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Resistance of *Aedes aegypti* (Diptera: Culicidae) in 2006 to Pyrethroid Insecticides in Indonesia and its Association with Oxidase and Esterase Levels

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Abstract: Three strains of *Aedes aegypti* collected in 2006 from three major cities in Indonesia, i.e., Bandung, Palembang and Surabaya, were tested to determine their resistance status to pyrethroid (permethrin and deltamethrin) and also the resistance mechanisms regarding three detoxifying enzymes, i.e., oxidase, esterase A and esterase B. The resistance level was expressed as Resistance Ratio (RR) compared to the susceptible VCRU strain. Results of this study showed that *Ae. aegypti* in Indonesia has developed tolerance to permethrin and deltamethrin, except in the Bandung strain, which was resistant to permethrin and deltametherin with RR_{90} 79.3 and 23.7, respectively. The study also suggested that detoxifying enzymes (oxidase, esterase A and esterase B) apparently were involved in the development of resistance in Bandung strain as indicated by the high level of activity of those enzymes in Bandung strain compared to other more susceptible strains. Although there is a possibility that other mechanisms, such as a target-site resistance mechanism, in Bandung strain were also involved.

Key words: *Aedes aegypti*, resistance, pyrethroids, oxidases, esterase, Indonesia

INTRODUCTION

Aedes aegypti is a primary vector for Dengue Fever/Dengue Haemorrhagic Fever (DF/DHF) virus, one of the major public health problems in many subtropical and tropical countries. For example, in Indonesia, a tropical country, several outbreaks of dengue have occurred since its first occurrence in 1968 and recently in 2006, 11173 cases with 1152 deaths were reported by the Ministry of Health Indonesia. In an effort to prevent or reduce dengue transmission, insecticides are used regularly to control *Ae. aegypti*. However the intensive and continuous use of insecticides to control this mosquito has caused the development of resistance of *Ae. aegypti* to many insecticides (Ponlawat *et al*., 2005; Macoris *et al*., 2003) and hinders the control programs (WHO, 1986). In Indonesia, organophosphates (temephos and malathion) and pyrethroids (permethrin and deltamethrin) have been widely used to control mosquito vectors, respectively since 1970s and 1980s. It is unfortunate that information on the susceptibility or resistance of *Ae. aegypti* to insecticides in Indonesia is limited. Among the few studies, Arief (2000) and Astari and Ahmad (2005) showed that *Ae. aegypti* collected in Bandung and several other cities in West Java, Indonesia (Ciamis, Purwakarta and Bogor) have become resistant to organophosphate and pyrethroids (deltamethrin and permethrin), unfortunately, however, their studies did not use a standard susceptible strain as control, resulting in the uncertainty of the resistant status. Whilst Brengues *et al*. (2003) reported a very high level of resistance to permethrin in *Ae. aegypti* collected from Semarang West Java Indonesia.

There are many reports detailing the role of metabolism in pyrethroid resistance in insects. For example, Vulule *et al*. (1999) reported elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae*, whereas Brengues *et al*. (2003) found that Indonesian strain *Ae. aegypti* collected from Semarang and Salatiga had elevated carboxyl esterase and monooxygenase levels associated with permethrin resistance.

This report describes the current resistance status of *Ae. aegypti* collected from three cities in Indonesia, i.e., Bandung (West Java), Palembang (South Sumatera) and Surabaya (East Java), to pyrethroids (permethrin and deltamethrin), as well as the possible mechanisms, which include the detoxifying enzymes, i.e., oxidase and esterase. However, different than previous workers, including Vulule *et al*. (1999) and Brengues *et al*. (2003),

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in the study reported here, we compare the activity levels of oxidase and esterase in four strains of *Ae. aegypti* before and after exposure to permethrin and deltamethrin.

MATERIALS AND METHODS

Mosquitoes: Three mosquito strains were tested in this study. They were collected from three cities located in three provinces in Indonesia in 2006, i.e., Bandung-West Java (BDG), Palembang -South Sumatera (PAL) and Surabaya-East Java (SBY). A pure susceptible Vector Control Research Unit (VCRU) strain was generously provided by Vector Control Research Unit, Universiti Sains Malaysia and was used as standard strain. All strains were reared in the Laboratory of Entomology, School of Life Sciences and Technology, Bandung Institute of Technology, before being used.

Insecticides: Insecticides used were permethrin 0.75% and deltamethrin 0.05%. Permethrin and deltamethrin were obtained from WHO in the form of insecticide impregnated paper.

Chemicals: Chemical substances used for biochemical assay were, among others, cytochrome-C, 3,3',5,5'-tetramethylbenzidine, α-naphthyl acetate, β-naphthyl acetate and O-Diamisidine (Fast Blue B) [Sigma-Aldrich, St. Louis, MO].

