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Entomological survey for identification of *Aedes* larval breeding sites and their distribution in selected rural villages of West and South Tripura, India

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A B S T R A C T

The present study aims to identify the breeding sites and distribution of *Aedes* immature forms in selected tribal villages in West and South Tripura. The survey of aquatic habitats harboring mosquitoes were undertaken in five villages in West Tripura and six villages in South Tripura from September to November 2021. All water-holding containers were inspected for the presence of immature mosquito forms using pipette or dipper net based on container sizes. Adults were counted and observed under a Stereomicroscope following systematic taxonomic keys. A total of 330 immature forms were collected during the study period. Interestingly, no *Aedes aegypti* was found during the study while, *Aedes albopictus* was found to be relatively the dominant species in the study area. The house index (HI), container index (CI) and breteau index of West Tripura were 21.6%, 36.6% and 26.4% respectively whereas South Tripura were 20.66%, 33.3% and 26.6% respectively. The present study indicates the dominance of *A. albopictus* in the rural villages of both West and South Tripura. A considerably larger proportion of artificial productive containers viz. damaged tyres and broken plastic items in the surrounding residential areas highlights the urgency of its reduction efforts. Immediate intervention is required to reduce the water staging in tyres and other products during the cultivation of dragon fruit. This may provide a lucrative alternative for preventing the transmission of dengue and other arboviruses.

Introduction

Dengue fever emerged as one of the most serious arboviral diseases affecting mankind and currently poses a risk for more than 128 countries globally (Sabchareon *et al.,* 2012). The disease has a major socio-economic and public health impact on the countries from where the epidemics are reported. *Aedes* mosquitoes are the most efficient vector of various arboviruses, including dengue, chikungunya, and Zika virus. The control of Aedes mosquitoes is the only available strategy to prevent dengue transmission and to decrease the disease burden. *Ae. aegypti*, the principal vector of dengue is highly adapted to all man-made and natural environments and is closely associated with human dwellings. *Ae. albopictus* is an invasive species, which can transmit a large number of arboviral diseases including

dengue and chikungunya (Das *et al.,* 2014). The *Aedes* species mostly breed in artificial water-holding containers. Vector control is the only option for dengue prevention, which can be done by preventing the transmission potential by reducing the emergence of adult mosquitoes (Ferdousi *et al.,* 2015). This could be achieved by targeting the immature stages of *Ae. Aegypti* through source reduction or biological or biocidal treatment.

The productivity of a container type depends on a range of factors, such as size and shape, the purpose of use, their locations, method of filling, the material of the container (plastic, metal, cement, etc.), temperature and competition among co-species. Various entomological indices are used to measure dengue vector infestation inside and surrounding the residential areas (Rahman *et al.,* 2021).

Examples of such indices are the house index (HI, the proportion of positive houses) and container index (CI, the proportion of positive containers), and the Breteau index (BI, the number of positive containers per surveyed houses) (WHO, 1971; Agbanrin, 2016). There is a lack of a sensitive vector surveillance tool to estimate the vector density in the outbreak areas. Therefore, the vector abundances is still expressed as house index, Breteau index, and container index (Padonou *et al.,* 2020).

A few studies on *Aedes* mosquito abundance and breeding preference were conducted in the urban districts of Tripura (Chowdhury *et al.,* 2021). However, there is no information about the rural areas of Tripura. A large number of dengue cases were reported from rural areas of West and South Tripura in September 2021.

Therefore, during the late monsoon (September to November) of 2021, we conducted a comprehensive entomological survey in five villages of West Tripura and six villages of South Tripura. The main objective was to identify the breeding sites of *Aedes* larvae and their distribution in rural areas of West and South Tripura.

Materials and Methods

Study area

The survey of aquatic habitats harboring mosquitoes were undertaken in five villages of West Tripura and six villages in South Tripura from September to November 2021. The field survey areas were selected merely on the basis of dengue cases reported for the first time.

