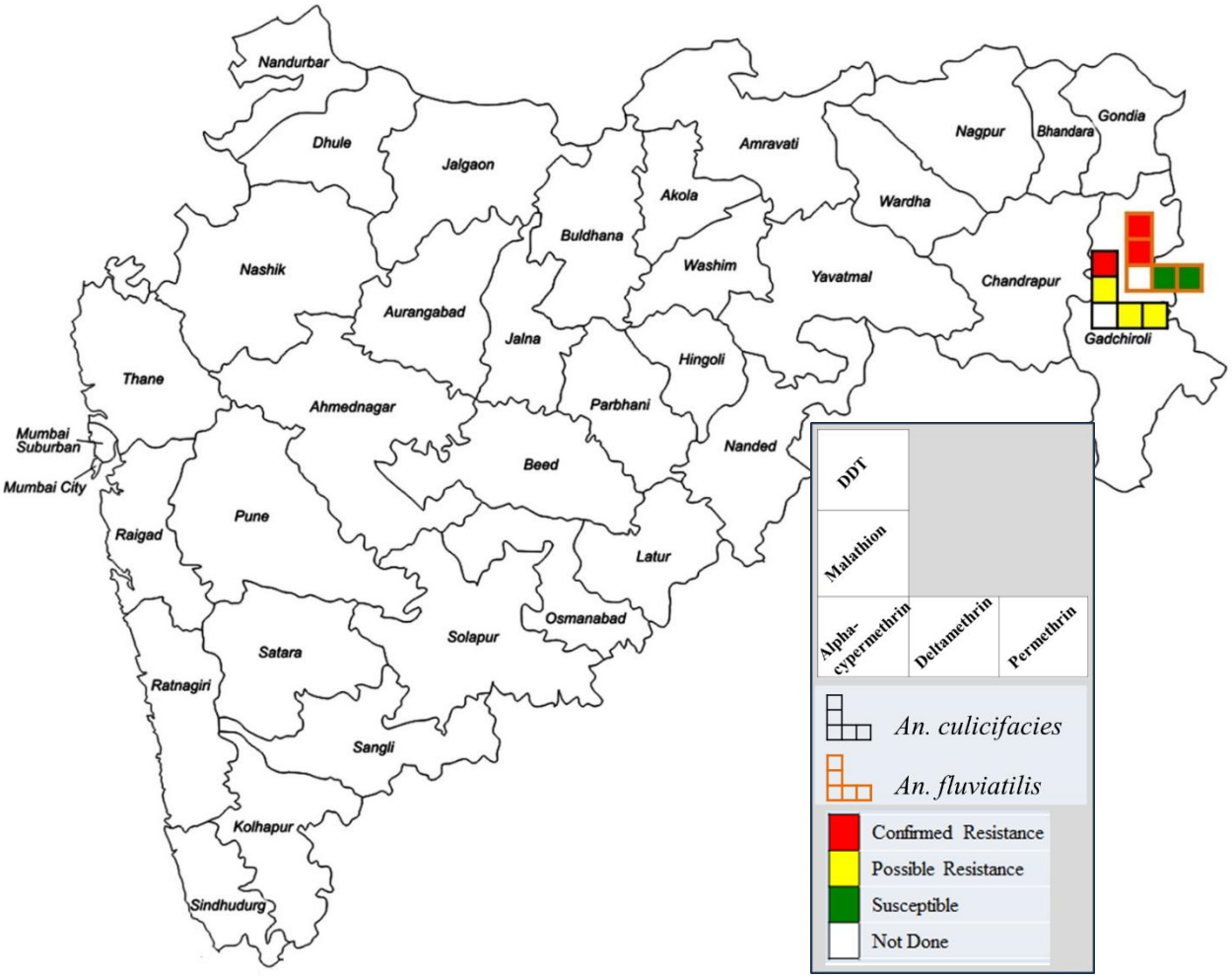
Kalaburgi
Bijapur
Yadgir
Bagalkot
Raichur
Belgaum
Koppal
Dharwad
Gadag
Bellary
Uttara Kannada
Haveri
Davanagere
Chitradurga
Shimoga
Udupi
Chikkamagalur
Tumkur
Chikkaballapur
Bangalore Rural
Kolar
Dakshina Kannada
Hassan
Bangalore Urban
Ramanagara
Mandya
Kodagu
Mysore
Chamarajanagar
DDT
Malathion
Alpha-cypermethrin
Deltamethrin
Permethrin
An. culicifacies
Confirmed Resistance
Possible Resistance
Susceptible
Not Done

