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Biochemical studies of insecticide resistance in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand

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Abstract. Biochemical analysis was performed on field caught *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) mosquitoes to determine activities of enzymes including mixed function oxidases (MFO), nonspecific esterases (α- and β-), glutathione-S-transferases (GST), and insensitive acetylcholinesterase (AChE). Biochemical tests were performed on F1 generation of *Ae. aegypti* field caught mosquitoes, while in *Ae. albopictus* F2 progenies were used. Twenty-six samples of *Ae. aegypti* mosquito were collected from areas across different parts of Thailand including Bangkok (central), and the provinces of Chiang Rai (north), Nakhon Sawan (north-central), Nakhon Ratchasrima (northeast), Chonburi (east), Chanthaburi (east), and Songkhla (south). Eight wild caught samples of *Ae. albopictus* were from Songkhla, Nakhon Sawan, Nakhon Ratchasrima and Kanchanaburi (west) provinces. The susceptibility to pyrethroids (deltamethrin, permethrin), organophosphate (fenitrothion) and carbamate (propoxur) insecticides were revealed in these samples. The biochemical test results were compared with those of the susceptible Bora (French Polynesia) strain. There was significant enhancement of MFO in pyrethroid resistant *Ae. aegypti* samples, except those from Songkhla and Hauykwang district in Bangkok. Biochemical assay results suggested that nonspecific esterases conferred fenitrothion resistance in *Ae. aegypti* in Nakhon Sawan, while insensitive AChE and/or nonspecific esterases could play role in fenitrothion resistance in Nakhon Ratchasrima. There was no consistent association of GST with pyrethroid resistance in *Ae. aegypti*. Low enzyme activities found in *Ae. aegypti* in Songkhla and in *Ae. albopictus* corresponded to their insecticide susceptibility status. The increased enzyme activity in field samples reflecting local history of insecticide employment was discussed.

INTRODUCTION

Dengue and dengue haemorrhagic fever (DHF) remain a serious transmitted disease in Thailand by which *Aedes (Stegomyia) aegypti* (Diptera: Culicidae) is incriminated as primary vector and, in recent years, *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) as a secondary vector. Vector control in Thailand is implemented using environmental management through decrease of potential breeding sites and insecticide-based control methods, by fogging or applying larvicides. For decades organophosphates (i.e. temephos, fenitrothion, malathion and chlorpyrifos) and carbamate (i.e. propoxur, bendiocarb) had been heavily used for vector control before being replaced by pyrethroids in 1992 for use in agriculture

and public health (Chareonviriyaphap *et al*., 1999). DDT has long been used for both insect control and in agricultural areas, while household insecticides containing pyrethroids are commonly used (Chareonviriyaphap *et al*., 1999).

Common insecticide resistance mechanisms include enhanced enzyme activities of non-specific esterases (α- and β-), glutathione S-transferases (GST) and P450-mediated monooxygenases or mixed function oxidases (MFO), and alteration of target sites (Oppenoorth, 1985; Hemingway & Ranson, 2000). MFO is shown associated with pyrethroid resistance (Zerba, 1988; Scott *et al*., 1998), GST plays a role in DDT resistance (Prapanthadara *et al*., 1993; Hemingway *et al*., 2004), while non-specific esterases mostly involve in resistance to organophosphates, carbamates and sometimes to pyrethroids (Hemingway *et al*., 2004). Target site resistance including knockdown resistance (*kdr*) and alteration in acetylcholinesterases (AChE) are associated with pyrethroid and DDT cross-resistance, and organophosphate and carbamate resistance, respectively (Hemingway & Ranson, 2000; Soderlund & Knipple, 2003). Numerous mutations in the para-type voltage dependent sodium channel gene identified in insects are associated with reduced channel sensitivity to target pyrethroids and DDT insecticides (Soderlund & Knipple, 2003), while a mutation in AChE results in a decreased sensitivity to inhibition by target insecticides (Weill *et al*., 2003).

In Thailand, insecticide resistance in *Ae. aegypti* has been recognized, but the associated detoxifying enzyme activities have been sparsely recorded. Studies on insecticide susceptibility of *Ae. aegypti* and *Ae. albopictus* revealed the occurrence of insecticide resistance in certain areas of Thailand (Somboon *et al*., 2003; Paeporn *et al*., 2004; Ponlawat *et al*., 2005; Yaicharoen *et al*., 2005). Our recent investigation revealed that most wild caught *Ae. aegpti* samples collected from northern, central, northern-central, northeastern, eastern, and southern Thailand were resistant to pyrethroids or together with organophosphate and/or carbamate, while in the south they remain susceptible to most test insecticides (Jirakanjanakit *et al*., 2007). In contrast *Ae. albopictus* was not resistant to all test insecticides (Jirakanjanakit *et al*., 2007). This study attempted to determine possible mechanisms responsible for insecticide resistance among these field *Ae. aegypti* and *Ae. albopictus* samples by biochemical analysis.

