4. Research report (Follow the guideline on the next page)

Annual Report of the LaMer Project FY 2022

1.Title of the research project:

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

2. Names and affiliations of members:

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2. Dr. Ashekul Islam, Assistant Professor and Principal Investigator of Vector Borne Diseases Laboratory at the Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail 1902, Bangladesh.
3. Mohammad Mosleh Uddin, PhD student, Molecular Ecology and Health (MEcoH) Laboratory at the Center for Marine Environmental Studies (CMES), Ehime University, Japan.
4. Jannatul Efte Ekra, MS thesis student, Vector Borne Diseases Laboratory at the Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail 1902, Bangladesh.

3. Purposes:

- Determine the distribution of mosquito species and disease prevalence in Tangail Sadar, Bangladesh.
- Subsequently, monitor the incremental trend in insecticide resistance among the mosquito population in Tangail to choose the best vector control strategies.

4.Methods:

Mosquito larvae collection and rearing:

Mosquito larvae from 17 different areas of Tangail Sadar in Bangladesh were collected and reared to get adult and maintained colonies independently in the insectary of Vector Borne Disease Laboratory (VBDL) in Mawlana Bhashani Science and Technology University (MBSTU), Bangladesh; with either 10% sugar or anesthetized mice blood meal with the accordance of the methods described previously by Min-Lin Zheng *et al.* [1].

Assessment of mosquito survivorship:

Never blood fed 50 female adult mosquitoes of 3-5 days old were allotted into four separate cages with four different experimental setups – i) with both blood and sugar meal,

ii) with only sugar meal, iii) with water meal only, and iv) with no meal (neither sugar nor blood nor water). Mortality was observed twice daily and recorded for further analysis.

Larvicide and Insecticide susceptibility test:

CDC Bottle Bioassay was conducted at different concentrations to find out resistance against insecticide in mosquitoes. Non-blood fed 3-5 days old female mosquitoes were used in assessing insecticide susceptibility using CDC bottle bioassay [2].

5.Result:

A portion of larvae collected from 17 different places was used to evaluate larvicidal efficacy to monitor the resistance in the larval stage. And the rest were allowed to develop adult mosquitoes. Female adults were further used in assessing survivorship and adulticide activity analysis.

Assessment of mosquito survivorship*:*

It was observed that among the mosquitoes with both blood and sugar meal (Fig. 1-A) had a longer lifespan (32 days) while mosquitoes with only sugar meal (Fig. 1-B), only water meal (Fig. 1-C) and neither water nor sugar meal (Fig. 1-D) died after 20 days, 15 days and 9 days respectively.

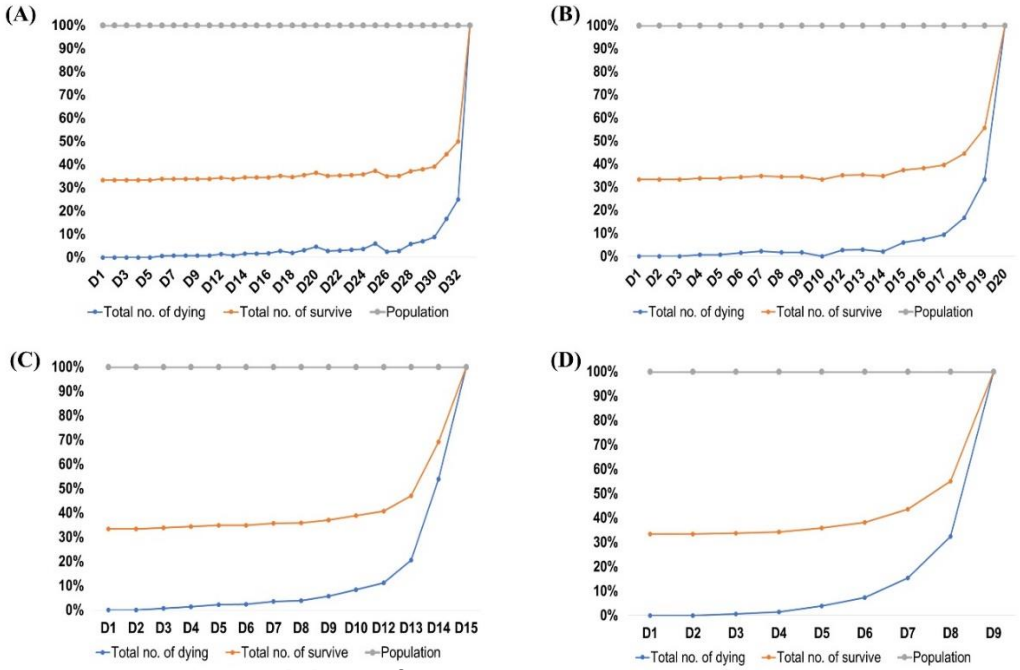

Fig. 1: Mosquito survivability at four separate experimental settings.

