Annual Report of the LaMer Project FY 2024

Title of the research project:

Insecticide resistance status and future insecticide choices for vector control strategies in Bangladesh.

Names and affiliation of members:

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Purposes:

To identify resistance *kdr* mutations in *Culex* sp. and *Aedes aegypti* using molecular tools, and to assess deltamethrin resistance in adult female mosquitoes of these species from Tangail and Dhaka City using the CDC bottle bioassay. This study aims to address the knowledge gap on genetic variations and associated risks, thereby informing targeted control strategies and promoting effective measures to mitigate mosquito-borne diseases in the region.

Methods

Mosquito larvae collection and maintenance

Mosquito larvae were collected in July 2023 from eight locations in Dhaka City and three locations in Tangail City, Bangladesh. In Dhaka, larvae were gathered from various breeding sites, averaging 20-30 larvae per site, whereas in Tangail, they were collected from open drains. The larvae were transported to the Laboratory of Vector-Borne Disease (LVBD) at MBSTU, Tangail, where they were reared under ambient conditions with fish food until they emerged as adults. Morphological identification revealed the mosquitoes from Tangail as *Culex* spp., while those from Dhaka were identified as *Aedes aegypti*. Due to minimal quantities, the *Aedes aegypti* specimens from Dhaka were pooled. The adult mosquitoes were maintained with a 10% sucrose solution and fed naive mice blood for egg production. Subsequently, F1 and F2 mosquito colonies were generated, with experiments conducted on the F2 generation of *Aedes aegypti* and F0 generation of *Culex* spp.

Susceptibility test

Bottle bioassays were conducted according to the CDC protocol using deltamethrin to assess mosquito susceptibility (Li et al. 2021). Briefly, transparent 250 ml glass bottles were coated with 1 ml of insecticide at varying concentrations (1X, 3X, 5X), then dried for 24 hours in the dark. Control bottles were coated with acetone. To avoid cross-contamination, both caps and bottles were labeled with the date and trial number. Adult female mosquitoes (10-25, aged 3-5 days, no blood-feeding history) were introduced into each bottle for 30 minutes. Mosquitoes that fell or had their ventral side up, unable to stand or fly post-exposure, were considered susceptible. Both susceptible and resistant mosquitoes were isolated and preserved at -20°C for further analysis.

Results:

The insecticide susceptibility assay was conducted to evaluate the efficacy of deltamethrin against *Aedes aegypti* and *Culex* sp., assessing mortality and survival rates following a standardized 30-minute exposure across varying concentrations (1X, 3X, 5X, and 10X). For *Aedes aegypti*, mortality rates at the 1X and 3X concentrations were recorded at 61.5%, with a corresponding survival rate of 39.5% at both doses (Fig. 1, Table 1).

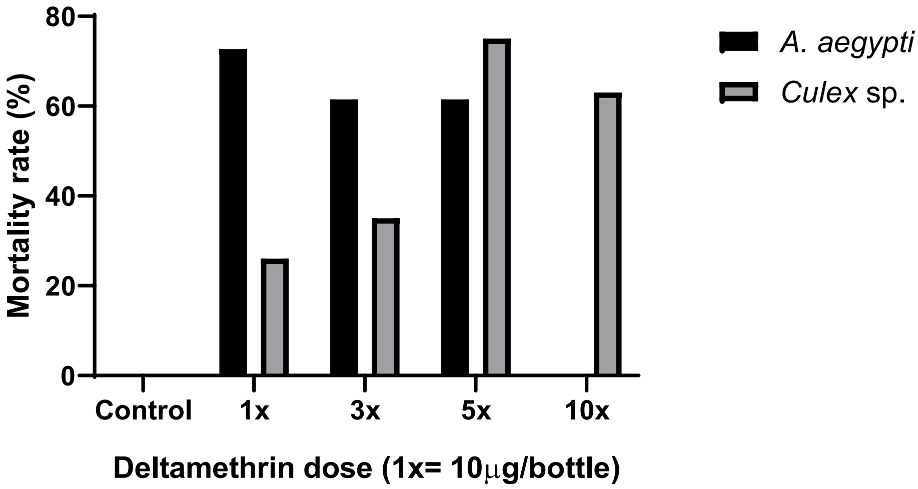

Fig. 1: Insecticide Susceptibility of *Aedes* aegypti and *Culex* sp. to deltamethrin

A further increase in insecticide concentration to 5X resulted in a mortality rate of 72.7%, reducing survival to 27.3%. However, the absence of a dose-dependent increase in mortality suggests a potential physiological threshold for toxicity, saturation of insecticide target sites, or enhanced metabolic detoxification at these exposure levels (Rahman et al. 2021). Additionally, genetic variability within the population or behavioral avoidance mechanisms may contribute to the observed plateau in mortality rates (Djiappi-Tchamen et al. 2021).

Table 1: Insecticide susceptibility test assay

Species	Expos	1X	3X	5X	10X

	ure (min)	Mortality rate (%)	Survival rate (%)	Mortality rate (%)	Survival rate (%)	Mortality rate (%)	Survival rate (%)	Mortality rate (%)	Survival rate (%)
A. aegypti	30	61.5	39.5	61.5	39.5	72.7	27.3	-	-
Culex sp.	30	26	74	35	65	75	25	63	37

1X=10µg/bottle

In contrast, *Culex* sp. exhibited lower susceptibility across all tested concentrations. At the 1X concentration, mortality was recorded at 26%, with a corresponding survival rate of 74% (Fig. 1, Table 1). Increasing the insecticide concentration to 3X resulted in a moderate increase in mortality to 35%, with 65% of individuals surviving. A significant increase in mortality was observed at the 5X concentration (75% mortality, 25% survival). However, at the highest concentration tested (10X), mortality unexpectedly decreased to 63%, with survival increasing to 37%, indicating a potential non-linear dose-response relationship.

The observed differences in susceptibility between *Aedes aegypti* and *Culex* sp. suggest species-specific physiological and biochemical responses to deltamethrin (Richards et al. 2018). The significantly higher mortality rates in Aedes sp. indicate greater susceptibility, whereas the reduced mortality and non-linear response in *Culex* sp. may point to potential resistance mechanisms.

Future Challenges

The emergence of insecticide resistance poses a critical challenge to the effectiveness of vector control programs. The observed variability in susceptibility, underscores the need for continued surveillance of resistance patterns and mechanisms. The potential for metabolic detoxification, target site mutations, or behavioral adaptations must be further investigated to inform effective resistance management strategies. Additionally, the non-linear response at higher deltamethrin concentrations highlights the complexity of insecticide interactions, necessitating the exploration of alternative control methods such as insecticide rotations, synergist applications, and integrated vector management approaches. Addressing these challenges will be essential for sustaining the efficacy of insecticidal interventions against mosquito vectors of public health significance.

Reference(s):

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