Species diversity and insecticide resistance of the primary vector of Japanese Encephalitis Virus in paddy cultivation areas

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Abstract

This study assessed the dynamics of mosquito larval populations and species diversity in paddy fields in Alappuzha district, Kerala, and investigated the pesticide resistance of *Culex tritaeniorhynchus* and *Culex sitiens* to malathion and deltamethrin. Mosquito larvae were gathered biweekly over the three-month study duration. The density and diversity of research were evaluated independently for each category using the traditional diversity index. The World Health Organization (WHO) tube test was utilized to evaluate the pesticide susceptibility of adult *Culex sitiens* and *Culex tritaeniorhynchus* mosquitoes to malathion and deltamethrin. A total of 2,426 immature mosquitoes were captured. Two genera and ten species were gathered and identified as follows: *Anopheles barbirostris*, *Anopheles peditaeniatus*, *Anopheles subpictus*, *Anopheles vagus*, and five species of *Culex: Cx. bitaeniorhynchus, Cx. gelidus, Cx. quinquefasciatus, Cx. sitiens, Cx. tritaeniorhynchus,* and *Cx. vishnui. Cx. tritaeniorhynchus* exhibited intermediate tolerance to both malathion and deltamethrin, but *Cx. sitiens* shown great sensitivity to both pesticides under identical exposure settings. The increasing population of *Culex tritaeniorhynchus* mosquitoes in rice farming regions may present considerable threats to human health. An improved understanding of mosquito variety and pesticide resistance would facilitate the formulation of successful, regionally tailored vector control methods.

Key words: Paddy field, *Cx. tritaeniorhynchus, Cx. sitiens,* pesticides, resistance

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INTRODUCTION

Vector-borne infections, mostly carried by hematophagous arthropods like mosquitoes, represent a considerable risk to global public health, disproportionately impacting low-income and developing nations (Balasubramanian and Nikhil, 2013). *Culex tritaeniorhynchus* is a significant vector for the transmission of Japanese encephalitis. Japanese encephalitis, a viral affliction common in Asia, is induced by the Japanese encephalitis virus (JEV) (Che-Liang *et al.,* 2017). Japanese encephalitis represents a considerable worldwide health threat, with approximately 100,000 cases and 25,000 fatalities per year (WHO, 2024). The JE virus is endemic in 24 states of India. https://ncvbdc.mohfw.gov.in/ The rising prevalence of pesticide resistance, fueled by negligent insecticide use and inadequate environmental stewardship, is intensifying this issue. India has experienced a significant increase in mosquito-borne diseases, accompanied by mounting indications of pesticide resistance in mosquito populations. Mosquitoes are prevalent worldwide, especially the Culex species (Bhattacharya *et al.*, 2016). These species are recognized as very opportunistic, preying on humans and animals, a behavior that enhances their capacity to spread mosquito-borne diseases and poses a public health risk (Kilpatrick *et al.*, 2005). Moreover, these species are proficiently adapted to thrive in diverse environmental situations (Carver *et al.*, 2009). The *Culex sitiens* complex is among the most significant groups within the Culex genus. *Culex sitiens* is prevalent in coastal environments, where favorable conditions induced by climate change and other reasons facilitate its development. *Culex sitiens* inhabits various brackish water environments, encompassing both temporary and permanent stagnant water bodies, and has become the predominant mosquito species in coastal regions, serving as a vector for numerous mosquito-borne diseases, including Japanese encephalitis (JE) (Vythilingam *et al.*, 2002 and Misra and Kalita, 2010). Iyengar (1953) documented the natural infection of *Cx. sitiens* with filarial parasites in India. *An. subpictus* has been identified as a malaria vector in several regions of India and other nations, predominantly in coastal zones (Sindhania *et al.*, 2020). Effective mosquito management necessitates comprehensive entomological understanding of the biology, ecology, and behavior of this vector (Donald *et al.*, 2014). Research on the correlations between environmental alterations and viral susceptibility in mosquitoes is essential for comprehending their role as vectors. Paddy agriculture areas serve as conducive environments for the brackish water mosquito *Cx. sitiens*, which is responsible for numerous health issues globally (Balasubramanian *et al.*, 2019; Chaiphongpachara and Sumruayphol, 2017). Irregular annual variations in climatic circumstances may influence the capacity of coastal mosquitoes to function as vectors, necessitating enhanced adaptation for survival (Relman *et al.*, 2008). *Culex tritaeniorhynchus* exhibits a documented history of pesticide resistance; however, the insecticide exposure history of *Cx. sitiens* remains unknown. This study aims to assess the pesticide susceptibility of *Culex tritaeniorhynchus* and *Culex sitiens*. This research will elucidate species composition, spatial distribution, and pesticide susceptibility, consequently augmenting understanding of these species as significant vectors of human diseases in paddy production regions.