MATERIALS AND METHODS

Mosquito test samples

Larval collection of *Ae. aegypti* mosquito was made in twenty-six collection sites across Thailand during October 2003 to December 2005. These were from five districts each in Bangkok (central), Nakhon Sawan (north-central), Nakhon Ratchasrima (northeast), and Songkhla (south) provinces, and four districts in Chonburi (east), a sample each from Chanthaburi (east) and Chiang Rai (north). In parallel, larval collection of eight *Ae. albopictus* samples were obtained from an area each in Nakhon Sawan, Nakhon Ratchasrima, and Kanchanaburi (west) provinces and five districts of Songkhla province. Field collections displayed in districts are shown in Tables 1 and 2. The F1 generations of field caught female mosquitoes upon subjection to insecticide susceptibility tests were used for biochemical assays. However, for most *Ae. albopictus* there was insufficient number of F1 progenies produced and F2 generation was used for assays. The Bora (French Polynesia) *Ae. aegypti* strain reared in the insectary at the Center for Vaccine Development, Mahidol University was for comparison with *Ae. aegypti* field mosquitoes.

Biochemical assays

The adult non-blood fed 2-3 day old female mosquitoes that were used for bioassays were subjected to biochemical analysis. Female mosquitoes were individually

homogenized in 100 µl of ice-cold 100 mM potassium phosphate buffer pH 7.2 and the volume was made up to 1.0 ml with the same buffer. Each sample was centrifuged at 700g, 4ºC for 5 min and the supernatant was used as enzyme source. Aliquots of 100 µl supernatant were then placed in microtiter plate held on ice. Enzyme assays were performed in the total 300 µl reaction mixture in each well at room temperature following addition of substrate solution, OD values were measured by Multiskan EX microtiter plate reader (Thermo Labsystems, Finland).

Assays of mixed function oxidases (MFO), glutathione-S-transferases (GST), nonspecific a-esterases and b-esterases in field-collected mosquitoes were performed following the methods previously described (Brogdon & Dickinson, 1983 ; Brogdon & Barber, 1990a; Brogdon *et al*., 1997) with some modification. For MFO assay, the OD values were measured at 620 nm after 5 min incubation of individual mosquito homogenate in each well with 200 µl of 2 mM 3, 3', 5, 5'-tetramethyl-benzidine dihydrochloride (TMBZ) and 25 µl of 3% hydrogen peroxide, the activity was determined from cytochrome c standard curve. Nonspecific α-esterases and β-esterases activities were assayed by 10-min incubation of mosquito homogenate in each well with 100 µl of 3 mM napthyl acetate (either α- or β-) at room temperature. The reaction was further incubated for 2 min with 100 µl of 2 mM *o*-dianisidine before the OD value at 540 nm was measured. Glutathione-S-transferases (GST) activity was measured in the reaction containing 2 mM reduced glutathione and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction rates were measured at 340 nm at 5 min, and the activity expressed as nmoles CDNB conjugated per min per mg protein using the extinction coefficient of 9.6 mM^{-1} cm^{-1}. Assay for acetylcholinesterase (AChE) activity (Hemingway *et al*., 1986) with some modification was carried out in the reaction mixture containing 2.6 mM acetylthiocholine iodide and 0.3 mM dithiobis-2-nitrobenzoic acid (DTNB), reaction rates were monitored at 414 nm at 20 min. Assay for insensitive AChE activity was undertaken with 100 mM propoxur as inhibitor, and inhibition was expressed as mean AChE activity remaining as a percentage of uninhibited activity. All chemicals used for assays were reagent grade or better. Propoxur was a gift from N. Jirakanchanakit, Center for Vaccine Development, Mahidol University.

Total protein content of each mosquito was determined in 300 µl reaction, using 20 µl homogenate diluted with 80 µl potassium phosphate buffer following the instruction provided with the protein assay kit (Bio-Rad, California, U.S.A). The absorbency was measured at 620 nm and transformed into protein concentration from bovine serum albumin standard curve. All enzyme specific activities were calculated by correcting for protein content. Means of enzyme activities in each *Ae. aegypti* mosquito sample were compared with the susceptible Bora strain by analysis of variance (ANOVA) using SPSS statistical program (SPSS Inc., 2001). Fisher's least significant difference (LSD) test was used to separate means at a = 0.001.