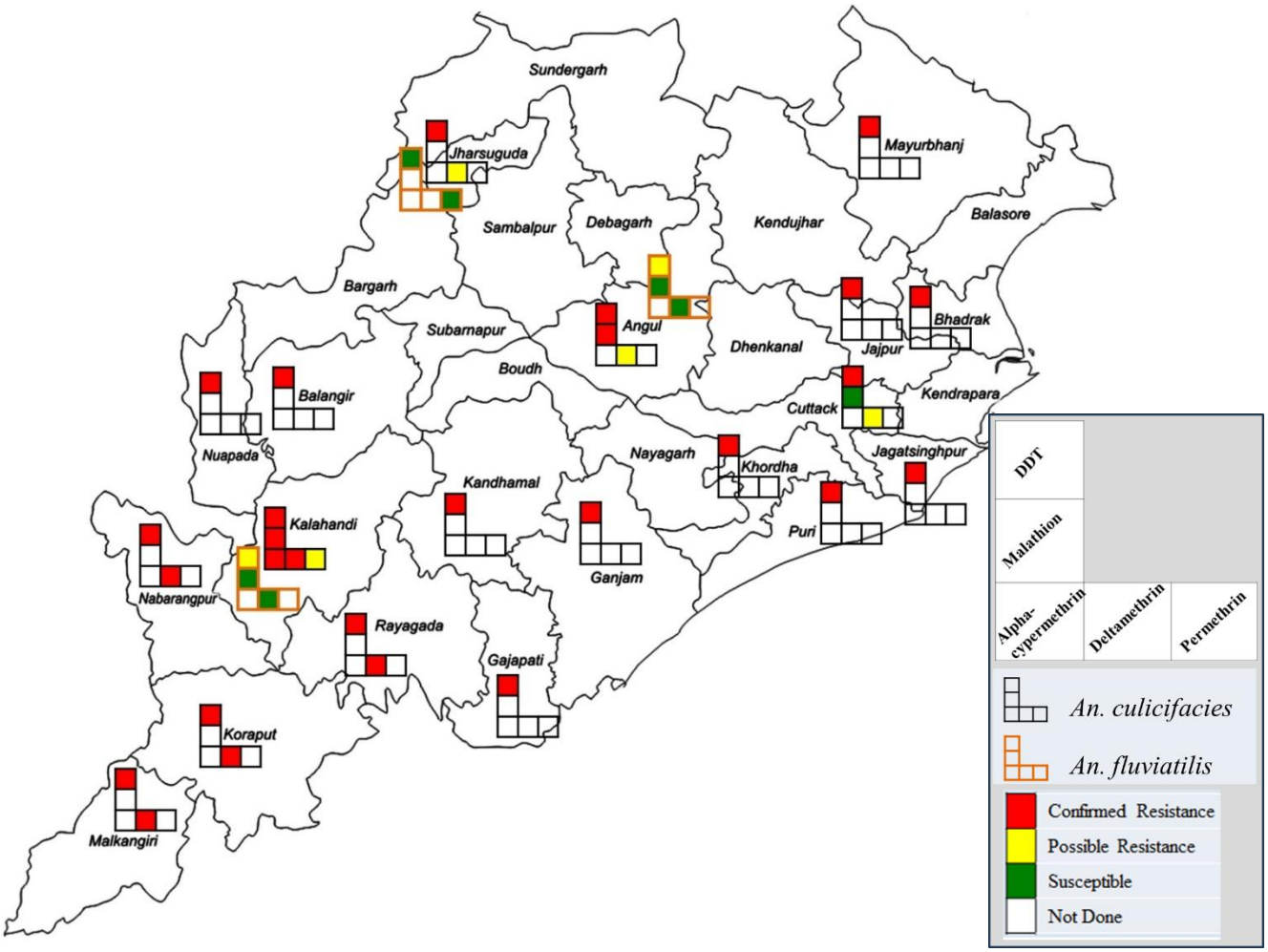
(e) Karnataka



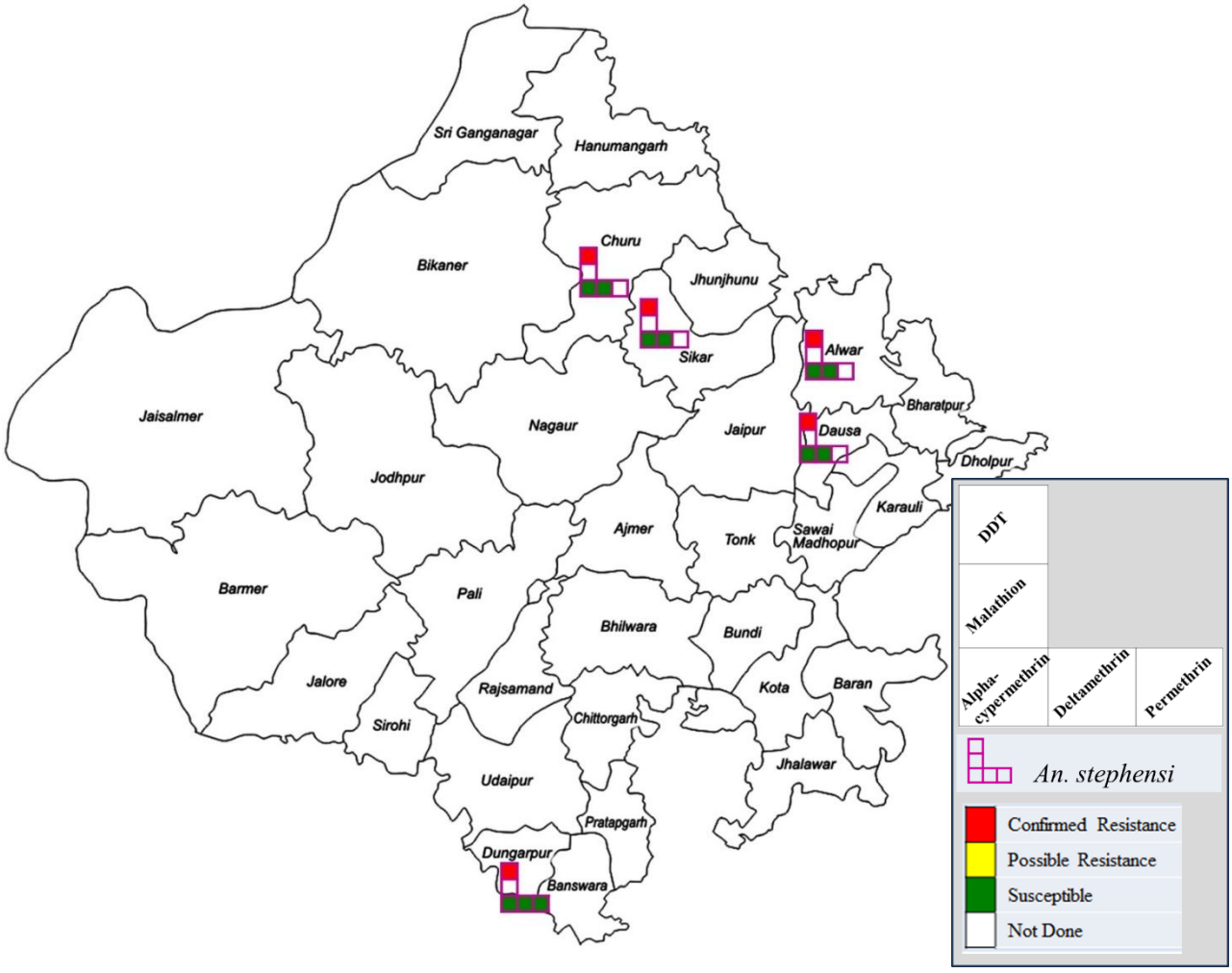
(f) Madhya Pradesh



(g) Maharashtra


Sundergarh
Jharsuguda
Mayurbhanj
Sambalpur
Debagarh
Kendujhar
Balasore
Bargarh
Angul
Subarnapur
Dhenkanal
Bhadrak
Jajpur
Boudh
Balangir
Cuttack
Kendrapara
Nuapada
Nayagarh
Khordha
Jagatsinghpur
Kandhamal
Kalahandi
Puri
Ganjam
Nabarangpur
Rayagada
Gajapati
Koraput
Malkangiri
DDT
Malathion
Alpha-cypermethrin
Deltamethrin
Permethrin
An. culicifacies
An. fluviatilis
Confirmed Resistance
Possible Resistance
Susceptible
Not Done