Resistance assay: Assay to determine resistance status of *Ae. aegypti* to permethrin and deltamethrin was conducted using method from WHO. Six groups of 15-25 adult male and female mosquitoes (2 days old) were exposed to insecticides for 5, 10, 15, 30, 45 and 60 min. The mosquitoes then were allowed to recover and after 24 h the number of dead mosquitoes was counted. The result was analyzed using probit analysis to determine LT_{90}. The resistance level was determined by comparing the LT_{90} values of mosquito tested to the LT_{90} values of VCRU strain.

Biochemical assay: Biochemical assay was conducted using two samples of mosquito, i.e., mosquitoes not exposed (control) and exposed for 60 min to permethrin and deltamethrin before treatment. The mosquitoes used were adult male and female mosquitoes (2 days old). Exposure time was based on maximum exposure time in resistance assay.

Total protein content was determined using the Bradford method. Individual mosquitoes were homogenized in 500 μL potassium phosphate buffer (PPB) 0.1 M pH 7.2 using pestle in 1.5 mL microtube. The homogenate was centrifuged at 13,000 rpm in 4°C for 10 min. The supernatant was stored in -80°C before use. To determine the total protein content, 25 and 50 μL from each mosquito sample were assayed and the results were compared to a standard curve using bovine serum albumin as protein standard.

Assay to determine the activity levels of oxidase, esterase A and esterase B were conducted using a method as described on the Center for Disease Control website (http://www.cdc.gov/ncidod/wbt/resistance/assay/microplate/index.htm) with modification. Samples containing 5 μg protein was used in each assay. In oxidase assay, TMBZ and H_2O_2 3% were added to the sample and the mixture was incubated for 5 min. Then, H_2SO_4 2 M was added as stop solution and the results were measured using Microplate Reader Spectrophotometer Model 550 (Biorad) at 450 nm. The results were compared to a standard curve of cytochrome-C. Activity level of oxidase was expressed in Unit/mg protein.

In esterase assay, α and β-naphthyl acetate were used as substrate for esterase A and esterase B, respectively. The mixture was incubated for 30 min and then O-diamisidine and SDS 1% were added as dye agent and stop solution, respectively. The resulted color can be measured using microplate reader spectrophotometer at 570 nm. The results were compared to standard curves of α and β-naphthol, the product of α and β-naphthyl acetate hydrolysis by esterase A and B, respectively. Activity levels of esterase A and esterase B were expressed in μmol-product/min.mg protein.

RESULTS AND DISCUSSION

Result of the assay showed that Bandung-West Java strain was the most resistant strain. It was resistant to permethrin and deltamethrin with RR_{90} 79.3 and 23.7, respectively (Table 1). Palembang-South Sumatera strain was slightly resistant to permethrin (RR_{90} 11.1) but still susceptible to deltamethrin (RR_{90} 2.2). Surabaya-East Java strain was still susceptible to permethrin and deltamethrin (RR_{90} 8.6 and 2.5, respectively). The definition of resistance was based on Valles *et al.* (1997), i.e., that a population is considered resistant if RR is more than 10. However, although Surabaya-East Java strain was considered susceptible to permethrin and deltamethrin and Palembang-South Sumatera strain was considered susceptible to deltamethrin, the higher RR compared to VCRU strain suggested that those strains have already developed tolerance to permethrin and deltamethrin and may develop resistance in the future. This is likely to

Table 1: Resistance levels of *Ae. aegypti* to permethrin and deltamethrin

	Permethrin		Deltamethrin	
Strains	LT_{90}	RR_{90}	LT_{90}	RR_{90}
VCRU	12.8	1.0	22.1	1.0
PAL	141.8	11.1	48.6	2.2
SBY	109.9	8.6	55.8	2.5
BDG	1015.1	79.3	524.5	23.7

VCRU: Vector Control Research Unit, a susceptible pure strain of *Ae. aegypti*, PAL: Palembang, SBY: Surabaya, BDG: Bandung, LT_{90}: Lethal Time 90%, min, RR_{90}: Resistance Ratio at LT_{90}

happen if pyrethroids keep being used to control the population, thus allowing the resistant individuals to survive and reproduce. Many studies have shown that resistance level of a population can increase in several generations when selection using insecticides was applied to the population (Chareonviriyaphap *et al*., 2002; Leksono, 1997).

The result of this study appears consonant with that of Brengues *et al*. (2003) interestingly however, reported a very high resistance ratio (296-fold) occurring in Semarang strain, whereas in the present study, the highest resistance ratio was only 79.3-fold, occurring in Bandung Strain. The reason for this difference resistance levels is probably due to the different frequency of insecticides use between those areas.