MATERIALS AND METHODS

Site selection

A three-month study was conducted to gather information on the bionomics of mosquitoes in both rural and paddy field regions. Mosquitoes were sampled from various habitats along the paddy field areas of Alappuzha district. All sites were visited during both the rice-growing and post-harvest seasons. Sampling was performed bimonthly throughout the three-month period. Larval collection was done using dipper samples taken along the margins of the rice fields.

Immature mosquito collections

Immature mosquitoes were collected using a white enameled dipper. The handle of the dipper is fitted with a 1 m-long aluminum pipe, if necessary. At least 10 dips were taken per habitat in different locations where mosquito larvae are expected to be found (edges of habitats, around vegetation, shallow and marshy areas, etc.). Dips of water were strained through a nylon sieve. For further studies, the laboratory collected larvae and pupae and transferred them using a bottle, plastic jars, or vials. The jars were labeled (date, name of sampling habitat) and then transported to the laboratory.

Pesticide susceptibility test

The World Health Organization (WHO) tube test was used to determine the insecticide susceptibility of adult mosquitoes to insecticides. It is a direct response to exposure tests and requires the use of a known standard concentration of insecticide for mosquito exposure. Malathion and Deltamethrin were used to determine the susceptibility status of these vector species. Diagnostic doses of Deltamethrin (0.05%) and Malathion (5.0%) were administered to individuals of each species for one hour, and observations were taken twenty-four hours later. The vector species were classified as susceptible (mortality >98%), resistant (mortality <80%), and intermediately resistant (mortality 80–98%) based on the WHO criteria.

RESULT

Larval presence and abundance

Two genera and ten species were gathered in total. A total of 445 dips were conducted throughout 12 larval habitats, resulting in the collection of 2426 immature mosquitoes. Ten mosquito species were identified: four Anopheles species (*Anopheles barbirostris, Anopheles peditaeniatus, Anopheles subpictus*, and *Anopheles vagus*) and six Culex species (*Culex bitaeniorhynchus, Culex gelidus, Culex quinquefasciatus, Culex sitiens, Culex tritaeniorhynchus*, and *Culex vishnui*). Table 1 presents the outcomes of the larval collections. Among the several mosquitoes collected, the saline-tolerant species *Cx. tritaeniorhynchus* was the most prevalent (56.51%), succeeded by *Cx. gelidus* (28.81%). *Cx. quinquefasciatus* (5.32%) and *Cx. sitiens* (4.82%) were predominantly confined to paddy field regions.

Table 1. The total number and percentage of larvae collected from the paddy field.

Mosquito Species	October	November	December	Total	Percentage (%)
Anopheles barbirostris	0	2	1	3	0.12
An. peditaeniatus	8	12	18	38	1.56
An. subpictus	10	24	5	39	1.60
An. vagus	0	1	0	1	0.04
Culex bitaeniorhynchus	2	0	0	2	0.08
Cx. gelidus	261	131	307	699	28.81
Cx. quinquefasciatus	0	45	84	129	5.32
Cx. sitiens	40	77	0	117	4.82
Cx. tritaeniorhynchus	747	378	246	1371	56.51
Cx. vishnui	0	27	0	27	1.11
	1068	697	661	2426	

Insecticide susceptibility experiment

The WHO tube bioassay was performed to evaluate mosquitoes susceptibility to different concentrations of malathion and deltamethrin. The findings revealed that *Cx. tritaeniorhynchus* had moderate resistance to malathion (5%) and deltamethrin (0.25%), with mortality rates of 86.7% and 93%, respectively. Conversely, *Cx. sitiens* exhibited extreme vulnerability, demonstrating 100% mortality to both pesticides under identical exposure conditions. Table 2 presents the findings of the pesticide susceptibility trial. *Cx. tritaeniorhynchus* mosquitoes were exposed to 5% malathion and 0.25% deltamethrin . After one hour of exposure, knockdown rates were recorded, and after twenty-four hours, mortality rates reached 86.7% and 93%, respectively, suggesting resistance to both insecticides. Conversely, *Cx. sitiens* mosquitoes subjected to identical amounts of malathion and deltamethrin exhibited 100% mortality after 24 hours, demonstrating susceptibility to both pesticides. Conversely, control mosquitoes (exposed to acetone) exhibited negligible mortality (3%), validating the effectiveness of the insecticide treatments. WHO guidelines stipulate that corrected mortality of 98–100% indicates complete susceptibility, 90–97% implies potential resistance, and below 90% denotes established resistance.

