6. SUMMARY

Plant-based insecticides for vector control are urgently needed for *Aedes albopictus*, *Anopheles barbirostris* and *Culex quinquefasciatus* which are the primary vectors of malaria, lymphatic filariasis, and dengue, respectively, in India and other South East Asian countries.

In the present study, larvicidal, adulticidal, oviposition deterrency, insect growth regulator and repellent activities of petroleum ether, acetone, chloroform, methanol and water extracts of thirty ethnomedicinal plants from Mizoram were tested against the larvae and adults of the three mosquito vectors.

**Larvicidal assay**

- Preliminary screening of larvicidal activity of 30 medicinal plants in five different solvents (petroleum ether, chloroform, acetone, methanol and aqueous extracts) were performed at 500ppm against three mosquito vectors - *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus*

- About fifteen plant extracts, petroleum ether extracts of *Blumea lanceolaria*, *Centella asiatica*, *Homalomena aromatic*ica, *Oroxylum indicum*, *Tagetes erecta*, *Elsholtzia communis*, *Melia azedarach*, *Syzygium aromaticum*, *Curcuma longa*; Chloroform extract of *S. aromaticum*, *C. longa*; acetone extracts of *E. communis*, *Hiptage benghalensis*, *S. aromaticum* and *C. longa* showed 100% mortality at 500 ppm against *Ae. Albopictus*. 
• Petroleum ether extract of *E. glandulosum*, *C. asiatica*, *H. aromatica*, *T. erecta*, *E. communis*, *M. azedarach*, *S. aromaticum*, *C. longa*; chloroform extract of *O. indicum*, *E. communis*, *C. longa*; acetone extract of *E. glandulosum*, *T. erecta*, *E. communis*, *H. benghalensis* and *C. longa*] showed 100% mortality at 500 ppm against *An. Barbirostris*

• In screening test against *Cx. quinquefasciatus*, 100% mortality were observed in *M. micrantha*, *C. asiatica*, *O. indicum*, *T. erecta*, *M. pachycarpa*, *E. communis*, *Polygonum plebium*, *M. azedarach*, *S. aromaticum*, *C. longa*; chloroform extract of *C. longa*; acetone extract of *C. reflexa*, *E. communis*, *H. benghalensis*, *S. aromaticum* and *C. longa*.

**Dose response bioassay**

• Dose response larvicidal bioassay was performed using *H. benghalensis*, *E. communis*, *H. aromatica*, *C. longa* and *S. aromaticum* extracts against *Ae. albopictus*, *An. barbirostris* and *Cx. quinquefasciatus* larvae.

• Larvicidal effect was found to be dose-dependent. *A. albopictus*, *A. barbirostris* and *C. quinquefasciatus* larvae were highly susceptible to petroleum ether extract of *C. longa* and *H. benghalensis*. 
Results of log probit analysis (at 95 % confidence level) and regression analysis of crude extracts of *H. benghalensis*, *E. communis*, *H. aromatica*, *C. longa* and *S. aromaticum* against *Ae. albopictus*, *An. barbiostris* and *Cx. quinquefasciatus* larvae revealed that lethal concentration (LC50) values gradually decreased with the exposure periods; lethal time (LT50) decreased with the concentration, and the mortality is positively correlated with the concentration.

The order of susceptibility of the three mosquito species was as follows: *A. albopictus>* *A. barbiostris>* *C. quinquefasciatus*.

**Disruption of nutrient profiles in larvae and plant extract**

Biochemical changes were also evidenced in third instar larvae of three mosquito species [*Ae. albopictus*, *An. barbiostris* and *Cx. quinquefasciatus*] following a sublethal exposure (10ppm) of *H. benghalensis* for 24 h. The level of sugar, glycogen, lipids, and proteins was significantly (*P*<0.05) reduced in larvae treated with *H. benghalensis*.

A significant reduction of sugar, glycogen and lipid was noticed when the three mosquito larvae were treated with sub-lethal concentration suggesting disruption of nutrient profiles in the larvae leading to eventual reduction in growth and development.
Insect growth regulators

- Insect growth regulatory effect of petroleum ether extracts of *M. azedarach* fruit was observed against *Ae. albopictus*.
- Treated larvae at 10 ppm and 20 ppm resulted in, malformed pupae and incomplete moulting adults.
- The total development period to eclosion was prolonged by 0.25, 0.75 and 1.25 days at the concentrations of 5 ppm, 10 ppm and 20 ppm respectively from 13.25 days in the control.

Adulticide bioassay

- The acetone root bark extracts of *H. benghalensis* is less toxic to adults and repelled laboratory-reared female *An. barbirostris*, *Ae. albopictus*, and *Cx. quinquefascitus* with the short median protection times of 57.66–135, 72.41–134.16, and 47.66–93 min, respectively.

Repellant bioassay

- Skin repellent activity of *G. arborea* leaf, *C. longa* rhizome and *H. aromatica* rhizome was performed against females of *An. barbirostris*, *Ae. albopictus*, and *Cx. quinquefascitus*. The results clearly show that repellent activity was dose dependent.
• The acetone extract of *G. arborea* leaf at 2000 ppm gave 50 minutes protection against *Ae. albopictus* female bite. The control provided only 2.2 minutes of protection.

• Petroleum ether extract of *C. longa* and *H. aromatica* provided 125 min and 90 min of protection at 20000 ppm against *Ae. albopictus* female bite.

• The repellency of *G. arborea* leaf extracts were also recorded against *An. barbirostris*. Concentration of 20000 ppm has shown longest protection time of 32.5 minutes only by the petroleum ether of *G. arborea*. The control provided 3.25 minutes of protection.

**Oviposition deterrent**

• Oviposition deterreny was observed against gravid *Ae. albopictus* preferred to lay eggs in the control cups than in the cups treated with petroleum ether extracts of *C. longa, E. communis* and *M. azedarach*.

• Significant reduction in the number of eggs laid was observed in all the test substances. The effective repellency ranged between 68.28 and 69.96%.

• The EC50 of the three plant extracts was between 15.39% and 20.35% and the oviposition active index was observed least in *C. longa* (-0.53) followed by *E. communis* (-0.52) and *M. azedarach* ( -0.25).
RAPD profile and DNA damage

- The random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in various exposed and unexposed *Cx quinquefasciatus* larvae to acetone and chloroform extracts of *C. longa* and *M. azedarach* at different concentrations (6.25, 12.5 and 25 ppm).

- This is the first report of an analysis of genomic alterations in plant extracts-treated mosquito larvae using RAPD-PCR fingerprinting.

- In comparison to the control larvae, larvae treated with the plant extracts caused greater changes in the RAPD patterns.

- DNA strand breakage was more in the treated larvae of *Cx. quinquefasciatus*.

The present investigation proves it as the medicinal plants used in this study are the potent larvicide, repellent, IGR and deterrents against *Ae. albopictus, An. barbirostris*, and *Cx. quinquefasciatus*, which can be recommended to control these mosquito species on its breeding site as well as in their habitats. However, further investigations are needed to confirm the lethal effects of these potent plant insecticides in field conditions and its impact on the nontarget organisms.