RESULTS

Table 1 shows enzyme activities of *Ae. aegypti* mosquito upon exposure to pyrethroid (deltamethrin, permethrin), carbamate (propoxur) and organophosphate (fenitrothion) insecticides. The enzyme activities were compared against the Bora *Ae. aegypti* laboratory reference strain.

There was significant increase in MFO activity in resistant pyrethroid samples of *Ae. aegypti* in Bangkok (except Hauykwang), Chonburi, Nakhon Sawan, Nakhon Ratchasrima, and Chantaburi Provinces. In Hauykwang of Bangkok where the mosquitoes were resistant to deltamethrin and permethrin, nonspecific esterases and GST activities were significantly higher than Bora. In pyrethroid-resistant samples in Bangkok,

Table 1. Mean (± SEM) values of enzyme activities of adult *Aedes aegypti* mosquito populations collected across Thailand

District of mosquito collection (number of mosquito)	Insecticide exposed (mortality rate)[a]		MFO[b] (nmole product/ min/mg protein)	α-Esterase[b] (nmole/α naphthol/ min/mg protein)	β-Esterase[b] (nmole/β naphthol/ min/mg protein)	GST[b] (nmole CDNB/ min/mg protein)	AChE[b]
	Pyrethroids	Organophosphate, Carbamate					
Bora (46)	NA		29.76 ± 1.60	366.92 ± 20.98	54.81 ± 3.08	23.50 ± 3.07	13.39 ± 0.53
Bangkok (Central)							
Bangkoknoi (116)	Del (87.9), Per (18.5)	Fen (100), Pro (99.0)	137.63 ± 16.12[c]	1239.84 ± 229.31[c]	97.46 ± 13.71[c]	62.97 ± 5.58[c]	13.93 ± 0.83
Hauykwang (133)	Del (52.0), Per (29.0)	Fen (100), Pro (99.5)	42.09 ± 11.55	896.69 ± 41.74[c]	98.88 ± 1.84[c]	71.55 ± 4.68[c]	11.08 ± 0.11
Laksi (45)	Del (75.7), Per (26.1)	Fen (100), Pro (98.6)	125.60 ± 15.83[c]	390.45 ± 9.59[c]	153.04 ± 20.37[c]	91.10 ± 18.88[c]	15.20 ± 2.10
Ladkrabang (45)	Del (52.1),Per (51.3)	Fen (100), Pro (99.5)	133.41 ± 39.56[c]	401.43 ± 45.04	58.25 ± 4.26	103.92 ± 15.40[c]	15.34 ± 2.51
Rasburana (29)	Del (73.0), Per (48.8)	Fen (99.5), Pro (100)	53.83 ± 2.31[c]	370.43 ± 21.69	58.32 ± 4.41	31.11 ± 5.45	11.98 ± 0.15
Chonburi Province (East)							
Muang (47)	Del (86.6) Per (85.4)	Fen (100), Pro (97.1)	48.93 ± 3.06[c]	412.97 ± 41.59	43.52 ± 3.05	46.05 ± 6.11[c]	11.13 ± 0.47
Panusnikom (71)	Del (52.9), Per (11.2)	Fen (100), Pro (82.4)	51.32 ± 2.77[c]	374.54 ± 18.78	66.94 ± 2.98[c]	40.26 ± 6.42[c]	14.24 ± 2.00
Banglamung (59)	Del (43.4), Per (5.0)	Fen (99), Pro (88.3)	52.14 ± 1.81[c]	389.53 ± 12.15	94.72 ± 2.76[c]	55.34 ± 1.22[c]	15.34 ± 2.18
Sriracha (47)	Del (80.5), Per (77.6)	Fen (100), Pro (100)	47.95 ± 6.33[c]	386.24 ± 23.27	29.54 ± 2.15	22.69 ± 2.30	11.98 ± 0.19
Nakhon Sawan Province (North-Central)							
Muang (86)	Del (85.1), Per (53.1)	Fen (60.0), Pro (82.0)	41.57 ± 2.92[c]	1102.60 ± 59.27[c]	109.00 ± 6.67[c]	28.79 ± 1.77	11.52 ± 0.50
Mae Wong (105)	Del (54.5), Per (47.9)	Fen (50.5), Pro (69.1)	55.16 ± 2.02[c]	1304.67 ± 75.45[c]	202.21 ± 12.31[c]	69.61 ± 2.61[c]	11.30 ± 0.20