Insecticides applied to manage agricultural pests in paddy fields can enter nearby backwaters, unintentionally affecting mosquito populations as non-target organisms. This exposure may contribute to the development of resistance, as evidenced by the intermediate resistance to malathion observed in *Cx. tritaeniorhynchus*. *Culex sitiens* typically breed in marshy ponds with high salinity, which are generally situated far from human habitation (Balasubramanian and Nikhil, 2015). As a result, the probability of these mosquitoes being exposed to insecticides is low. Consequently, *Culex sitiens* show a high mortality rate when subjected to different doses of insecticides.

Table 2. Pesticide susceptibility of adult mosquitoes

Mosquito species exposed	Pesticide used	No. of mosquitoes exposed	Exposure time (Hrs)	Mortality after 24 hours	Status
Cx. tritaeniorhynchus	Malathion 5%	120	1.0	86.70	Resistant
	Deltamethrin 0.25%	120	1.0	93.00	Resistant
Control	Acetone	120	1.0	3	NIL
Cx. sitiens	Malathion 5%	120	1.0	100	Susceptible
	Deltamethrin 0.25%	120	1.0	100	Susceptible
Control	Acetone	120	1.0	2	NIL

DISCUSSION

Understanding the quantity and species composition of vectors is essential for assessing the distribution and potential risk of vector-borne illness transmission (Sumarnrote *et al.*, 2020; Rathinam and Sidhik, 2022). The flexibility of the mosquito species community is significantly constrained. Nevertheless, certain species possess the ability to acclimate to varying environmental conditions. The results facilitate the emergence of salt-tolerant mosquitoes and impede the proliferation of natural predators and competitors incapable of enduring saline environments (Freitas *et al.*, 2012). The rising populations of these mosquitoes may pose significant health risks to humans, as they are aggressive feeders on both avian species and humans. It can therefore serve as a vector for encephalitis viruses in both coastal and paddy field regions. The principal vectors of Japanese Encephalitis, including *Culex tritaeniorhynchus, Culex gelidus,* and *Culex vishnui*, predominantly reproduces in rice paddies and freshwater environments (Arunachalam *et al.*, 2009). The current study revealed that among the collected mosquito larvae species, *Cx. tritaeniorhynchus* and *Cx. gelidus*, the two principal vectors of Japanese encephalitis, were prevalent. The prevalence of these mosquitoes was significantly elevated during the summer season. *Culex tritaeniorhynchus* has been identified as the primary vector of Japanese encephalitis in numerous regions globally. Isolations of the Japanese encephalitis virus have also been obtained from *Cx. tritaeniorhynchus* and *Cx. gelidus* in India (Balasubramanian *et al.*, 2019).

Chemical control remains the most effective primary strategy for managing mosquitoes. Resistance to most chemical pesticides has been reported due to their prolonged and extensive application to reduce mosquito vectors. The extensive use of agricultural pesticides markedly diminished the mosquito vector, hence decreasing the disease incidence (Sahayaraj *et al.*, 2007). *Culex tritaeniorhynchus* mosquitoes exhibited resistance to the two insecticides tested, deltamethrin and malathion, as per WHO tests. Researchers assert that the ecological characteristics of *Cx. sitiens* render them less vulnerable to pesticide exposure compared to other species. These species generally reside in brackish or coastal waters, and due to their frequent inaccessibility to humans, are more susceptible to pesticides.

This study consistently subjects other mosquito species to a range of insecticides. This prolonged and continuous exposure to pesticides results in elevated resistance levels. The findings indicate that this species exhibits significant resistance, probably attributable to the application of crop protection herbicides (Handayani *et al.*, 2019). Consequently, it is clear that the risk of pesticide resistance is increasing, necessitating the integration of appropriate resistance management measures in all mosquito control operations.

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