International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2021; 8(3): 10-15

www.dipterajournal.com
Received: 07-03-2021
Accepted: 09-04-2021

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# Susceptibility status of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) to insecticides in Southern Odisha, India

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**Abstract**
Insecticides are a valuable resource in vector control programmes. The programmes primarily rely on the susceptibility of the vectors against the insecticides. The current study determined the insecticide susceptibility of two major dengue vectors, *Aedes aegypti* and *Ae. albopictus* in Koraput district of Odisha. Immature stages of dengue vectors were collected from domestic and adjoining mosquito breeding habitats and were reared to adult stage ($F_1$), then exposed to insecticide impregnated papers. The treatments used were diagnostic concentration of organochlorines (4% DDT), pyrethroids (0.05% deltamethrin) and organophosphates (5% malathion). We found *Ae. aegypti* was resistant to DDT, deltamethrin and developed a possible resistance to malathion, while *Ae. albopictus* was resistant to DDT but susceptible to both malathion and deltamethrin. Hence there is an urgent need for implementation of proper insecticide resistance management strategies to control dengue vectors during outbreak.

**Keywords:** *Aedes aegypti, Ae. Albopictus,* insecticide susceptibility, resistance

## 1. Introduction
Dengue is a mosquito-borne viral disease transmitted by the *Aedes* mosquitoes to humans. Dengue virus causes acute fever when it is spread to humans through the bite of a carrier mosquito which can soon manifest into fatal Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Approximately, 2.5 billion people around the world are at risk to dengue and 50 million cases are reported annually [1]. The burden of dengue is too high in South East Asian countries. The first case of dengue was reported in the State of Delhi, India [2] in the year 1996. Consequently, many cases were reported from various parts of India in subsequent epidemics caused by four different dengue virus (DENV) serotypes [3, 4]. During the last 5 years (from 2015 to 2019), the total number of reported dengue cases in India ranged from 99913 (2015) to 188401 (2017) [5]. The East Central Indian State Odisha, reported its first dengue occurrence with six deaths in 2010, followed by more dengue outbreaks in 2011 affecting more than 52 people (Source: Chief District Medical Officer, Malkangiri). The total number of dengue cases in the State ranged from 2450 to 8380 and the number of deaths due to dengue ranged from 2 to 11 during the last 5 years [5]. *Aedes aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) are the prime vectors of dengue virus (DENV) across the world [6, 7]. With no vaccine or specific treatments available so far for DENV, vector containment remains to be one of the basic methods in prevention of dengue outbreaks. Many strategies to prevent dengue spread have been implemented such as elimination of mosquito breeding sources like water-holding containers [8], maintenance of proper sanitization, urbanization planning, awareness programs etc., but the use of insecticides has been proved to be one of the most potent preventive strategies against the disease [9]. Space spraying (pyrethroids or organophosphates) of insecticides is commonly employed during outbreaks [10] in case the population of adult mosquitoes cannot be controlled by larval source management/larvicidal measures. Insecticide resistance is an inherent problem associated with repeated use of insecticides [11, 12] which negatively affected the efficacy of vector-control programmes [13]. Information regarding the susceptibility/ resistance level in dengue vectors to insecticide is

important for executing vector control strategies. Several studies conducted worldwide showed the occurrence of insecticide resistance in both the dengue vector species, *Ae. aegypti* and *Ae. Albopictus* [11,14-17].

*Ae. albopictus,* followed by *Ae. aegypti* are the most abundant vector species found in Odisha State, India [18]. No data are available so far on susceptible/resistant status of these vectors in southern region of Odisha State. Recent dengue transmission has radically stretched to many urban, semi-urban and rural areas, where earlier it was not reported and has become an important public health crisis in the State [18]. Extensive monitoring and vector crisis management are required in this area due to increased dengue outbreaks in every year. To apply an efficacious and viable vector control program, the susceptibility/resistance status of the dengue vectors to different routinely used insecticides has to be assessed. Hence, the current study was carried out to determine the susceptibility of adult *Ae. aegypti* and *Ae. albopictus* to three insecticides using World Health Organization Pesticide Evaluation Scheme (WHOPES) approved procedures [19-20] in order to facilitate developing an effective control measures against dengue vectors in the southern region of Odisha State.