Larval sampling and rearing

Five villages of West Tripura (Khuntia para, Khirod Thakur para, Bahumani para, Takajala, Gabordi) and six villages of South Tripura (Krishnapur, Nachirnagar, Batisha, Kamalpur, Chandrapur, Rajnagar) were selected and larval collections were carried out in 25 houses per village. Standard dipper isused as a survey tool for sample collection. Occasionally 5 ml graduated plastic pipettes proved helpful alternatives of standard dippers for small breeding sites like tyres, plastic containers and the latter were pooled to get the required water sample for larval collection. Properly labelled containers of larvae and pupae were brought and maintained under controlled and ideal laboratory conditions.

Immature larvae culture was fed with artificial larval food containing yeast and dog biscuits (3:1 ratio). Adults who emerged from pupae were transferred to the wooden framed cage and provided 10%sucrose. Later on emerged adults were picked with the help of mouth aspirator and pinned on corks in glass vials for Identification (Malla and Jebanesan, 2022; Selvan *et al.,* 2016; Vanlaruia *et al.,* 2014).

Identification

Adults were counted and observed under a Stereomicroscope following systematic taxonomic keys. Morphological differences among genera and species can be determined on the basis of respiratory siphon, proboscis, head, thorax, abdominal segments, wing scales and hind legs (Tyagi *et al.,* 2015).

Results and Discussion

In the present study, a total of 330 mosquito specimens recorded from the selected breeding habitats of eleven villages were identified morphologically into three genera viz. *Anopheles*, *Culex* and *Aedes*. The collected mosquito species were *Aedesal bopictus* (52.4%), Culex sp. (31.2%) and *Anopheles sp.* (16.4%) were prevalent in the aquatic breeding points (Figure 1). In India, *Aedes culex* and *Anopheles* genera of mosquitoes are commonly known as vectors and are capable of transmitting pathogens. Mosquito larval forms harboring aquatic breeding habitats are crucial to determining the status of adult distribution and abundance (Mwangangi *et al.*, 2012). Different aquatic habitats were observed across five villages of West Tripura and six villages of South Tripura of the study area for the distribution and abundance of mosquito species at rural areas of Tripura. The present investigation reveals the mosquito species in the study area belonging to the three genera. Among *Aedes albopictus* was abundantly found in the aquatic habitats and is considered a major vector. Similar results were found in previous epidemiological investigations carried out in the Northeast (Das *et al.*, 2014; Chowdhury *et al.*, 2021).

The key container with the highest *Aedes* population was discarded tyres and broken plastic containers (53.2% and 28.6% respectively). In rural areas, dragon fruit cultivation is known for economic sources and its cultivation is increased since 2017 (Panday, 2020). Damaged or old tyres were used to support the tree and increase the yield (Figure 2). These tyres were the source of breeding habitats for the mosquitoes. Immediate intervention is required to reduce the water staging in tyres and other products during the cultivation of dragon fruit. This may provide a lucrative alternative for preventing the transmission of dengue and other arboviruses.

The house index (HI), container index (CI) and breteau index of West Tripura were 21.6%, 36.6% and 26.4% respectively. A Similar pattern of results was found in South Tripura (HI - 20.66%, CI - 33.3% and BI - 26.6% respectively (Table 1).

Table.1 House Index (HI), Container Index (CI) and Breteau Index (BI) for each study villages.

Village	No. of Houses Positive	No. of Container inspected	No. of Container positive	HI%	CI%	BI%
West Tripura & Sepahijala						
Khuntia para	2	5	3	8	60.0	12
Khirod Thakur para	5	18	7	20	38.9	28
Bahumani para	7	22	8	28	36.4	32
Takarjala	6	23	6	24	26.1	24
Gabordi	7	22	9	28	40.9	36
South Tripura						
Krishnapur	8	32	9	32	28.1	36
Nachirnagar	6	24	8	24	33.3	32
Batisha	5	19	6	20	31.6	24
Kamalpur	3	8	3	12	37.5	12
Chandrapur	5	23	8	20	34.8	32
Rajnagar	4	14	6	16	42.9	24