Resistance of Bandung-West Java strain to permethrin and deltamethrin may be cross resistance, which is resistance to different insecticides that have similar structure or mode of action. Permethrin and deltamethrin differ mainly by the α-cyano in deltamethrin that is the characteristics of type II pyrethroids. The α-cyano gives steric effect on deltamethrin that causes deltamethrin to be more stable and difficult to be hydrolyzed on its ester bond (Kerkut and Gilbert, 1985).

Cross resistance to several types of pyrethroid insecticides have been found in many mosquito species from many countries, for example *An. stephensi* and *An. culicifacies* from India (Ganesh *et al*., 2003). However, there were no indications of cross resistance in other strains, because of the low levels of resistance in those strains to permethrin as well as deltamethrin. Resistance of Bandung strain to permethrin and deltamethrin may be due partly to the increased level of activity of detoxifying enzymes. Many studies have found that elevated levels of activity of those enzymes (oxidases, esterase A and esterase B) can be related to an increase in tolerance to insecticides in mosquitoes, suggesting the role of detoxifying enzymes in the development of resistance to pyrethroids and organophosphate insecticides in mosquitoes (Chareonviriyaphap *et al*., 2003; Macoris *et al*., 2003; Yaicharoen *et al*., 2005). The results of biochemical assay in control mosquitoes (not exposed to insecticides) showed that activity levels of detoxifying

Table 2: Activity levels of oxidase of four strains of *Ae. aegypti* before and after exposure to permethrin and deltamethrin

	Oxidase (Unit mg^{-1} protein)					
Strains	N	Control	N	Permethrin	N	Deltamethrin
VCRU	30	0.777±0.410	22	0.786±0.089	30	0.932±0.417
PAL	27	0.843±0.153	29	0.851±0.131	29	0.787±0.245
SBY	38	0.839±0.353	39	0.949±0.537	39	0.965±0.395
BDG	29	1.153±0.248**	30	1.072±0.240	42	0.563±0.121*

*: Significantly different ($p<0.05$) from control (not exposed) of the same strain, **: Significantly different ($p<0.05$) from VCRU in the same column, N: Number of mosquitoes tested

Table 3: Activity levels of esterase A of four strains of *Ae. aegypti* before and after exposure to permethrin and deltamethrin

	Esterase A (μmol-product min^{-1} mg^{-1} protein)					
Strains	N	Control	N	Permethrin	N	Deltamethrin
VCRU	29	0.087±0.035	28	0.168±0.045*	40	0.164±0.053*
PAL	25	0.138±0.038**	29	0.147±0.031	27	0.147±0.041
SBY	34	0.100±0.062	35	0.122±0.055	37	0.153±0.049*
BDG	41	0.138±0.054**	40	0.141±0.051	45	0.147±0.057

*: Significantly different ($p<0.05$) from control (not exposed) of the same strain, **: Significantly different ($p<0.05$) from VCRU in the same column, N: Number of mosquitoes tested

Table 4: Activity levels of esterase B of four strains of *Ae. aegypti* before and after exposure to permethrin and deltamethrin

	Esterase B (μmol-product min^{-1} mg^{-1} protein)					
Strains	N	Control	N	Permethrin	N	Deltamethrin
VCRU	28	0.057±0.013	26	0.127±0.018*	39	0.108±0.031*
PAL	27	0.068±0.019**	29	0.077±0.016	28	0.081±0.014*
SBY	22	0.031±0.006**	36	0.086±0.043*	19	0.085±0.022*
BDG	36	0.112±0.034**	36	0.090±0.026b	45	0.119±0.046

*: Significantly different ($p<0.05$) from control (not exposed) of the same strain, **: Significantly different ($p<0.05$) from VCRU in the same column, N: Number of mosquitoes tested

enzymes (oxidases, esterase A and esterase B) before exposure to insecticides were significantly higher in Bandung strain compared to the susceptible VCRU strain (Table 2-4), suggesting that those enzymes might be involved in the development of resistance in Bandung strain. Both oxidase and esterase are capable of detoxifying pyrethroids through different mechanisms. Esterases detoxify pyrethroids through hydrolysis of ester bond, while oxidases detoxify pyrethroids through hydroxylation (adding -OH) or oxidation of ester bonds of pyrethroids (Kerkut and Gilbert, 1985). The high level of resistance in Bandung strain presumably was caused by a combination of various detoxifying enzymes available in the insect's body, which together can readily detoxify the insecticides entering the insect's body before reaching their target site.

Exposure to permethrin and deltamethrin caused a change in the activity levels of the enzymes, as can be seen in Table 2-4. Exposure to permethrin and deltamethrin increased the activity levels of the enzymes in all strains, except for oxidase in the Bandung strain,

which decreased after exposure to deltamethrin. Overall, increase in the more resistant strain (Bandung) was lower than the more susceptible strain (VCRU, Surabaya and Palembang). It is hypothesized that some of the insecticides entering the insect's body can be detoxified directly by the detoxifying enzymes, while the rest are bound by receptors and induce the production of more enzymes. In Bandung strain, it is probable that because the activity level of the enzymes were higher than in other strains, more insecticides could be detoxified and less insecticides were available to induce the production of the enzymes. A plausible explanation is that in Bandung strain, another mechanism of pyrethroid resistance is involved, i.e., changes in target site sensitivity. For example, Brengues *et al*. (2003) reported that in a highly resistant *Ae aegypti* strain from Semarang (269-fold levels of resistance to permethrin), the activity level of esterases and monooxygenases were not significantly higher than those of controls.