Mae Pern (127)	Del (52.0), Per (37.8)	Fen (18.4), Pro (99.0)	302.03 ± 46.68[c]	3876.68 ± 163.18[c]	442.59 ± 118.45[c]	168.04 ± 51.00[c]	10.46 ± 0.78
Krok Pra (82)	Del (85.3), Per (72.1)	Fen (59.6), Pro (86.0)	48.91 ± 4.50[c]	645.28 ± 49.48[c]	162.34 ± 9.75[c]	32.13 ± 5.18	11.78 ± 1.72
Taklee (93)	Del (74.3), Per (36.3)	Fen (98), Pro (100)	504.96 ± 106.38[c]	894.58 ± 27.05[c]	163.87 ± 36.61[c]	31.51 ± 2.48	12.70 ± 1.52
Nakhon Ratchasrima Province (Northeast)							
Prathai (75)	Del (81.6), Per (89.9)	Fen (41.8), Pro (100)	44.92 ± 4.76[c]	1102.89 ± 298.57[c]	186.92 ± 16.41[c]	34.33 ± 11.03	18.55 ± 0.54[c]
Kornburi (54)	Del (78.8), Per (80.0)	Fen (93.7), Pro (100)	54.08 ± 1.95[c]	404.32 ± 36.14	68.00 ± 11.36	34.87 ± 9.37	19.81 ± 0.69[c]
Kangsanamnang (50)	Del (89.6), Per (94.7)	Fen (68.3), Pro (100)	33.34 ± 4.36	400.19 ± 45.29	171.20 ± 10.81[c]	45.72 ± 5.28[c]	22.21 ± 1.05[c]
Serngsang (75)	Del (60.6), Per (69.3)	Fen (61.6), Pro (100)	71.10 ± 5.89[c]	434.66 ± 76.86	60.49 ± 11.49	42.19 ± 8.48[c]	24.34 ± 1.19[c]
Seekhew (55)	Del (96.9), Per (96.0)	Fen (58.8), Pro (100)	56.45 ± 2.26[c]	654.57 ± 82.34[c]	82.25 ± 5.26[c]	54.76 ± 14.07[c]	24.74 ± 1.52[c]
Songkhla Province (South)							
Muang (53)	Del (81.4), Per (61.4)	Fen (100), Pro (95.9)	29.36 ± 1.0	400.25 ± 10.52	55.85 ± 1.67	19.41 ± 0.88	15.96 ± 2.6
Singhanakorn (39)	Per (99.0), Per (94.2)	Fen (100), Pro (100)	32.06 ± 1.88	177.20 ± 7.94	47.05 ± 2.29	23.73 ± 0.77	13.86 ± 0.38
Bangklum (35)	Del (96.8), Per (98.0)	Fen (100), Pro (100)	25.79 ± 1.04	267.31 ± 10.86	45.42 ± 1.56	20.63 ± 0.78	9.19 ± 1.01
Chana (45)	Per (99.0), Per (94.1)	Fen (100), Pro (100)	28.39 ± 1.88	177.52 ± 9.32	49.39 ± 1.53	24.96 ± 1.12	12.50 ± 0.42
Hadyai (40)	Del (88.9), Per (84.0)	Fen (100), Pro (99.0)	31.57 ± 2.91	349.42 ± 27.01	49.01 ± 1.73	17.35 ± 0.84	11.39 ± 1.91
Chanthaburi Province (East)							
Muang (21)	Del (61.5), Per (80.4)	Fen (95), Pro (99.1)	118.14 ± 1.20[c]	265.19 ± 27.83	72.046 ± 5.62[c]	53.24 ± 2.39[c]	11.39 ± 0.83
Chiang Rai Province (North)							
Phan (30)	Del (97), Per (100)	Fen (100), Pro (100)	39.28 ± 11.04	414.88 ± 69.68	155.02 ± 19.21[c]	24.95 ± 3.54	13.91 ± 0.83

[a] Mortality rate are referred to Jirakanjanakit *et al.*, 2007. Del, deltamethrin, Per, permethrin, Fen, Fenitrothion, Pro, propoxur

[b] Enzymes assayed: MFO: mixed function oxidases, α- and β-esterases, GST: glutathione S-transferases, AChE: percent acetylcholinesterase activity after propoxur inhibition

[c] Significant increase in mean differences compared to the Bora susceptible strain ($p < 0.001$, Fisher's least significant difference test)

NA, not applicable

although there were increases in non-specific esterases in some districts, there was no consistent correlation with pyrethroid resistance.