## 2. Materials and Methods

### 2.1. Study area

Larval and pupal stages of *Ae. aegypti* and and *Ae. albopictus* were collected from 10 different locations in two urban (Koraput N 18.80988°, E 082.71581° and Jeypore N 18.86522°, E 082.57555°) and four rural (Kundariguda N 18.86287°, E 082.57874°; Kenduguda, N 18.87443°, E 082.56639°; Gadapadar N 18.89382°, E 082.59222°; Janiguda N 18.80088°, E 082.69944°; and Chindri N 18.81713°, E 082.73133°) areas of Koraput district from June 2020 to August 2020 (Fig.1). In each location, the breeding sites such as, tyres, flower pots, plastic cups, storage tanks, unused mud pots, cut bamboos, tree holes, drains and construction sites if available were physically searched for the presence of water, mosquito larvae and pupae. From all positive containers both indoors and outdoors *Aedes* larvae and pupae were collected with dipper and strainer. In each sampling site, *Aedes* larvae or pupae or both collected from different breeding habitats were mixed together, labeled and kept in plastic containers. These immature were reared to adults in the laboratory. The emerged adults were identified to species following standard identification keys [21]. The emerged vector species from urban and rural areas were kept separately to identify the species composition in two sites. The adults were fed on 10% sucrose solution-soaked cotton pads.