In conclusions the results of this research suggested that the resistance level of *Ae. aegypti* to pyrethroids (permethrin and deltamethrin) in Indonesia is still low, except for Bandung strain. However, the other strains tested already showed increased tolerance to both insecticides, indicating that a portion of the population has already developed resistance to the insecticides. If treatment using insecticides is still applied continuously, there is the possibility that those populations will become resistant in the future. Moreover, the involvement of oxidase and esterase and possibly changes in target sensitivity in the development of resistance, especially in Bandung strain, may pose a threat to the possibility of cross resistance to other classes of insecticides, such as temephos (organophosphate), which is widely used in Indonesia to control mosquitoes. Therefore, continuous monitoring of resistance levels of mosquito to insecticides is very important and must be done to prevent the development of resistance in the future that consequently might lead to more severe DF/DHF outbreaks.

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REFERENCES

Arief, R., 2000. Resistance test of *Aedes aegypti* from four cities in West Java to the Propoxur, Malathion, Permethrin and Esbiothrin. B.Sc. Thesis, Bandung Institute of Technology (In Indonesian).

Astari, S. and I. Ahmad, 2005. Insecticide resistance and effect of piperonyl butoxide as a synergist in three strains of *Aedes aegypti* (Linn.) (Diptera: Culicidae) on Insecticides permethrin, cypermethrin and d-allethrin. Bull. Health Stud., 33: 70-73.

Brengues, C., N.J. Hawkes, F. Chandre, L. McCarroll, S. Duchon, P. Guillet, S. Maguin, J.C. Morgan and J. Hemingway, 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* correlated with novel mutations in the voltage-gated sodium channel gene. Med. Vet. Entomol., 17: 87-94.

Chareonviriyaphap, T., P. Rongnoparut and P. Juntarumporn, 2002. Selection for pyrethroid resistance in a colony of *Anopheles minimus* species A, a malaria vector in Thailand. J. Vector Ecol., 27: 222-229.

Chareonviriyaphap, T., P. Rongnoparut, P. Chantarumporn and M.J. Bangs, 2003. Biochemical detection of pyrethroid resistance mechanisms in *Anopheles minimus* in Thailand. J. Vector Ecol., 28: 108-116.

Ganesh, K.N., J. Urmila, P. Guillet and L. Manga, 2003. Pyrethroid susceptibility and enzyme activity in two malaria vectors, *Anopheles stephensi* (Liston) and *A. culicifacies* (Giles) from Mysore, India. Indian J. Med. Res., 117: 30-38.

Kerkut, G.A. and L.I. Gilbert, 1985. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 12, Insect Control Pergamon Press, Oxford.

Leksono, A.S., 1997. Perubahan tingkat toleransi larva *Aedes aegypti* (L.) (Diptera: Culicidae) terhadap malathion dengan seleksi 8 generasi. B.Sc. Thesis, Bandung Institute of Technology (In Indonesian).

Macoris, M.L.G., M.T.M. Andrighetti, L. Takaku, C.M. Glasser, V.V. Garbeloto and J.E. Bracco, 2003. Resistance of *Aedes aegypti* from Sao Paolo Brazil to organophosphate insecticides. Mem. Inst. Oswaldo Cruz., 98: 703-708.

Ponlawat, A., J.G. Scott and L.C. Harrington, 2005. Insecticides susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. J. Med. Entomol., 42: 821-825.

Valles, S.M., P.G. Koehler and R.J. Brenner, 1997. Antagonism of fipronil toxicity by Piperonyl butoxide and S,S,S-tributyl phosphorotrithioate in the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol., 90: 1254-1258.

Vulule, J.M., R.F. Beach, F.K. Atieli, J.C. McAllister, W.G. Brogdon, J.M. Roberts, W.A. Hawley and R.W. Mwangi, 1999. Elevated oxidase and esterase levels associated with permethrin Tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-impregnated nets. Med. Vet. Entomol., 13: 1-6.

WHO (World Health Organization), 1986. Resistance of vectors and reservoirs of disease to Pesticides. WHO Tech. Ser. 737.

Yaicharoen, R., R. Kiatfuengfoo, T. Chareonviriyaphap and P. Rongnoparut, 2005. Characterization of deltamethrin resistance in field populations of *Aedes aegypti* in Thailand. J. Vector Ecol., 30: 144-150.