Significant increases in nonspecific esterases activities were found among *Ae. aegypti* mosquitoes in Nakhon Sawan where they were resistant to pyrethroid and fenitrothion (except Taklee that was pyrethroid resistant, but fenitrothion sensitive) and some samples were either resistant or incipient resistant to propoxur. In Nakhon Ratchasrima province the fenitrothion resistance was observed with higher remaining AChE activity upon propoxur inhibition, Prathai and Seekhew also had significantly increased non-specific α- and β-esterases. The present study could not consistently point to a possible mechanism conferring propoxur carbamate resistance in Chonburi and Nakhon Sawan provinces. For GST activity, it was found elevated among *Ae. aegypti* samples without consistent correlation with pyrethroid resistance nor organophosphate resistance. In the south and Chiang Rai (north), there were low enzyme activities in *Ae. aegypti* mosquitoes, corresponding to low level resistance to pyrethroids and susceptibility to fenitrothion and propoxur.

It can been seen that the activity level of MFO increases were high in three out of five districts of Bangkok, when comparing to the Bora strain. In Nakhon Sawan, the high level activity increases were found for non-specific esterases, and high level MFO activity was found in two (Mae Pern and Taklee) out of five districts. Whereas high level increase in non-specific esterase activity was found only for Prathai district in Nakhon Ratchasrima province, other enzyme activities were not highly elevated. Existent enzyme activities within the local mosquito samples in different provinces could contribute to the different levels of activity increases.

Table 2 displays enzyme activities of *Ae. albopictus*, but there was no laboratory reference strain of *Ae. albopictus* available for comparison with field samples. However, it could be noted that there were less enzyme activities in *Ae. albopictus* when compared to *Ae. aegypti* samples. This was in accordance to the susceptibility of *Ae. albopictus* to all test insecticides and the low level fenitrothion resistance. Interestingly *Ae. albopictus* in Nakhon Sawan had noticeably low enzyme activities comparing to those in other areas.

DISCUSSION

The present study demonstrates that MFO could be predominant enzyme responsible for pyrethroid resistance in *Ae. aegypti* in Thailand. However, nonspecific esterases could play a role in pyrethroid resistance, particularly in Hauykwang of Bangkok. Esterase metabolism contributed to pyrethroid resistance in *An. albimanus* and pyrethroid tolerance in *An. gambiae* (Brogdon & Barber 1990b; Vulule *et al.*, 1999) and elevation of a-esterase is correlated to permethrin tolerance in *Ae. aegypti* (Flores *et al.*, 2005).

We observed no correlation of the enhanced GST activity in *Ae. aegypti*, although high GST activity could be due to DDT resistance as seen when samples from the pyrethroid resistant Bangkok and Nakhon Ratchasrima were tested (Jirakanjanakit, N.). Among these DDT-pyrethroid cross-resistance samples, there was no detected mutation in the sodium channel gene that would generate altered amino acids (unpublished data), thus the resistance was not due to knock down resistance.

There was significantly increased nonspecific esterase activity in fenitrothion organophosphate resistance in *Ae. aegypti* in Nakhon Sawan (north-central), while in Nakhon Ratchasrima (northeast) resistance could be conferred by insensitive AChE. Both nonspecific esterases and insensitive AChE are documented to play role in organophosphate resistance. For example, esterase-based mechanism was reported responsible for temephos organophosphate resistant *Culex quinquefasciatus* and *Ae. aegypti* (Ranasinghe &

Table 2. Mean (± SEM) values of enzyme activities of adult *Aedes albopictus* mosquito populations collected in Thailand