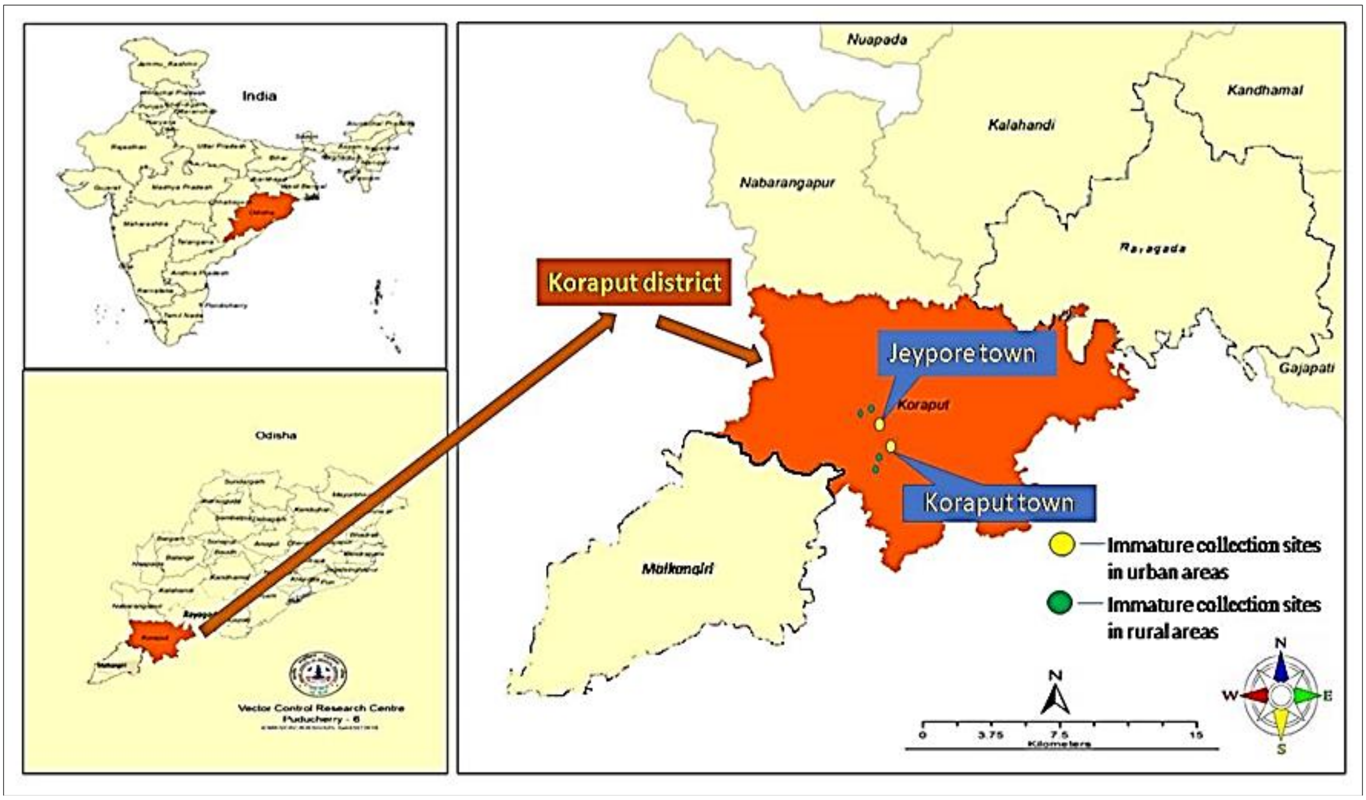


**Fig 1:** Map showing immature collection sites

### 2.2. Study design

Susceptibility tests were performed using World Health Organization (WHO) test kits [19]. Optimum laboratory conditions of 27°C ± 2°C with 75% ± 10% relative humidity were maintained during the test [19-20]. Insecticide papers sprayed with 4% DDT, 0.05% deltamethrin and 5% malathion and their respective control papers impregnated with risella, olive and silicone oil used in the susceptibility tests were obtained from Vector Control Research Unit, University Sains Malaysia, Penang, Malaysia for use in the susceptibility tests [22-24]. Non-blood-fed two days old emerged *Ae. aegypti* and *Ae. albopictus* females were grouped in four batches of 25 each. Each batch was then introduced into WHO exposure tubes with insecticide treated papers for one hour. Before exposure, the mosquitoes were held in holding tubes having white paper for one hour to acclimatize them inside the tubes. Stagnant, injured or dead mosquitoes were removed after the acclimatization and replaced with fresh one to maintain the count to 25. Two control replicates with 25 mosquitoes each were maintained parallel for comparison. 50% ($KDT_{50}$) and 95% knock down ($KDT_{95}$) time was determined by recording the number of knock downs every 10 minutes up to one hour of the exposure. After the exposure, the surviving mosquitoes were transferred to the WHO recovery tubes having 10% sugar solution for their food for the next 24-h. The number of dead mosquitoes (mortality) was scored to record the susceptibility status. Re-examination of the, exposed mosquitoes was carried out to confirm the species.

### 3. Data analysis

Mortality rate of the tests and the controls was calculated, following the WHO guidelines [19-20]. As the control mortality in all the tests was below 5%, correction of test mortality compared to control mortality using Abbott's formula was not required[26]. Insecticide susceptibility/resistance status of the two vector species was classified as per the WHO criteria, i.e., corrected mortality (CM) of $\geq$98% is 'susceptible', <90% is 'resistant' and 90-97% is 'possible resistance' that needs verification. Probit analysis with log transformation was used to estimate $KDT_{50}$ and $KDT_{95}$ values. Chi-square test was used to compare each species mortality between insecticides. IBS SPSS statistics version 25.0 was used to analyze statistical data and a P-value$\leq$ 0.05 was considered statistically significant.

### 4. Results and Discussion

### 4.1 Species composition

A total of 1616 mosquitoes belonging to three *Aedes* species, including the two recognized dengue vectors species *Ae. aegypti* (34.7%) and *Ae. albopictus* (48.2%) emerged from the immature collections (Table 1). The third species emerged was *Ae. vittatus* (Bigot) (17.1%). Among the 560 *Ae. aegypti* emerged from the immature samples, 347 (61.9%) were from urban areas and the remaining 213 (38.1%) were from rural areas. Similarly, out of 780 emerged *Ae. albopictus*, 21.0% (n = 164) was from urban areas and 79.0% (n = 616) was from rural areas (Table 1). The major breeding habitats of *Ae. aegypti* in urban areas were plastic containers used to store water and tyres, where as for *Ae. albopictus*, unused mud pots were the preferential breeding habitats in rural areas.

**Table 1:** Number of Aedes mosquitoes emerged from urban and rural areas of Koraput district

| Aedes species | Number emerged | | |
|---|---|---|---|
| | Urban | Rural | Total |
| *Ae. aegypti* | 347 | 213 | 560 |
| *Ae. albopictus* | 164 | 616 | 780 |
| *Ae. vittatus* | 75 | 201 | 276 |
| Total | 586 | 1030 | 1616 |

The results of susceptibility/resistance status of *Ae. aegypti* and *Ae. albopictus* to diagnostic dosages of DDT, malathion and deltamethrin are given in Table 2. In total, six susceptibility tests were carried out exposing 450 (300 treated and 150 control) $F_1$ adults of each of the two dengue vector species to the three insecticides. Mortality of *Ae. aegypti* was 12%, 94% and 24% against DDT, malathion and deltamethrin, respectively. Malathion caused significantly higher mortality than the other two insecticides ($\chi2$ = 159.75, p<0.001).

