CHAPTER IV

DISCUSSION

Permethrin resistance in PMD-R has recently been demonstrated to be associated mainly with a homozygous mutation in the voltage-gated sodium channel gene (Yanola *et al.,* 2010). They showed in the larval bioassay that the resistance level of the heterozygous forms (determined by the permethrin LC_{50}) was reduced ~ 8-fold compared to the homozygous form (25.64 ppb), but that this reduced level was still ~3-fold higher than that of the PMD strain (1.03 ppb). The present results show that the LC_{50} of PMD-R was reduced only ~3-fold (from 25.42 ppb to 8.39 ppb) by PBO (Table 7 and Figure 10). This therefore implies that the resistance ability of PMD-R is due mainly to the *kdr* gene (about three-quarters) and the rest due to oxidase-based resistance. These results agree with a previous (unpublished) observation that the addition of PBO in the larval bioassay of the heterozygous larvae reduced the LC_{50} close to the susceptible PMD strain, commented in Yanola *et al.,* (2010). The LC_{50} of PMD was reduced 2.27-fold in the presence of PBO which was similar to PBO's synergistic effect on PMD-R (3.03-fold) (Table 7). The level of total P450s was similar in both strains (Figure 12), agreeing with a previous study showing that both strains had an activity of P450s 4-fold higher than the susceptible Rockefeller strain (Prapanthadara *et al.,* 2002). These results reflect the in vivo involvement of P450s in detoxification of permethrin in both strains, agreeing with the result in the in vitro assay (Table 19).

The effect of esterase inhibitor, BNPP, was not distinct in the larval bioassay, but was observed in the in vitro assay in which a significant reduction of PBCOOH was detected (Table 19, Reactions 2, 5). These results suggest a role for esterase enzymes in the hydrolysis of permethrin in both strains. In mosquitoes, elevated esterase enzymes are the primary mechanism for organophosphorus insecticide resistance as well as a secondary mechanism for carbamate resistance. Additionally, elevated levels of esterases also confer pyrethroid resistance in some insect species, including *Ae. aegypti*, (Flores *et al.,* 2005; Hemingway and Karunaratne 1998; Jagadeshwaran and Vijayan, 2009). Studies in several areas in Thailand found that elevated esterases in *Ae. aegypti* were associated mainly with organophosphorus insecticide resistance (Pethun *et al.,* 2007). The PMD and PMD-R strains probably had only a slightly increased esterase activity (Prapanthadara *et al.,* 2002), because they originated from an area where resistance to organophosphorus insecticides was not observed (Somboon *et al.,* 2003). No resistance to organophosphorus insecticides (temephos, fenthion and malathion) was also observed in India (Tikar *et al.,* 2008). However, one should be aware that in vitro experiments with model substrates do not necessarily reflect the in vivo situations of insect metabolizing insecticide molecules.

Interestingly, PMD-R produced more PBCOOH than PMD in most reactions, particularly when PBOH and PBCHO were the substrates (Tables 20, 21). This was observed in both NADPH and NAD^+ generating systems. These results have suggested a difference in the oxidative enzyme system between the two strains. NADPH is known to be cofactor of P450s which can also oxidize alcohol and aldehyde in insects and mammals (Asai *et al.,* 1996; Guo *et al.,* 2010). Although both strains had an equivalent level of P450 activity, it does not necessarily mean they

have identical P450 genes, because resistance can occur due to detoxification by only a few P450s (Scott 1999; Nakamura *et al.*, 2007). Of the 160 P450 active genes found in *Ae. aegypti*, Strode et al. (2008) reported that 23 genes were over-expressed in the PMD-R strain relative to a susceptible strain; however, only a few P450s genes may be involved in pyrethroid resistance. The P450 genes in the PMD strain are still not known and require further study.

Replacement of the NADPH generating system with NAD^+ resulted in a considerably higher production of PBCOOH, suggesting that, besides P450s, there were other oxidative enzymes playing a role in oxidizing the substrates. The enzymes that are known to metabolize PBOH and PBCHO are ADHs and ALDHs, respectively, and both groups require NAD^+ as a cofactor (Devlin 2006; Gibson and Skett 1994). These enzymes are involved in detoxifying alcohol and aldehyde in mammals and insects (Guo *et al.*, 2010), but it has not yet been shown that they are involved in permethrin metabolite oxidation in insects. The present study demonstrates for the first time in insects that oxidation of PBHO and PBCHO in *Ae. aegypti* can also be mediated by ADHs and ALDHs, respectively, as previously reported in mammals (Choi *et al.*, 2002; Hodgson 2003).

It has been demonstrated that PBO can also inhibit the activity of ADHs in insects (Guo *et al.*, 2010) and this might occur in our larval bioassay (Table 7). The ongoing study revealed that the ALDH genes (AAEL014080 and AAEL009948 in VectorBase) were over-expressed in the PMD-R larvae compared with the PMD larvae (Lumjuan N., unpublished data). This may partially explain the difference of PBCOOH production between the two strains. The in vitro results suggest that over-expression of ADH genes might also occur in both strains studied (Tables 20, 21).

In conclusion, the permethrin resistance mechanism in the *Ae. aegypti* PMD-R strain is conferred mainly by the *kdr* gene and partially by oxidative enzymes involving P450s, ADHs and ALDHs. It is likely that both types of resistance mechanism are present in many Thai populations of *Ae. aegypti*, occurring either singly or in combination. In this situation, the reduction of vector populations using pyrethroid will be difficult. It is important that care is taken in the appropriate choice of insecticide, using those that are less affected by *kdr* and oxidase based resistant mechanisms where necessary.