(A) Survival curve of female mosquitoes (*n*=50) with both blood and sugar meal; (B) Survival curve of female mosquitoes (n=50) with only sugar meal; (C) Survival curve of female

Larvicide action under laboratory conditions:

Chlorpyrifos was found to be highly effective against collected larvae tested under laboratory conditions (Fig. 2-A). According to Fig. 2-A, however, at diagnostic or recommended

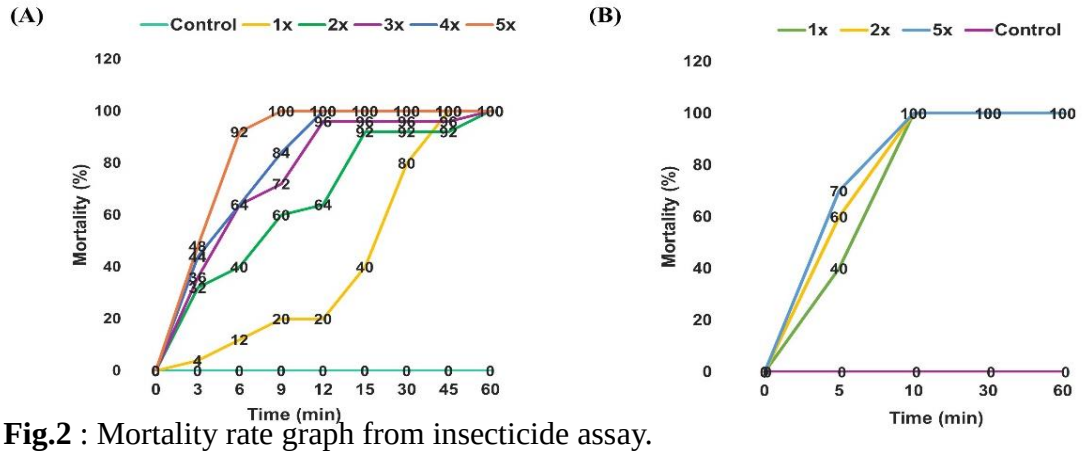

Fig.2 : Mortality rate graph from insecticide assay.

(A) Larvicidal assay using Chlorpyrifos; (B) CDC bottle bioassay with Dimefluthrin

Dose (1x), the mortality rate was < 90% (80%) after 30 minutes. At two times and three times of recommended dose, after 30 minutes of exposure to chlorpyrifos, the mortality rate is still below 98% (92% and 96% respectively). However, within one hour, the mortality rate was 100% for all concentrations of Chlorpyrifos. The highest efficacy in the laboratory was observed for chlorpyrifos at four and five times the concentration of recommended dose.

Adulticide action under laboratory conditions:

Dimefluthrin was found highly effective against all colonies of mosquitoes tested under laboratory conditions (Fig. 2-B). The highest efficacy (about 70%) in the laboratory was observed at five times concentration within 5 minutes of its application compared with the recommended dose, presenting it as highly effective.

6.Future Challenges

There is increasing data on insecticide resistance in mosquito vectors evaluated in laboratory bioassays. However, these data do not inform the various stakeholders about effective vector control strategies. For practical resistance, such a need is to be standardized at the local level so that the national control programs can determine whether control tools are increasing or decreasing in effectiveness over time. The actual impact of insecticide use must therefore be determined by a thorough study that takes into account surveillance, disease prevalence, and resistance mechanisms.

References

1. Zheng ML, Zhang DJ, Damiens DD, Lees RS, Gilles JRL. Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors Aedes aegypti and Aedes albopictus (Diptera: Culicidae) - II - Egg storage and hatching. Parasites and Vectors. 2015;8:1–7. doi:10.1186/S13071-015-0951-X/TABLES/3.

2. Al-Amin HM, Johora FT, Irish SR, Hossainey MRH, Vizcaino L, Paul KK, et al. Insecticide resistance status of Aedes aegypti in Bangladesh. Parasit Vectors. 2020;13. doi:10.1186/S13071-020-04503-6.