**Table 2:** Responses of *Ae. aegypti* and *Ae. albopictus* to DDT, malathion and deltamethrin in southern Odisha

| Insecticide | Species | Number of mosquitoes: Exposed T | Exposed C | Dead T | Dead C | CM (%) | $KDT_{50}$ (min) (95% CI) | $KDT_{95}$ (min) (95% CI) | $\chi2$ (p) | Slope+SE | Status |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DDT 4% | *Ae. aegypti* | 100 | 50 | 12 | 1 | 12.0 | 975.78 (Not estimable) | 5983.32 (Not estimable) | 4.24 (0.37) | 2.09$\pm$2.54 | R |
| | *Ae. albopictus* | 100 | 50 | 20 | 1 | 20.0 | 158.40 (103.38- 494.10) | 699.20 (287.63 - 7903.14) | 0.69 (0.95) | 2.55$\pm$0.61 | R |
| Malathion 5.0% | *Ae. aegypti* | 100 | 50 | 94 | 1 | 94.0 | 52.36 (46.40 - 63.84) | 92.27 (72.05 - 180.87) | 12.6 (0.013) | 6.68$\pm$0.66 | PR |
| | *Ae. albopictus* | 100 | 50 | 100 | 1 | 100.0 | 45.39 (38.65 - 55.12) | 76.78 (60.82 - 156.25) | 22.4 (<0.001) | 7.20$\pm$0.60 | S |
| Deltamethrin 0.05% | *Ae. aegypti* | 100 | 50 | 24 | 0 | 24.0 | 139.98 (97.20- 305.10) | 814.50 (353.71 - 5118.75) | 1.22 (0.87) | 2.15$\pm$0.4 | R |
| | *Ae. albopictus* | 100 | 50 | 99 | 1 | 99.0 | 62.88 (55.90 - 74.42) | 210.11 (152.30 - 350.03) | 5.21 (0.27) | 3.14$\pm$0.37 | S |

T- test; C- control; CM- corrected mortality; KDT- knock-down time; CI- confidence interval; R- resistant (CM <90%); PR- possible resistance (CM 90–97%); S- susceptible

Mortality of *Ae. albopictus* was 20%, 100% and 99% against DDT, malathion and deltamethrin, respectively. Malathion 5.0% and deltamethrin 0.05% produced significantly higher mortality compared to DDT ($\chi2$ = 213.80, *p*<0.001).

For *Ae. aegypti*, the $KDT_{50}$ and $KDT_{95}$ against DDT was not estimated due to the fact that only two mosquitoes were knocked down at 60 min. The $KDT_{50}$ was 52.36 min against malathion and 139.98 min against deltamethrin. In case of *Ae. albopictus*, the $KDT_{50}$ against DDT, malathion and deltamethrin was 158.40; 45.39 and 62.88, respectively (Table 2). The $KDT_{95}$ for *Ae. aegypti* against malathion and deltamethrin was 92.27 and 814.50 min, respectively and $KDT_{95}$ for *Ae. albopictus* against DDT, malathion and deltamethrin was 699.20, 76.78 and 210.11 min, respectively (Table 2).

The major approaches to combat dengue involve the reduction of vector density by eliminating the breeding sources or using insecticides or both. Accordingly, evaluation of the resistance status of dengue vectors towards different insecticides is important and a prerequisite for dengue control programmes to make sure that a suitable insecticide is selected for effective implementation. Till date, no information is available on the susceptibility/resistance status of dengue vectors in endemic southern region of Odisha State. The current study assessed the insecticide susceptibility/resistance of the two dengue vector species in the southern region of Odisha State, which are affected by repeated outbreaks of dengue recent past [5] and to support the control activities. The southern districts of Odisha State have been hyper endemic for *falciparum* malaria since many decades [28] and during the last 8 years, outbreaks of both dengue and Japanese encephalitis (JE) have also been reported in this region [5, 28]. No specific vector control programme is available for containment of these two diseases in India. Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) with DDT/deltamethrin used for controlling malaria is considered as assured methods for the control of dengue vectors. Further, to decrease the population of dengue/JE vectors, thermal fogging with synthetic pyrethroids or malathion is regularly done in dengue/JE affected localities [28]. Hence, the susceptibility/resistance status of dengue vectors to DDT, deltamethrin and malathion was determined in southern region of Odisha State.

