CHAPTER V

CONCLUSION

In this study, we investigated the sodium channel mutation associated with the pyrethroid resistance in two *Ae. aegypti* PMD-R and PMD strains, which are the laboratory strains of permethrin resistance and susceptible, respectively. The nucleotide sequences of three *Ae. aegypti* voltage-gated sodium channel regions from the PMD-R and PMD strains were aligned with those sequences from Liverpool and China susceptible stains. The nucleotide alignment of four strains showed a single nucleotide substitution, T to G, in the IIIS6 coding region of the sodium channel sequence in permethrin resistance PMD-R strain. This nucleotide substitution resulted in an amino acid substitution, phenylalanine (F) to cysteine (C), at the amino acid position 1552 based on the *Ae. aegypti* voltage-gated sodium channel protein. The F to C mutation was referred as F1534C in reference to the equivalent mutation at the amino acid position 1534 in the housefly *Vssc1* sequence.

The genetic inheritance of the F1534 and C1534 alleles in permethrin resistance was examined by using the crossing experiments and larvae bioassay showed an incompletely recessive phenotype of permethrin resistance. The permethrin resistant level (LC_{50}) of F1 hybrids with the heterozygous mutation (F/C1534) was reduced eight-fold compared to the homozygous mutation (C/C1534). This result suggested that the homozygous mutation (C/C1534) is highly associated with permethrin resistance and probably plays a major role in the resistant

characteristics of PMD-R. Our backcrossing experiment indicated a number of unlinked gene contribute to resistance which suggested the involvement of other resistant mechanisms. This suggestion was tested by adding the oxidase inhibitor, piperonyl butoxide (PBO), in the larvae bioassays. The results showed the F1 hybrid strains were decrease in permethrin resistance level being close to the susceptible strain. Therefore, we consider that the permethrin resistance in PMD-R strain is conferred mainly by the F1534C mutation of the sodium channel gene and partially by the oxidative enzymes.

We have successfully developed the TaqMan SNP and AS-PCR assays for monitoring the F1534C mutation in *Ae. aegypti* populations. Depending on the available facilities, these assays are equally useful tools for the rapid detection of the F1534C resistance mutation which is essential for the development of resistance management strategies. The AS-PCR method was extremely useful to reveal the high frequency of the F1534C mutation throughout Thailand as well as indicating this may be a problem for resistance in the neighbouring countries of Myanmar and Cambodia. The AS-PCR of the permethrin exposed samples showed a very closely association of the mutant C1534 allele and the resistant phenotype. However, 19 permethrin resistant individuals were homozygous for the wild type F1534 allele. DNA sequencing demonstrated that all the individuals were homozygous for two other mutations in domain II, V1016G and S989P, which are known to confer pyrethroid resistance. Therefore, the F1534C mutation and other mutations underlie a high prevalence of pyrethroid resistance in Thailand.

The spread of pyrethroid resistant *Ae. aegypti* could have serious implications for the successful use of pyrethroid as a control measure and this

problem must be closely monitored. Recently, the F1534C mutation was confirmed to reduce the sensitivity of the cockroach sodium channel to type I pyrethroid but not type II pyrethroid (Hu *et al*., 2011) which suggested that type II pyrethroid could be recommended to use for vector control in the permethrin resistant populations which found the F1534C mutation.