District of mosquito collection (number of mosquito)	Insecticide exposed (mortality rate)[a]		MFO[b] (nmole product/ min/mg protein)	α-Esterase[b] (nmole/α naphthol/ min/mg protein)	β-Esterase[b] (nmole/β naphthol/ min/mg protein)	GST[b] (nmole CDNB/ min/mg protein)	AChE[b]
	Pyrethroids	Organophosphate, Carbamate					
Songkhla Province (South)							
Singhanakorn (28)	Del (98.8), Per (100)	Fen (100), Pro (100)	19.75 ± 1.27	338.68 ± 4.96	46.39 ± 1.32	23.82 ± 6.67	13.91 ± 0.86
Chana (24)	Del (100), Per (100)	Fen (96.0), Pro (100)	12.91 ± 1.44	230.56 ± 3.68	25.61 ± 1.25	24.23 ± 7.70	11.40 ± 1.31
Hadyai, subdistrict Kor Hong (24)	Del (100), Per (100)	Fen (98), Pro (100)	32.82 ± 3.44	365.67 ± 1.09	38.22 ± 1.27	28.17 ± 4.12	10.57 ± 0.18
Hadyai, subdistrict Tungtumsao (32)	Del (100), Per (100)	Fen (100), Pro (100)	33.67 ± 4.55	312.42 ± 3.02	38.64 ± 1.76	25.72 ± 6.59	11.63 ± 0.17
Bangklum (22)	Del (100), Per (100)	Fen (100), Pro (100)	21.15 ± 2.01	311.69 ± 4.85	44.57 ± 1.68	24.47 ± 5.55	9.92 ± 0.52
Khanchanaburi Province (West)							
Tamaka (36)	Del (100), Per (100)	Fen (91.3), Pro (98.1)	31.20 ± 1.00	217.75 ± 3.07	27.03 ± 1.00	20.02 ± 2.69	11.29 ± 1.57
Nakhon Sawan Province (North-Central)							
Muang (12)	Del (98.9), Per (98.0)	Fen (100), Pro (100)	6.20 ± 1.00	97.70 ± 1.07	8.55 ± 0.19	22.02 ± 1.69	9.29 ± 0.53
Nakhon Ratchasrima Province (Northeast)							
Seekhew (19)	Del (100), Per (100)	Fen (98.6), Pro (100)	23.48 ± 1.25	212.07 ± 2.08	20.01 ± 6.37	20.09 ± 6.37	9.49 ± 0.11

[a] Mortality rate are referred to Jirakanjanakit *et al.*, 2007. Del, deltamethrin, Per, permethrin, Fen, Fenitrothion, Pro, propoxur

[b] Enzymes assayed: MFO: mixed function oxidases, α- and β-esterases, GST: glutathione S-transferases, AChE: percent acetylcholinesterase activity after propoxur inhibition

Georghiou, 1979; Rodríguez *et al.*, 2002), while in *C. tritaeniorhynchus* and *C. pipiens* the resistance was predominantly due to the insensitive AChE (Raymond *et al.*, 1986; Takahashi & Yasutomi, 1987).

The existent enzyme overproduction in mosquito through prior insecticide or chemical pressure in the area could constitute resistance against alternate insecticides. As previously noted, fenitrothion resistance in *An. albimanus* had selected for elevated esterase mechanism resulting in resistance against pyrethroids (Beach *et al.*, 1989; Brogdon & Barber, 1990b). In the present study, high mean values of esterases activity resulting in fenitrothion resistance in Nakhon Sawan could be explained by its history of insecticide uses of pyrethroids, temephos and malathion in the area. In Bangkok, high mean values of MFO, nonspecific esterases and GST could be attributed to the indiscriminate uses of household insecticide products, generating existing enzyme amounts. The districts of Banglamung, Panusnikom and Sriracha in Chonburi have the history of moderately pyrethroid use, also consistent with the resultant higher MFO in the area. In another instance, low usage of insecticide and agricultural chemicals in southern Thailand resulted in low enzyme activity among *Ae. aegypti* and rendered them susceptible to most test insecticides.

Our study demonstrated low detoxifying enzyme activity in the susceptible *Ae. albopictus*, even in the same area where *Ae. aegypti* was found resistance to pyrethroids and fenitrothion. However, exceptional low activity in Muang of Nakhon Sawan could reflect the limitation of sample size tested. The enzyme activity of *Ae. albopictus* was mostly lower than the Bora susceptible strain reflecting different basal activity in different species, and/or of different geographic origin as was also demonstrated for *Ae aegypti* in Songkhla comparing to the Bora strain.

It is evident in this study that *Ae. aegypti*, and to a lesser extent in the south, have developed pyrethroid and fenitrothion resistance with the increase of associated enzyme activity. The elevated MFO activity found covering throughout different regions where pyrethroid resistance was found could limit new insecticide candidates for use in *Ae. aegypti* control programs, potentially in Bangkok where mean values of all enzyme activities were mostly high. In Nakhon Sawan located in northeastern Thailand, elevated MFO activity and high production of nonspecific esterases could complicate the resistance management program of *Ae. aegypti* in the area. These findings should be informative and have important implications for effective control of dengue vector in Thailand.

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