According to the tentative recommendation of WHO, for carrying out bioassays with *Aedes* mosquitoes, the discriminating concentrations of deltamethrin and malathion are 0.03% and 0.8% [19-20]. Since, it is tentative recommendation; the susceptibility tests of *Aedes* mosquitoes were also carried out in many parts of the world even recently against DDT 4%, malathion 5% and deltamethrin 0.05% [22-25]. In the current study, DDT 4%, deltamethrin 0.05% and malathion 5.0% was used for the bioassays. The results showed increased level of phenotypic resistance to DDT in the field populations of *Ae. aegypti* and *Ae. albopictus*. The mortality of *Ae. albopictus* was 20% when exposed to DDT and 12% in case of *Ae. aegypti*. The recognition of DDT resistance in both the dengue vectors in the current study is not surprising, as DDT resistance in these vector species has been reported widely throughout the world [29-31]. The current study also observed that *Ae. albopictus* was susceptible to malathion and deltamethrin indicating that this insecticide could still be used for controlling dengue vector where *Ae. albopictus* is known to transmit the disease. The tests conducted with *Ae. aegypti* against deltamethrin and malathion showed that this species is resistant to both the insecticides in this area. A similar finding was observed in a recent study conducted at New Delhi that *Ae. aegypti* has developed resistance against most of the currently used insecticides[23]. Insecticide resistance management (IRM) is crucial to maintain vector control sustainable. Studies have been undertaken by many investigators to assess insecticide susceptibility status of dengue vectors in different parts of India [32, 33]. The high resistance found in *Ae. aegypti* to the commonly used adulticides and larvicides necessitates continuous susceptibility monitoring for effective vector control programme [22, 34, 35].

The reason for the resistance detected in both the dengue vectors to DDT in the current study could be due to prolonged use of DDT in public health programs for more than seven decades in Odisha State. It has been reported that in Thailand, intense use of DDT for a period of two years to control dengue vector resulted in development of resistance to this insecticide [36]. In the same way, resistance to DDT in *Aedes* vector mosquitoes has been widely reported in Sri Lanka [37] and many parts of India [38]. DDT resistance in *Ae. aegypti* was also observed in Cape Verde archipelago and Senegal [39], Central Africa [40], Malaysia [41] and Grand Cayman [42].

In Odisha State, information on insecticide susceptibility/resistance status of dengue vectors is limited and, hence, could be an obstruction to achieve the desired results from dengue vector control activities. The current study provides the baseline data on insecticide susceptibility/resistance status of dengue vectors for the dengue endemic southern region of Odisha. Similar to our findings, a study conducted in 2017 in Odisha State showed that *Ae. albopictus* was susceptible to the synthetic pyrethroid, Cyfluthrin [43]. The results of the current study revealed that *Ae. albopictus* remains to be susceptible to malathion and deltamethrin. The density of *Ae. albopictus* was comparatively higher in rural areas, where this species has been exposed to DDT as this organochlorin insecticide has been extensively used in malaria control program. Though malathion is not used in the routine public health program, but during JE outbreak period and at the time of rising dengue cases in rural areas, malathion is only being used to control the respective vector mosquitoes. Moreover, universal coverage of long-lasting treated nets is in use in the rural villages of Odisha State since last four years only. Therefore, *Ae. albopictus* is still susceptible to malathion and deltamethrin. However, because of current use of synthetic pyrethroids in IRS and universal distribution of LLINs (incorporated with synthetic pyrethroids) in rural areas, there is a chance of development of resistance in *Ae. albopictus* to synthetic pyrethroids in future. In case of *Ae. aegypti*, its population is high in urban areas, where malathion and synthetic pyrethroids are extensively used in thermal fogging by municipalities every year during monsoon and post monsoon months to control dengue vectors. This could be the reason that *Ae. aegypti* has developed resistance to malathion and synthetic pyrethroids.

## 5. Conclusion

This is the first report of development of insecticide resistance in *Ae. aegypti* and *Ae. albopictus* in southern dengue endemic region of Odisha State. Recent rise in outbreaks of both dengue and JE in this area requires a strong vector surveillance and management. The observations made in the current study could facilitate guiding the programme in selection and implementation of appropriate insecticide-based strategy to curb vector borne dengu disease.

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