Fig.1 Distribution mosquito genera during the study area

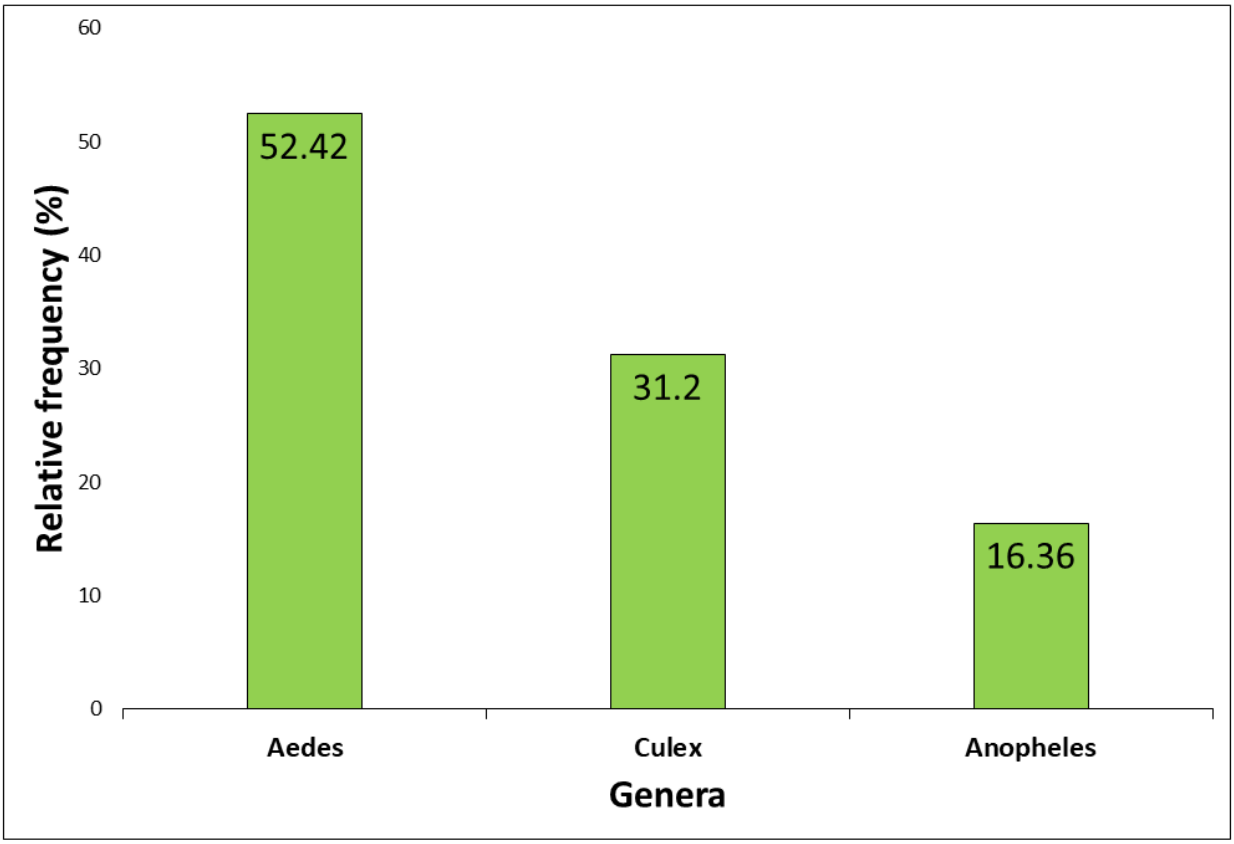

Fig.2 Use of tyres in dragon fruit cultivation

In the present study, CI indicates high epidemic risk whereas HI and BI indicates moderate epidemic risk as per the entomological surveillance indicators defined by WHO (WHO, 1972). In West Tripura, high house index rates in Bahumani Para (28%) and Gabordi (28%) whereas in South Tripura, Krishnapur (32%) and Nachirnagar (24%). The high Container index rates in Khuntia Para (60%) and

Rajnagar (42.9%) of West and South Tripura respectively.

In the present study, the investigation on the mosquito larval survey was conducted at five villages of West Tripura and six villages in South Tripura.

A total of 330 mosquitoes specimens belonging to three genera were recorded from all kinds of selected breeding habitats. *A. albopictus* mosquitoes were documented as the most dominant species in the present study exhibiting almost all breeding sites of all villages. A considerably larger proportion of artificial productive containers viz. damaged tyres and broken plastic items in the surrounding residential areas highlights the urgency of its reduction efforts.

Conflict of interest statement

We declare that we have no conflict of interest.

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