(h) Odisha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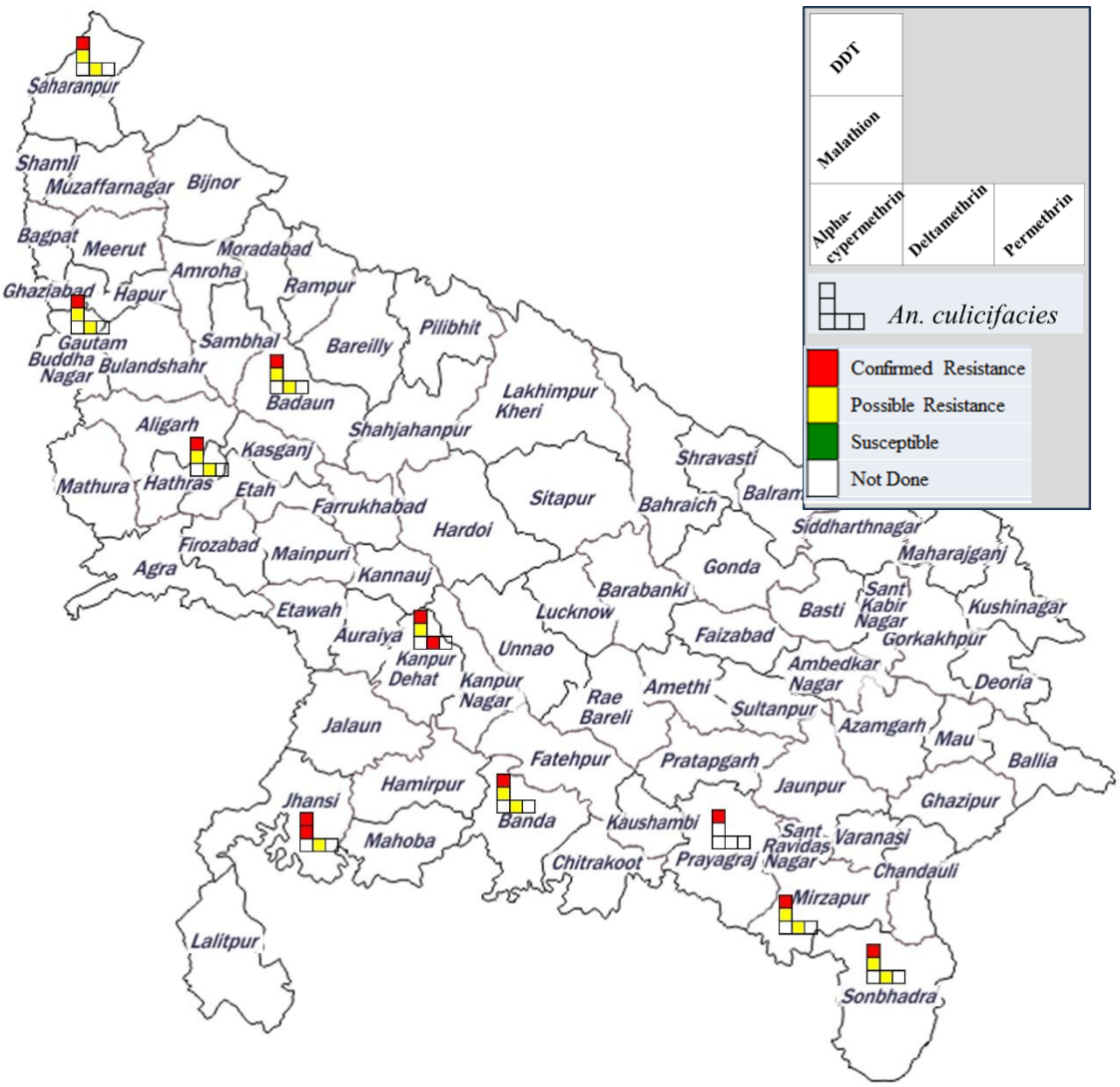
Hanumangarh
Bikaner
Churu
Jhunjhunu
Sikar
Alwar
Bharatpur
Jaisalmer
Nagaur
Jaipur
Dausa
Dholpur
Jodhpur
Karauli
Ajmer
Tonk
Sawai Madhopur
Barmer
Pali
Bhilwara
Bundi
Jalore
Rajsamand
Kota
Baran
Sirohi
Chittorgarh
Udaipur
Jhalawar
Pratapgarh
Dungarpur
Banswara
DDT
Malathion
Alpha-cypermethrin
Deltamethrin
Permethrin
An. stephensi
Confirmed Resistance
Possible Resistance
Susceptible
Not Done

(i) Rajasthan



(j) Uttar Pradesh

Supplementary Figure 2. Insecticide resistance status of anophelines in northeast states of India (2017-2024) and up to June 2025.

